An investigation of GTN and NO related therapeutics in the treatment of acute stroke

By

Mark Willmot, MBChB, MRCP

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Abstract

Background: High blood pressure is common in acute stroke and has been linked with poor outcome. Hence, outcome might be improved by lowering blood pressure. This thesis investigates the potential for glyceryl trinitrate, a nitric oxide donor, for lowering blood pressure in acute stroke. Methods: A systematic review was employed to clarify the relationship between outcome and BP in observational studies. Next two systematic reviews of animal studies using nitric oxide therapeutics in experimental stroke were performed to assess the effects on infarct volume and cerebral perfusion. Finally, two randomised controlled clinical trials of glyceryl trinitrate were performed in acute stroke patients to measure the systemic and cerebral haemodynamic effects. Results: In observational studies high blood pressure in acute stroke was associated with subsequent death, death or dependency, and death or deterioration. In experimental stroke nitric oxide sources and selective nitric oxide synthase inhibitors significantly reduced stroke volume. Glyceryl trinitrate lowered peripheral and central blood pressure and increased aortic compliance when given <48 hours from stroke. Glyceryl trinitrate did not alter quantitative measures of cerebral perfusion despite significantly lowering blood pressure <5 days from stroke. Conclusion: High blood pressure is a therapeutic target in acute stroke and animal data support the use of nitric oxide sources for lowering blood pressure. It is feasible to use glyceryl trinitrate for this purpose since it does not compromise cerebral perfusion. Trials now need to urgently assess the effect of lowering BP on outcome.

Chapter 1.

Background

1.1 Stroke – the problem

Stroke may be defined as a sudden neurological deficit of presumed vascular origin which lasts for >24 hours or leads to death.[1] There are 4.5 million deaths a year from stroke and more than 9 million stroke survivors worldwide.[2] In addition, approximately one in four men and one in five women aged over 45 will have a stroke if they survive to 85 years.[2] As a consequence, the impact of stroke on society is enormous. In the UK stroke services account for 4-6% of the NHS budget, excluding social service and carer costs.[2] This burden is likely to increase in the future, as incidence increases with age and the population ages.[3] In the past, both the medical profession and the general public have considered stroke to have a low priority. However in recent years this perception has changed as awareness of the impact of stroke on society has increased. As a result, UK stroke services have been identified as an important area for development in the National Service Framework for Older People.[4]

1.1.1 Risk Factors

Both 'modifiable' and 'non-modifiable' risk factors have been linked to stroke in observational studies. Age is one of the strongest 'non-modifiable' risk factors; for example, stroke incidence rises from ~3 per 10,000 population in the 30 – 40th decades to ~300 per 10000 in the 80 – 90th decades.[5] In addition, males have a higher risk, although because women tend to live longer they are more likely to die from stroke.[6] Also, UK mortality is worst in Black Africans, Black Caribbean's and Asians[7], partly due to higher prevalence of diabetes and hypertension in these populations.[8] Elevated blood pressure (BP) is the strongest of the 'modifiable' factors.[8] In one meta-analysis every 7.5mmHg increase in diastolic BP increased stroke incidence by 46%.[9] Other 'modifiable' factors that have been linked to stroke include; smoking[10], diabetes[11], atrial fibrillation (AF)[8], alcohol consumption[12], high salt intake[13], low social status, obesity[14], plasma

homocysteine[15], elevated white cell count[16], fibrinogen levels[17] and haematocrit.[18] Some of these associations have not been widely accepted as causal.[19]

1.1.2 Types of stroke

Stroke can be classified by pathology, aetiology, duration, location, or clinical syndrome.[5] There are three main pathological types; ischaemic stroke (IS), primary intracerebral haemorrhage (PICH) and sub-arachnoid haemorrhage (SAH).

a) IS

Arterial occlusion accounts for 85% of stroke presentations.[20] IS may be further classified by aetiological sub-type according to the TOAST classification:[21]

i) Large artery atherosclerosis. The major brain arteries and carotids are prone to atherosclerotic narrowing, especially at sites of origin or bifurcation. This can cause infarction by reducing blood flow distal to the point of stenosis or by acting as a nidus for platelet aggregation leading to distal embolisation.

ii) Small vessel occlusion. Lipohyaline degeneration or micro-atheroma in deep perforating arteries can lead to infarcts within sub-cortical areas, such as the basal ganglia, thalamus and internal capsule. So called ‘lacunar strokes’ account for up to 25% of IS.[22]

iii) Cardioembolism. Embolism from thrombi that form in the heart and aorta account for approximately 30% of IS.[22] A variety of cardiac sources have been identified, including valvular lesions, rhythm disturbances and hypokinesis of the left ventricle. Non-rheumatic AF is the commonest cause of cardioembolic stroke in the west. Untreated AF results in an absolute risk of stroke of 5% per year.[8]

iv) Stroke of undetermined aetiology. When no likely aetiology can be determined despite a complete evaluation.

b) PICH

10% of strokes are due to PICH. The major causal factors can be divided into 3 categories:[8]

i) Anatomical factors. Bleeding caused by defects in the brain vasculature, for example; microaneurisms, arteriovenous malformations, amyloid angiopathy.

ii) Haemodynamic factors. Hypertension is the most powerful risk factor for both ischaemic and haemorrhagic stroke. Chronic high BP underlies microaneurism formation and rupture, leading to PICH at well-defined sites, such as the basal ganglia, pons and sub-cortical white matter.

iii) Haemostatic factors. Bleeding caused by problems with platelet or coagulation function; for example, with thrombolysis or anticoagulant usage.

c) SAH

Leakage of blood from vessels as they transverse the subarachnoid space. Although the World Health Organisation (WHO) definition of stroke includes SAH,[23] for the purposes of this thesis the term ‘stroke’ refers to IS and PICH only.

1.1.3 Bamford classification

The Bamford classification defines several clinical syndromes.[24] This system works well at the bedside[25] and may be used to predict prognosis;[20,26]

i) Total Anterior Circulation Syndrome (TACS). A triad of a unilateral weakness (hemiparesis), a visual field defect (hemianopia) and higher cerebral dysfunction (e.g. speech difficulty, sensory neglect, difficulty performing complex tasks). Usually a result of damage to a large part of a cerebral hemisphere involving the proximal anterior cerebral circulation. The prognosis is poor with 60% mortality and 35% dependency at 1 year.[20]

ii) Partial Anterior Circulation Syndrome (PACS). Patients present with 2 out of 3 of hemiparesis, hemianopia or higher cerebral dysfunction. Alternatively, an isolated defect in higher cerebral dysfunction or a weakness in one area out of face, arm or leg. Usually a result of stroke involving a distal portion of the anterior cerebral circulation. Prognosis is better than TACS with 20% mortality and 30% dependency at 1 year.[20]

iii) Lacunar Syndrome (LACS). Present with hemiparesis and / or sensory symptoms alone in at least two regions of face, arm and leg. Caused by stroke located in sub-cortical areas (see above). Often associated with hypertension and diabetes. The prognosis is relatively good with10% dead and 25% dependent at 1 year.[20]

iv) Posterior Circulation Syndrome (POCS). Patients present with visual problems (isolated visual field defect or loss of eye movement), poor swallowing, coordination problems (ataxia) or bilateral weakness. Usually due to a lesion in the posterior circulation, involving the occipital lobes, cerebellum or brainstem. 1year mortality and dependency are both 20%.[20]

1.1.4 Pathophysiology of IS

An IS begins with thrombotic occlusion of a cerebral artery, or less often a reduction in perfusion due to a severe stenosis. Typically the thrombotic process is initiated by rupture of an atherosclerotic plaque and injury to the vascular endothelium.[27] Exposed sub-endothelial structures activate platelet aggregation and the blood coagulation cascade, leading to thrombus formation and vessel occlusion. The brain is especially vulnerable to vascular occlusion because it requires a constant supply of oxygenated blood and glucose to meet it's high energy demands (equivalent to a 20 Watt light bulb).[8] If cerebral perfusion falls below ~20ml/min/100g there is a rapid loss of neuronal electrical function.[28] Restoration of blood flow at this stage can reverse the situation because the neurones involved remain viable. However, if cerebral blood flow (CBF) falls below a critical level (~10ml/min/100g) the situation becomes irreversible.[29] At this point aerobic respiration fails and neurones become unable to generate sufficient adenosine triphosphate (ATP) to maintain energy dependent cellular ion homeostasis. Intracellular calcium levels rise because of failure of ATP reliant ion pumps, increased cellular permeability and release of excitatory neurotransmitters (glutamate).[30] Calcium then triggers cell death by activating enzymes, such as phospholipases, proteases and nitric oxide synthase (NOS, see section 1.3.3). Furthermore, tissue hypoxia leads to the development of acidosis (through lactate formation) and the formation of free radicals (O^{2-}, OH•) that further contribute to irreversible neuronal damage.[30]

In theory, the presence of 2 separate CBF thresholds leads to the development of an irreversibly damaged infarct core surrounded by an area of brain, called the ischaemic penumbra, which is in electrical failure.[29] Possibly penumbra could be salvaged if perfusion is restored (e.g. thrombolysis) or if agents that protect the vulnerable neurones from further damage are administered (neuroprotection).[31] Positron Emission

Tomography (PET) studies in humans suggest that the penumbra may remain viable for up to 17 hours.[32]

1.1.5 Pathophysiology of PICH

Several factors can lead to PICH (see section 1.1.2b). In young patients arteriovenous malformations and microangiomas are the commonest causes, whereas in older people hypertension and amyloid angiopathy are more frequently responsible.[8] When an intracranial vessel ruptures, blood immediately disrupts neurones in the vicinity of the bleed. In addition, the surrounding zone becomes ischaemic because of direct mechanical compression, oedema, loss of cerebral autoregulation and the presence of vasoconstrictor substances in extravasated blood.[33] Since the brain is encased in a rigid skull the presence of a large haematoma mass can lead to a fatal rise in intracranial pressure.

1.2 Treatment of Stroke – the current situation

1.2.1 Acute medical treatment of IS

a) Antiplatelet therapy

Two large trials found that aspirin (160-300mg daily) given within 48 hours of onset of IS reduces the risk of subsequent death or dependency.[34,35] However, the effect of aspirin is small (13 less patients dead or disabled per 1000[34] and probably reflects reduced risk of early recurrence rather than a direct effect on the stroke itself. Other antiplatelet agents that are effective in secondary prevention have not been studied in large trials.

b) Thrombolysis

Thrombolytic agents have been long established as a treatment for acute myocardial infarction. By contrast, evidence for efficacy in acute IS has only recently become available. Commonly used agents include: streptokinase, recombinant tissue plasminogen activator (rTPA) and urokinase. A recent systematic review of 18 trials found a significant reduction in death or dependency at 3 months (odds ratio, OR 0.84, 95% confidence interval, CI 0.75 to 0.95) when these drugs were administered up to 6 hours after onset.[36] However, there was a net increase in deaths during the first 10 days due to secondary cerebral haemorrhage. Interestingly, administration within 3 hours of onset was more effective and did not have an adverse effect on death.[36] Hence, it seems reasonable to use thrombolytic agents within the 3-hour time window on patients that are similar to those included in the trials. Further studies are required to determine the patients most likely to benefit and the latest time window for administration.[37]

c) Neuroprotection

Many compounds that provide effective neuroprotection in animal models have progressed to full human studies. However, as of 2001 more than 37 potential neuroprotective agents had been studied in over 114 clinical trials and yet none is in routine clinical use.[38]

1.2.2 Acute treatment of PICH

There is no acute medical treatment for PICH, although agents that reduce intracranial pressure are often given, e.g. osmotic diuretics. In a recent phase II trial, recombinant activated factor VII (eptacog alfa) reduced haematoma expansion, mortality, and disability when given within 4 h of ICH onset; a phase III trial (the FAST trial) is now in progress.[39] Craniotomy and evacuation of supratentorial PICH was found to be harmful in one systematic review.[40] However, this did not include findings from a recent randomised

controlled trial (1033 patients) that assessed a policy of initial conservative treatment against early surgery.[41] Using a prognosis based dichotomous outcome, assessed at 6 months follow up, there was no significant difference between treatment groups. Pre-specified sub-group analysis revealed that early surgery conferred an advantage when the lesion was located superficially (<1cm deep). It is widely accepted that selected patients with infratentorial haemorrhage benefit from craniotomy, however this has not been formally tested in a trial.[8]

1.2.3 Secondary Prevention for IS and PICH

In the first year after IS or TIA there is an 8% risk of recurrent cerebrovascular events.[42] There are several pharmacological and non-pharmacological ways of reducing this threat:

a) Antiplatelet therapy

Treatment with an antiplatelet agent is an essential part of the armamentarium for secondary prevention of IS. Three drugs in common use have independent modes of action on platelets: Aspirin irreversibly inhibits cyclo-oxygenase, clopidogrel is an indirect antagonist of the adenosine diphosphate (ADP) receptor, and dipyridamole prevents the uptake by red cells of adenosine (which has antiplatelet properties). A recent meta-analysis (21 trials) found that allocation of antiplatelet therapy to stroke patients resulted in 36 fewer serious vascular events per 1000 treated.[43] This benefit included a highly significant reduction in non-fatal stroke (25 fewer per 1000) that exceeded the estimated excess risk of bleeding (1-2 per 1000).[43] Aspirin was the most widely studied antiplatelet drug, with doses of 75-150 mg as effective as higher daily doses. Interestingly, clopidogrel was slightly more effective than aspirin in a large trial.[44] However it is not cost effective for initial treatment and is usually reserved for patients who are aspirin

intolerant. Combination of aspirin with other antiplatelet agents may be an alternative since it is presumed that the individual effects on platelets will be additive. Aspirin and dipyridamole were found to have an additive effect as compared with aspirin alone in two trials.[45,46] However, the combination of aspirin with clopidogrel did not confer any additional benefit and led to increased bleeding in the MATCH study.[47] Further work is ongoing in this area with the CHARISMA and PROFESS trials.

b) Anticoagulant therapy

Several trials have demonstrated superiority of full anticoagulation with warfarin over aspirin or placebo for the prevention of cardioembolic IS. A recent meta-analysis of antithrombotic therapy in AF showed that adjusted dose warfarin reduced stroke by about 60%, with absolute risk reductions of 3% a year for primary prevention and 8% per year for secondary prevention.[48] In addition, warfarin reduced the risk by about 40% relative to aspirin therapy. Treatment with full dose anticoagulation carries with it the risk of inducing major bleeding, although in the European AF Trial (EAFT) this was only 2.8% per year.[49] Titration to an International Normalised Ratio of 2.0 – 3.0 is generally accepted as the optimum and safest level, however patients with mechanical heart valves require a higher range (2.5 – 4.5).[42]

c) Surgery for carotid stenosis

Large-scale trials have provided data that allow selection of appropriate stroke patients for carotid endartrectomy.[50,51] Both these studies found that the immediate risk of surgery was worth trading off against the long-term risk of stroke (without surgery) when high-grade stenosis was present (70 to 99%). The benefits were found to persist with surgery up to 12 months after the most recent cerebral event.[42]

d) BP reduction

Treatment with antihypertensive agents not earlier than one week after the onset of IS or PICH was associated with reduced risk of subsequent stroke (fatal and non-fatal), myocardial infarction and total vascular events in one systematic review.[52] The majority of the included studies recruited patients irrespective of their BP, suggesting that there may be no treatment threshold. In addition, one trial found that dual therapy was superior to monotherapy, implying that the benefits relate, in part, to the magnitude of BP reduction.[53] By contrast with primary prevention studies[54], significant differential effects were observed between drug classes.[52] For example, diuretics and (angiotensin converting enzyme (ACE) inhibitors appeared to be beneficial, whereas β blockers had no effect on any outcome. This could be attributed to paucity of data, although studies with β blockers in the acute stroke period had similarly disappointing findings.[55]

e) Cholesterol Reduction

Individual studies have shown that interventions which reduce cholesterol levels, especially 'statins', are of benefit in reducing coronary heart disease and stroke events in those with a history of cardiovascular disease. Current clinical practise in IS patients is based on extrapolations and subgroup analyses from these studies. A recent systematic review of 5 trials involving 1700 patients found no evidence of a difference in stroke recurrence between the treatment and placebo groups for those with a previous history of stroke or TIA.[56] However, this review did not include the Heart Protection Study[57], which subsequently found a significant reduction in vascular events in a subgroup of 3280 patients with cerebrovascular disease. Ongoing trials are continuing to examine this question.[58]

f) Management of risk factors

'Modifiable' risk factors should be identified and corrected. Smoking should be strongly discouraged since the risk of stroke in a smoker reduces to that of a non-smoker around 3 to 5 years after stopping.[42] In addition, patients should normalize weight, exercise regularly, moderate alcohol intake and eat a healthy diet.[20]

1.2.4 Rehabilitation

Rehabilitation involves the coordinated use of medical, social, educational and vocational measures to train or retrain individuals to the highest levels of functional ability.[59] For hospitalised patients this is best achieved in an organised (stroke unit) setting, since this environment is associated with the best outcome.[60] However, it is not clear which components of 'organised stroke unit care' account for these findings. A recent systematic review identified significant improvements in primary activities of daily living (ADL), extended ADL, and social participation for patients receiving comprehensive occupational therapy (OT).[61] Likewise, greater physiotherapy input results in reduced death and deterioration.[62] However, cardiovascular exercise interventions[63] and physiotherapy using the Bobath technique both failed to alter stroke outcome in other meta-analyses.[64] Similarly, speech and language therapy was found not to be effective in one systematic review of 12 trials.[65] More rehabilitation research needs to be conducted.

1.3 Nitric Oxide – a potential therapeutic target in stroke

The therapeutic modulation of nitric oxide (NO) has generated considerable interest in recent years as a new strategy for the treatment of many disease processes where NO is implicated. The purpose of this section is to discuss the theoretical basis behind the use of NO related therapeutics for treatment of stroke.

1.3.1 NO synthesis

In 1980 it was demonstrated that endothelial cells release a potent vasodilating substance called endothelium derived relaxing factor (EDRF).[66] It was later established that NO accounted for the biological activity of this substance[67], and that many other types of eukaryotic cells were also capable of synthesising it.[68] NO is a gas that is water-soluble and can freely diffuse across biological membranes. It is synthesised from L-arginine (L-Arg) by the enzyme NOS in the presence of several co-factors, including nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleide (FMN), and tetrahydrobiopterin (BH_4).

Three distinct isoforms of NOS have been isolated, and these can be divided into constitutive and inducible subtypes. The constitutive forms of NOS are expressed under 'basal' conditions and are labelled neuronal NOS (nNOS, NOS I) and endothelial NOS (eNOS, NOS III). These are calcium dependent enzymes and are preferentially, but not exclusively, associated with the nervous system and endothelium respectively. In contrast, a calcium independent, inducible NOS (iNOS, NOS II) exists that is produced by many cell types in response to endotoxins or cytokines. Expression of this protein occurs over a number of hours and results in higher concentrations of NO than is produced by the constitutive forms of NOS.[69]

1.3.2 General functions of NO

NO is a 'multimodal' molecule with complex actions that can be either detrimental or beneficial in differing pathological and physiological situations. The main physiological effects of NO are mediated through activation of soluble guanylate cyclase (SGC) in smooth muscle cells, platelets, leucocytes, neurones and other cells to increase levels of the secondary messenger cyclic GMP (cGMP)[70] (Figure 1.1). However, NO is a free radical and by virtue of it's unpaired electron it can interact directly with other molecules to produce cGMP independent effects. For example, NO can react with reactive oxygen species like superoxide anion radical ($O2^{\cdot-}$) to produce peroxynitrite. This can disrupt many cellular processes through direct oxidising effects and by altering protein conformation through a process termed nitration.[71]

Under physiological conditions, and when released in small controlled amounts, NO is involved in the regulation of several important biological processes. In the cardiovascular system, NO acts as a mediator of vascular tone and blood flow.[72] The release of NO within the vascular tree primarily occurs in response to increased shear stress on blood vessels.[73] Vasodilation then occurs via activation of cGMP in smooth muscle cells. In this way NO not only participates in the control of vascular tone, but contributes to BP control[74] and regulates blood flow distribution in vascular beds[75] (e.g., brain, skeletal muscle[76], penile erection[77]). In addition, NO acts to protect the vascular endothelium against atherosclerosis. It may achieve this through several processes including; suppression of leukocyte migration and adhesion to endothelium[78,79], antiproliferative effects on vascular smooth muscle cells[80], and reducing lipoprotein oxidation and absorption into blood vessels.[81] Moreover, NO has antiplatelet properties, and protects against thrombosis by inhibiting platelet adhesion and aggregation.[75]

NO has many additional roles outside of the cardiovascular system. Interestingly, it appears to be able to either promote or prevent cellular inflammation and death. Evidence shows that NO can be anti-inflammatory through several activities including; inhibition of the maturation of cytokines, such as interleukin -18 and interleukin -1ß[82], blocking effects of interferon γ[83], and preventing the expression of cellular adhesion molecules via effects on nuclear transcription factor - κB (NF-κB).[83,84] Alternatively, NO can be pro-inflammatory by increasing expression of certain cytokines like tumour necrosis factor (TNFα) and interleukin – 6.[83] Such disparities are also present in the case of cell death. For instance, NO is known to promote apoptosis by activation of caspases[85] and by direct damage to mitochondria, in association with peroxynitrite species.[86] Alternatively, however, NO can enhance neuronal survival by attenuation of Ca^{2+} influx via antagonism of the NMDA glutamate receptor.[87]

The reasons for the dual effects of NO are unclear, although it may be that where there are high local concentrations of NO or where it is derived from a particular source (such as iNOS or nNOS) the toxic effects predominate. Similarly, the redox status of NO could be an important contributory mechanism since NO in the form NO· is neurodestructive via peroxynitrite formation as described above, whereas NO+ (which has one less electron) and NO- (with an extra electron) can interact with thiol groups on NMDA to reduce calcium influx[88] (although interestingly, it is still controversial as to whether NO- and NO+ have any role at physiological pH).

The toxic effects of NO are employed to our benefit by the immune system. In particular iNOS derived NO, produced in high concentrations, can attack several targets in micro organisms. In viruses, there is evidence that NO interferes with enzymes containing cysteine residues (e.g. reverse transcriptase) and viral encoded transcription factors.[89]

Moreover, NO has a pivotal role in defence against parasites, e.g. malaria[90] and is bactericidal against a large range of bacterial pathogens in vitro and in vivo.[89]

Finally, NO has a role in cell signalling and neurotransmission. Several studies have demonstrated that as well as activating soluble guanylate cyclase NO can stimulate other cellular signalling proteins, e.g. poly (ADP-ribose) synthetase, or inhibit others, e.g. adenylate cyclase type 1, protein kinase C, and cytochrome P450.[83] Furthermore, NO released from nitrergic neurones has a role in modulating the release of neurotransmitters at the neuromuscular junction.[91] Likewise, some actions of the autonomic nervous system are mediated by NO. In particular, NO is thought to participate in the non-adrenergic, non-cholinergic (NANC) control of gut motility through a direct action on intestinal smooth muscle.[92]

Altogether, when released in controlled amounts it is evident that NO plays a fundamental role in many biological processes. However, when this critical NO homeostasis is disturbed, leading to too much or too little NO, then tissue injury and disease can occur. Excess NO production has been linked to septic shock[93] and arthritis. Similarly, NO deficiency states have been associated with acute stroke,[94] hypertension[95] hypercholesterolemia, diabetes and atherosclerosis.[96]

1.3.3 Role of NO in stroke

Calcium influx plays a major part in neuronal death following stroke by up-regulating many detrimental intracellular signalling pathways and enzymes, including nNOS and eNOS. Consistent with up-regulation of NOS are findings of increased NO concentration in the cerebrospinal fluid (CSF) of patients with recent stroke.[97,98] NO release in stroke appears to have positive as well as negative effects (Table 1.1). For instance, transgenic

mice which do not express eNOS develop larger infarcts after middle cerebral artery (MCA) occlusion and show decreased blood flow at the periphery of the ischaemic lesion[99], supporting the notion that eNOS derived NO is neuroprotective. NO from this source probably exerts its beneficial effects in the vasculature, through maintaining CBF and by inhibiting platelet aggregation and leukocyte adhesion.[100] However, these beneficial effects may be outweighed by synthesis of very large amounts of NO derived from nNOS. The detrimental effects of nNOS derived NO were confirmed by the observation that nNOS deficient transgenic mice showed reduced infarct volumes compared with controls after focal MCA occlusion.[101] Several destructive properties of high local concentrations of NO may be involved here, such as production of peroxynitrite reactive oxygen species and direct DNA and mitochondrial damage.

The molecular cascade that follows excitotoxicity also includes several delayed neuro-destructive processes, including inflammation and programmed cell death (apoptosis).[102] NO is known to contribute to both of these processes, but appears to have a major role in neurotoxicity due to inflammation. In particular NO derived from iNOS, which starts to be expressed around 12 hours post stroke and is maximal after 48 hours[103], is neurotoxic. Indeed, transgenic mice that are deficient in iNOS have smaller infarcts and better outcomes than controls when subjected to MCA occlusion.[104] The mechanism by which iNOS derived NO causes damage is unclear, but may relate to the attraction of increasing numbers of inflammatory cells and release of damaging cytokines as well as the direct neurotoxic effects of uncontrolled NO production.[105]

1.3.4 NO as a treatment for acute stroke

A useful strategy might be to increase NO availability and thereby exploit its beneficial haemodynamic, anti-thrombotic, anti-inflammatory and neuroprotective properties.

Potentially useful sources of NO are its essential amino acid substrate L-Arg and pharmacological donors. Exogenous L-Arg appears to increase NO levels partly via the NOS pathway, but also by the release of other vasoactive substances and arginase enzyme.[106,107] In contrast, NO donors are drugs that generate NO through mechanisms that are independent of NOS. Commonly used agents are the organic nitrates (e.g. glyceryl trinitrate, GTN, isosorbide dinitrate, ISDN), sodium nitroprusside (SNP), sydnonimines (e.g. molsidomine, SIN-1), S-nitrosothiols (e.g. s-nitrosoglutathione), NONOates (e.g. SPERMINE-NONOate, DETA-NONOate), and hybrid donors (e.g. nitroaspirins, nicorandil). Pre-clinical studies of these agents have given variable results for effects on lesion size and CBF in animal models of cerebral ischaemia (table 1.2)

An alternative strategy is to pharmacologically reduce NO availability and therefore limit its pro-inflammatory and neurotoxic properties. A large number of compounds exist that can reduce NO by inhibiting the three forms of NOS enzyme (table 1.3). The first NOS inhibitors were the guanidino aminoacids, many of which act competitively at the NOS active site. Examples include, NG-nitro-L-arginine (L-NNA), NG-nitro-L-arginine methyl ester (L-NAME, a methyl ester pro-drug that is activated to become L-NNA) and NG-monomethyl-L-arginine (L-NMMA). Both L-NAME and L-NNA exhibit greater in-vitro potency than L-NMMA in inhibiting nNOS and eNOS versus iNOS. However, none of the guanidino aminoacids discriminate sufficiently to enable them to be used to target a single NOS isoform. By contrast, some inhibitors possess higher affinity against one or other isoform and are commonly referred to as ‘selective’ inhibitors, although this term is used rather indiscriminately.[108] Agents that are used to target iNOS include: aminoguanidine, NG-iminoethyl-L-lysine (L-NIL), NG-iminoethyl-L-ornithine (L-NIO), the bis-isothioureas (PBITU)[109], 1400W (N-[3-(aminomethyl)benzyl]acetamidine), GW273629 and GW274150.[110] Other agents are used to target nNOS and include: 7-nitroindinazole

(7-NI), tri(fluoromethylphenyl)imidazole (TRIM)[111], ARL 17477, AR-R18512[112], BN 80933[113], S-ethyl and S-methyl thiocitrulline and vinyl L-NIO. Recent in-vitro studies have suggested that in some cases the distinction between selective iNOS and selective nNOS inhibitor may not be straightforward. For example, aminoguanidine is only mildly selective against nNOS in-vitro (~5 fold) and probably affects other molecular targets.[108] Similarly, 7-NI has been found to be an equipotent inhibitor of all three isoforms of NOS at the isolated enzyme level[108,114] although it has more selectivity for nNOS in vivo, possibly a consequence of cell specific effects (neuronal verses endothelial).[108] Again, pre-clinical studies of NOS inhibitors have given variable results for effects on lesion size and CBF in animal models of cerebral ischaemia (table1.2)

1.4 High BP – a potential therapeutic target in acute stroke

Disturbances of physiological parameters are common in the setting of acute stroke. 75-82% of strokes present with high BP (systolic BP > 140mmHg).[35,115] Subsequently BP normalises in about 60% of patients over a period of 4 to 10 days.[116,117] The causes of high BP are multifactorial and relate in part to activation of the sympathetic nervous system, renin-angiotensin axis, previous hypertension, increased CO, cortisol / stress response and 'white coat hypertension'.[118-122] By contrast, low BP (systolic BP < 140 mmHg) is less common, occurring in approximately 18-25% of acute stroke patients.[35,115]

1.4.1 Relationship of acute BP with stroke outcome

In observational studies both high[23,123-134] and low BP[135] have been linked to poor outcome. Hence, BP might be related to stroke recovery by a 'U' or 'J' shaped curve. Evidence in favour of this comes from observational studies[136,137] and the International Stroke Trial, where the best outcome occurred at a systolic pressure of 140-160mmHg.[138] Why high BP should relate to poor outcome is not clear.[139] In IS it may involve early re-infarction[138,140], or promotion of cerebral oedema[138,141], although interestingly not haemorrhagic transformation.[138] In haemorrhagic stroke high BP might promote continued intracerebral bleeding.[142] Low BP may be related to poor outcome in IS through increased coronary events.[138] Other studies directly contradict these findings and suggest that high BP might be protective in the acute phase.[143-146] Possibly this is because acute stroke impairs cerebral autoregulation[147,148], vascular reactivity[149] and the autonomic nervous system[150], making the brain prone to hypoperfusion when exposed to low BP. More studies are required to better understand the relationship between BP and outcome in acute stroke.

1.4.2 Acute BP lowering

If high BP is associated with increased mortality and dependency then lowering it might lead to improved outcome. However, there is a risk that actively lowering BP could result in cerebral hypoperfusion because of impaired cerebrovascular control. Also, case reports of negative outcome in treated patients and findings from some trials, like the INWEST trial of nimodipine[151], have raised additional concerns. Recent work is beginning to strengthen the case for a more aggressive approach with the discovery that some classes of antihypertensive drugs do not have detrimental effects on CBF in acute stroke (section 1.4.4). Results are urgently required from large trials of BP lowering in acute stroke to help settle this debate. Similarly, trials are required to determine whether the 40% of stroke patients who are admitted on prior antihypertensive agents should stop or continue therapy.

1.4.3 Indications for BP lowering

Indications for intervention in hypertensive stroke patients vary in available guidelines.[152,153] Stable patients should be observed for a period of time since BP tends to fluctuate in acute stroke and can spontaneously fall.[116] Therapy is recommended when there is associated hypertensive encephalopathy, heart failure, myocardial ischaemia, aortic dissection or continued intracerebral bleeding.[152,153] A specific BP threshold requiring intervention is not known. There are many suggestions in the literature, e.g. systolic pressures greater than 180-240mmHg and diastolic pressures from 105-130mmHg in different guidelines. Where thrombolysis for IS is planned several trials have used the lower thresholds in these ranges.[154] This is because there was evidence of increased rates of PICH in hypertensive stroke patients treated with alteplase.

1.4.4 Which agent?

There is still no definitive, large randomised controlled trial (RCT) that favours any particular agent and the existing data argues both for and against different antihypertensive classes.

Calcium antagonists. These were investigated as potential neuroprotective agents. A recent meta-analysis of 11 trials revealed a non-significant trend to increased case fatality and disability (OR 1.28, 95%CI 0.98, 1.67) in the presence of significant reductions in BP.[155] Additionally, a post-hoc analysis of data from the INWEST trial found an association between diastolic BP reduction and neurological deterioration following i.v. nimodipine administration.[156] Furthermore, some evidence suggests that calcium antagonists reduce regional CBF in stroke patients[157] and have mild antiplatelet effects that might preclude their use in PICH.[158] Hence, calcium channel blockers are not recommended.

Beta blockers. Beta blockers are commonly used in the treatment of hypertensive emergencies, although it is less clear whether these drugs are appropriate for use in acute stroke. In one case series labetolol was given to PICH patients with no apparent detrimental effects.[159] The only controlled trial of these agents was disappointing in that it found a non-significant increase in mortality and a decrease in neurological outcome in patients receiving the active treatment.[55]

Diuretics. Thiazide diuretics are of proven benefit in primary and secondary stroke prevention. However, they did not alter BP in the immediate post stroke phase in one clinical trial.[160]

NO donors. Drugs that donate exogenous NO, like GTN, have been used for more than a century in vascular medicine. Some of these agents have been investigated for their therapeutic potential in animal stroke models because of possible neuroprotective qualities (table 1.2). Clinical trials in patients have been concerned more with possible haemodynamic benefits. NO donors may be particularly efficacious antihypertensive agents in stroke because of their concomitant cerebral vasodilatory properties.[161] In one clinical study SNP was given to 15 acute stroke patients within 12 hours of ictus at a dose sufficient to reduce mean arterial BP by 10mmHg.[162] Effects on regional CBF (using single photon emission computerised tomography - SPECT) and platelet function (whole blood aggregation and flow cytometry) were assessed. SNP significantly reduced platelet aggregation. In addition, in 3 out of the 4 patients who underwent SPECT scanning, there appeared to improvements in penumbral CBF related to treatment with SNP. However, there are limitations to the widespread use of SNP in stroke patients. Most importantly, its effects on BP require close monitoring since high doses can cause hypotension[163] and can actually be detrimental in stroke.[164] Similarly, infusions that last longer than 72 hours can lead to production of cyanide metabolite.[165] More recently, two clinical trials have studied 5mg-10mg GTN patches given transdermally. A patch is a very practical means of delivering NO therapy to patients who can't swallow safely. In one study, a 12-day course of transdermal GTN (5mg) or placebo was administered within 5 days of stroke onset to 37 patients.[166] GTN significantly reduced 24 hour systolic and diastolic BP at days 1 and 7 respectively, and had no antiplatelet effect as measured by platelet aggregation studies and expression of platelet adhesion molecules. In the second study[167] 90 patients were randomised to a 10-day course of transdermal GTN (5mg, 5-10mg or 10mg) patches or control. There was a similar antihypertensive effect on 24 hour systolic and diastolic BP, which were lowered by 6.3% and 4.8% respectively in those on the 5 mg patch. In addition, there was no effect on indirect measures of CBF,

assessed by transcranial Doppler (TCD), suggesting that cerebral perfusion was maintained during the treatment period. Furthermore, in both studies the active drug was well tolerated, for example only one patient stopped GTN prematurely due to headache in the second trial, although there was evidence of limitation of therapeutic effect (nitrate tolerance) by the end of the treatment periods. Both studies were too small to assess effects on clinical outcome (death or death and dependency) and further assessment on regional CBF is required.[168] A phase III trial (Efficacy of Nitric oxide in Stroke trial – ENOS) of 5mg GTN patches in ischaemic and haemorrhagic stroke is currently underway.[169] Finally, another NO donor, better known as a treatment for ischaemic heart disease, has been investigated in stroke. Nicorandil is a hybrid compound consisting of an organic nitrate attached to N-[2-hydroxyethyl] nicotinamide vitamin group. It has a dual action, firstly via activation of cGMP to cause smooth muscle relaxation and secondly by opening K^+ channels, leading to a shortening of muscle action potential and an indirect Ca^{2+} channel blocking effect.[170] Taken orally, it is absorbed rapidly with minimal hepatic 'first pass' effect and it has the advantage of avoiding the problems of nitrate tolerance. Furthermore, in addition to its coronary vasodilating effect, nicorandil was shown to significantly increase CBF and reduce BP in one study of 9 stroke patients.[171]

Angiotensin Converting Enzyme (ACE) inhibitors. Whilst ACE inhibitors (in combination with diuretics) are now well established for secondary prevention of cerebrovascular disease[172,173], there is currently limited data available in the setting of acute stroke. In one study (12 patients) captopril was administered within 5 days of onset and the effects on regional CBF were monitored with SPECT.[174] No significant changes in hemispheric blood flow or mean arterial BP were documented. These findings were subsequently confirmed in another small trial.[157] In a further controlled trial the

administration of perindopril 2-7 days after IS did not alter total CBF, despite significant reductions in BP.[175] None of these studies found an association with reduced death or death and dependency, however they were all too small to accurately assess this. A medium-sized RCT is presently investigating lisinopril.[56]

Angiotensin receptor blockers (ARB). A recent but relatively small trial evaluated the use of candesartan in hypertensive IS patients (n=342).[176] There was a significant 47.5% reduction in a secondary outcome comprising all-cause, cerebral and cardiovascular mortality, but there was no effect on the primary outcome (combined death and dependency) at 3months. Another trial assessed the effect of losartan 2-7 days after admission with stroke on 24 patients without occlusive carotid disease but with mean arterial BP between 110 and 145 mmHg.[177] The effects on mean arterial BP, internal carotid artery (ICA) flow and CBF (using hexamethylpropyleneamine oxime SPECT) were measured. No change occurred in ICA flow, or cortical or hemispheric CBF despite a reduction in mean arterial BP of 9.5 mmHg in the treated patients. Further clinical trials of ARBs are now warranted, with the main emphasis being on clinical stroke outcome.

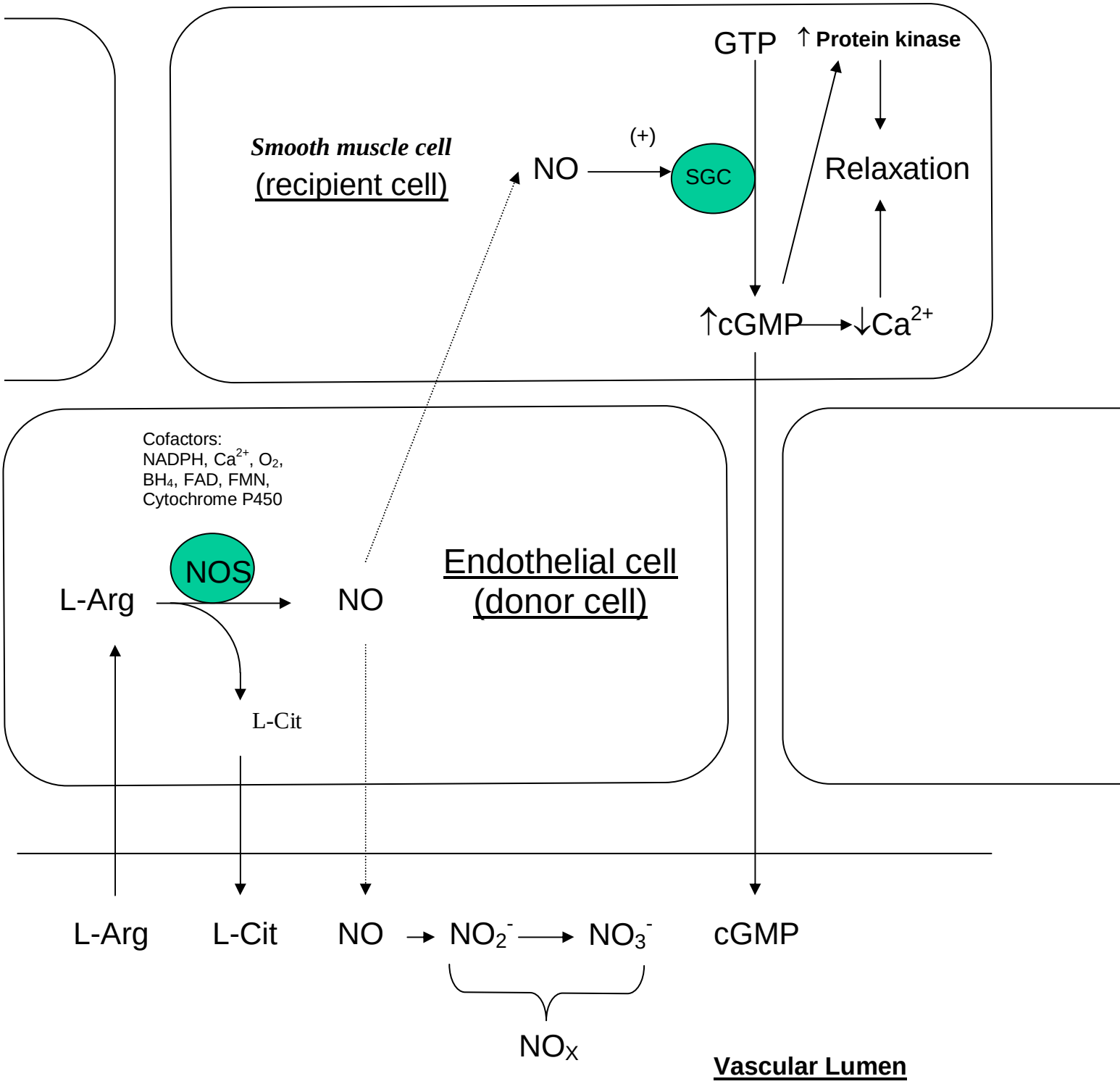
Other antihypertensives. There is little evidence on the merits of other classes of antihypertensives, such as the alpha-blockers, hydralazine or centrally acting agents.[178] Work in animals suggests that the alpha-blockers might reduce recovery.[179]

1.4.5 Acute BP elevation

An alternative therapeutic strategy is the pharmacological elevation of BP in an attempt to improve stroke outcome by increasing intraluminal hydrostatic pressure, opening collaterals and improving penumbral perfusion.[180] There are no definitive outcome studies that can justify the routine use of such therapy, although promising results were

reported in case series with dobutamine[181], phenylephrene[180], norepinephrine[182] and levarterenol.[183] However, other studies that used interventions which raise BP, including haemodilution[184], aptiganel[185], and diaspirin cross-linked haemoglobin[186] were disappointing. In any case, prompt treatment with fluids should be instigated in any episode of hypotension relating to under-hydration, since increased plasma osmolality is a marker of increased mortality in stroke.

Figure 1.1 Schematic diagram of NO synthesis and action on smooth muscle cells.



Abbreviations: L-Arginine (L-Arg), L-citrulline (L-Cit), nitric oxide (NO), nitric oxide synthase (NOS), nicotinamide adenine dinucleotide phosphate (NADPH), calcium (Ca^{2+}), oxygen (O_2), flavin adenine dinucleotide (FAD), flavin mononucleide (FMN), tetrahydrobiopterin (BH_4), soluble guanylate cyclase (SGC), nitric oxides (NO_X), nitrate (NO_3^-), nitrite (NO_2^-), guanosine triphosphate (GTP), cyclic guanosine monophosphate (cGMP).

Table 1.1 Summary of effects of NOS isoforms in stroke.

Isoform	eNOS	nNOS	iNOS
Main source	Endothelium	Neurones	Macrophages
Role in acute stroke	Beneficial	Detrimental	Detrimental
Result of gene knockout in stroke model	Larger infarct	Smaller infarct	Smaller infarct
Result of pharmacological knockout in stroke model	Larger infarct	Smaller infarct	Smaller infarct

Table 1.2 NO donors and stroke.

TYPE OF DONOR	EXAMPLES	COMMENTS
Organic nitrates	Glyceryl trinitrate (GTN)	Cerebral vasodilatory properties in normal volunteers.[161] Beneficial haemodynamic effects demonstrated in stroke patients.[166,167] Conveniently administered as a patch but tolerance is a problem. A definitive phase III trial is ongoing.[169]
	Isosorbide dinitrate (ISDN)	Neuroprotective effect demonstrated in one stroke model.[187]
Organic nitrites	Amyl nitrite	No studies in stroke
Inorganic nitroso-compounds	Sodium nitroprusside (SNP)	Reduces infarct size in animal models.[163,188,189] Several case series in humans show protection against SAH induced vasospasm. One small clinical trial demonstrated an antiplatelet effect in stroke patients.[162] Unlikely to have wide utility because of several therapeutic limitations.
Sydnonimines	Molsidomine	An oral NO donor. Does not improve outcome post MI.[190] Not studied in stroke clinically.
	3-Morpholinosydnonimine (SIN-1)	An i.v. NO donor with short duration of action. Has antiplatelet and anti-atherogenic effects in animal models. Reduced infarct in one pre-clinical study[189] but didn't in another.[191]
	CAS754	Reduced infarct size in one pre-clinical study.[192]
S-nitrosothiols	S-nitrosoglutathione (GSNO)	Has a significant antiplatelet effect with relatively little haemodynamic compromise. Evidence in two trials of clinical benefit in patients with carotid artery disease.[107,193]
	S-nitroso-N acetylpenillamine (SNAP)	No studies in stroke. Reverses vasospasm in experimental SAH.[194]
	S-nitrosocysteine (SNOC)	No studies
Diazeniumdiolates (NONOates)	DETA NONOate	Improved neurological outcome and increased neurogenesis in rats[195], but did not effect infarct size in another pre-clinical model.[191]

	Spermine NONOate	Reduced infarct volume and increased blood flow in one stroke model.[163]
	DEA NONOate	Not studied in stroke
Mesionic oxatriazole derivatives	GEA-3162	Not studied in stroke
	GEA-3175	Not studied in stroke
Hybrid NO donor drugs	Nitroaspirins (NCX-4016, NCX-4215)	Effective antiplatelet agents with less gastric toxicity than aspirin.[196-198] Neuroprotective in one animal model.[199]
	Nicorandil	Increased CBF in one small study of stroke patients.[171]
Others	Iron sulphur nitrosyls (sinitrodyl)	No studies in stroke
	FK-409	No studies in stroke

Table 1.3 NOS inhibitors and stroke

TYPE OF INHIBITOR	EXAMPLES	COMMENTS
L-Arg analogues	NG-monomethyl-L-arginine (L-NMMA)	Non-specific inhibitor causing hypertension, systemic vasoconstriction and reduced CBF in normal humans.[200-202] Unlikely to be of benefit in stroke.
	NG-nitro-L-arginine methyl ester (L-NAME)	Widely used non-specific inhibitor. Contradictory effects demonstrated in stroke models.[188,203-210] No clinical stroke studies.
	NG-nitro-L-arginine (L-NNA)	Widely used non-specific inhibitor. Contradictory effects demonstrated in stroke models.[211-221] No clinical stroke studies.
L-citrulline analogues	S-methyl-L-thiocitrulline	No stroke studies
	S-ethyl-L-thiocitrulline	No stroke studies
Guanidines	Aminoguanidine	Partially selective iNOS inhibitor which exhibits neuroprotection up to 24 hours after experimental stroke.[222-224] No clinical stroke trials but well tolerated in humans.
Mercapto-alkyl-guanidines	2-Mercaptoethyl guanidine	No stroke studies
Isothioureas	S-isopropyl-isothiourea	No stroke studies
	S-methyl-isothiourea	No stroke studies
Bis-isothioureas	N-(3-(Aminomethyl)benzyl)acetamidine (1400W)	Selective iNOS inhibitor which significantly reduces stroke volume and improves outcome in animal models.[225]
Indazoles	7-Nitroindazole (7-NI)	Partially selective nNOS inhibitor. Reduces infarct in animal models[210,226,227], but can also reduce CBF.[228] No clinical studies.
	Trimethylphenylfluoroimidazole	No stroke studies
Heterocyclic substituted amidines	AR-R17477	A nNOS inhibitor. Pre-clinical studies show a bell shaped dose response curve for infarct volume reduction.[112] Some detrimental effects on CBF.[112]

Acetamidine derivatives	GW273629, GW274150	Selective iNOS inhibitors. No studies in stroke
Neuroprotectants which also influence NOS	Lubeluzole	Development discontinued
	PBN derivatives (CPI-22)	Promising in phase II studies. Further development expected.

Chapter 2.

General Methods

2.1 Cardiac Haemodynamics

2.1.1 BP Measurement

Background

Early electronic BP monitors were inaccurate[229], but recent models have shown good agreement with intra-arterial and Korotkoff sound measurements.[230,231] In 2001 23 devices had been tested against the British Hypertension Society (BHS)[232] and Association for the Advancement of Medical Instrumentation (AAMI)[233] protocols, however only 5 passed.[232] Nevertheless there are considerable advantages to using validated monitors. For instance, no transducer needs to be placed over the brachial artery, so cuff placement is not critical. In addition, the lack of a requirement for auscultation makes it a suitable technique for ambulatory monitoring. Lastly, inaccuracies can occur with conventional sphygmomanometry due to observer error and terminal digit preference. These can be avoided using electronic monitors.[234]

Method

The patient was sat at 45^{o} and the non-hemiparetic arm was held horizontally at midsternal level. All restrictive clothing was removed. An appropriate sized cuff was then applied to the arm so that the tubing was over the position of maximal pulsation of the brachial artery. The cuff was connected to a validated electronic device (Omron HEM-705CP, Omron Corp, Tokyo, Japan, figure 2.0). This digital readout oscillometric device, which inflates and deflates the cuff at the push of a button, was selected because previous work has shown that it is reliable and complies with AAMI and BHS standards.[235] Two or three readings were taken and mean values of systolic BP and diastolic BP were determined.

2.1.2 Cardiac Output Measurement

Background

The blood volume of the finger varies with each cardiac cycle because of changes in arterial BP. An infrared plethysmograph can detect this variation. If a pneumatic finger cuff can be inflated and deflated quickly enough to maintain a constant finger blood volume (when the arterial wall is said to be 'unloaded') then the cuff pressure must be equal to the intra-arterial pressure.[236] The first device that exploited this principal, known as the volume-clamp technique, was the Finapres. Validation studies have demonstrated comparable BP values to intra-arterial recordings.[237,238] A new portable version of the Finapres has subsequently become available, called the Portapres. This device can be used to measure 24-hour ambulatory beat to beat BP since it can be worn on a belt.

The 7 channel version of Portapres records continuous non-invasive finger BP waveform, height compensation data, beat to beat BP (systolic BP, diastolic BP, mean arterial BP), heart rate and interbeat interval.[236] BP measurements from Portapres have been validated against intrabrachial measurements.[239] Both Finapres and Portapres can also be used to non-invasively measure cardiac output (CO) by the Modelflow method. This computes stroke volume (SV) from the arterial pressure wave, taking into account pressure and age dependent compliance of the aorta, as well as heart rate to correct for wave reflections.[240] CO is then calculated by multiplying SV by heart rate (CO = SV x HR). Studies in patients with cardiovascular disease show that Modelflow values from arterial pressure accurately track changes in CO when compared with thermodilution based estimates.[240-242]

Method

Patient information (sex, age, weight and height) was entered into the main unit of the Portapres model-2 (TNO-TPD Biomedical Instrumentation, figure 2.1). The subject was sat at 45^{o} and the front-end box was applied to the non-hemiparetic arm. Next, the height correction unit was fixed at the level of the apex beat (usually 5th left mid clavicular line) and an appropriate sized finger cuff was selected and applied to the middle phalanx of the middle finger (figure 2.2). Measurements were taken for 5 minutes and were monitored using the BeatScope 2 software (TNO-TPD Biomedical Instrumentation) on a laptop computer (Toshiba, Japan). The same software was used to calculate CO by the Modelflow method from the arterial pressure waveform. All subsequent measurements were taken using the same arm and with the same sized finger cuff.

2.1.3 Pulse wave analysis

Background

BP measurements from the brachial artery are widely used in clinical trials, however the brachial artery is not subject to the adverse effects of atherosclerosis like the major central arteries. Moreover, the onset of left ventricular damage is more likely to be influenced by the pressure in the aorta rather than in the arm. Hence it can be argued that peripheral BP measurement might not be the best way to assess future risk of cardiovascular disease. Instead, measurement of central haemodynamics derived from the aortic pulse waveform may be better indicators.

The aortic arterial pressure wave may be simply and rapidly assessed by means of applanation tonometry. This technique involves compressing a peripheral artery against its underlying structures with a micromanometer tipped probe. The intra-arterial pulse pressure is transmitted through the arterial wall to the sensor and can be displayed on a sphygmograph. A computer, which is calibrated using brachial BP, calculates the central

aortic pressure and waveform from the peripheral waveform. This requires the use of a mathematical equation called the 'generalised transfer function'. This has been validated in a number of studies[243-245], although there are some concerns that it may not be accurate when calibration is done non-invasively.[246]

Once the aortic pulse wave has been calculated it is possible to determine central BP values and a measure of central compliance called the augmentation index (AI). The AI is calculated as the difference between peak 2 (P2) and peak 1 (P1) of the aortic pulse (see figure 2.3), expressed as a percentage of pulse pressure (PP). AI is negative in healthy young people, but increases becoming positive with aging or increasing cardiovascular risk. It is strongly influenced by pulse wave velocity (PWV), since this determines the timing of arrival of P2. It is also affected by mean arterial BP[247], heart rate[248] and height.[249] Various cross-sectional studies have demonstrated increases in AI in populations with high cardiovascular disease risk, such as type-1 diabetes[250], hypercholesterolemia[251] and increased age.[252] As of yet there are no data on the prognostic value of AI when derived from the radial artery. However, carotid artery measurements independently predict all cause and cardiovascular mortality in patients with renal failure.[253]

In addition to AI, Buckberg et al have demonstrated that the ratio of the area of the diastolic to the systolic phase in the aortic pulse has a close correlation with the blood supply to the subendocardium in animals.[254] This ratio was designated as the subendocardial viability ratio (SEVR) or Buckberg index (BI). Low SEVR is associated with the presence of coronary heart disease risk factors[255] and predicts death after coronary surgery.[256]

Method

Initially, brachial BP was recorded twice in the non-hemiparetic arm using a validated automatic sphygmomanometer (Omron 705CP, Japan). The mean systolic BP and diastolic BP were then entered into the pulse wave analysis software (Sphygmocor Pulse Wave Analysis System, Sydney, Australia) along with standard patient demographics. Next the non-hemiparetic wrist was rested on a stable surface and gently extended to expose the radial artery. The strongest location of the radial pulse was found by placing the index and ring finger over the artery. A micromanometer tipped probe (a tonometer) was then used to compress the radial pulse against the underlying bone until a characteristic sphygmograph was found (figure 2.4). Once a consistent pressure waveform was displayed a recording was made for 10 seconds before the data were captured. Measurements were taken until two satisfied the quality control system built in to the software (pulse height and variability ≤ 10%). All subsequent readings were taken from the same arm and the mean values for central systolic BP, diastolic BP, mean arterial BP, BI and AI were extracted for analysis.

2.2 Cerebral Haemodynamics

2.2.1 Transcranial Doppler (TCD)

Background

TCD uses an ultrasound transducer to emit high frequency sound waves that reflect off the surfaces of moving erythrocytes in blood vessels. The transducer detects the frequency shift in the reflected waves and a computer displays the data. Before the signal can be viewed it is processed so that the three-dimensional Doppler data (time, frequency and signal intensity) can be displayed in a colour coded two-dimensional format (Spectral analysis). A 'Fast Fourier Transformation' is used to make this conversion.

In a typical examination several blood flow parameters are measured directly, such as peak systolic velocity (PSV), end diastolic velocity (EDV) and direction of blood flow. Other parameters can be derived through calculations, such as mean flow velocity (MFV), pulsatility index (PI) and resistance index (RI). The PI (or Gosling Index) is used to describe the shape of the waveform and is calculated by the formula PI = (PSV-EDV)/MFV. Likewise, the RI (or Pourcelot Index) is calculated by the formula RI = (PV-EDV)/PV. Both of these parameters act as an index of vascular resistance distal to the point of insonation, and in general they decrease as resistance decreases. As humans age PI tends to increase and velocity gradually decreases, probably because of reduced vascular compliance.[257] Typical values for MFV and PI in healthy elderly (>60yrs) volunteers are shown in table 2.1.[257]

Haemodynamic abnormalities on TCD have been found to correlate well with the presence of intracranial stenosis or occlusion.[258] Common anomalies are focal increases

in MFV (~ >30% of normal) and dampened or blunted waveforms. For example, a PSV above 90 cm/sec is 99% specific for the presence of MCA stenosis.[259] In contrast, the relationship between velocity determined by TCD and CBF is much less certain. One article found a good correlation between the absolute velocity and absolute CBF measured by Xe 133 SPECT[260], however this was not supported by subsequent studies.[261,262] Similarly, several investigators have studied the relationship between changes in blood velocity and changes in regional CBF. Again, the results were variable with some in favour[262-265] and some against.[161,260,266] Hence, blood velocity is probably not a good indicator of CBF, although research is still ongoing in this area.

Method

A Nicolet EME Companion TCD system was used connected to a 2 Mhz probe. Initially the patient was placed in a supine position in bed with the Doppler equipment near to hand. A small amount of ultrasound gel (Aquasonic 100, Parker laboratories USA) was applied to the patient's skin in the trans-temporal window. This area is located over the temporal bone, just superior to the zygomatic arch (figure 2.5). Next the TCD was adjusted to record at a depth of 56mm using a 10mm sample size. The signal power was adjusted to the lowest possible setting according to FDA guidelines. Then the probe was placed flat against the skin and gently manoeuvred until an audible Doppler signal originating from as near as possible to the M1 segment of the MCA was found. The TCD settings were adjusted in order to obtain the highest possible velocity before measurements of PSV, EDV, MFV, PI and RI were made. Finally the procedure was repeated on the contralateral MCA. A typical MCA waveform is shown in figure 2.6.

2.2.2 Xenon CT (XeCT)

Background

Stable (non-radioactive) xenon (Xe) is a highly lipid soluble gas which can freely cross the blood brain barrier. The radio-density of Xe was first recognised in 1966[267], however it took another decade before it was first used as a contrast agent for CT.[268] Like most methods for measuring CBF, the XeCT technique is based on the Fick principal of indicator dilution.[269] This states that the concentration of an indicator absorbed in a tissue per unit time can be deduced from the difference in concentrations of the indicator in arterial and venous blood. Determination of the venous concentration is not necessary in XeCT since the CT scan can directly measure the time dependent concentration of Xe in the brain (CxeBr(t)) instead. This is done by initially obtaining two baseline scans at up to 8 levels. Six enhanced scans are then taken at each level during a 4.5 minute Xe inhalation. The pairs of baseline scans are averaged and then subtracted from each of the enhancement images to yield CxeBr(t). Likewise, the time dependent concentration of Xe in the arterial blood (CxeArt(u)) can be determined from the (expired) end-tidal Xe measured by a thermoconductivity analyser. Both CxeBr(t) and CxeArt(u) are related mathematically to the affinity of the brain for Xe (λ - the blood brain partition coefficient) and the brain uptake flow constant (k), in a formula known as the Kety-Schmidt equation (modified for Xe):

$$CxeBr(t) = \lambda k \int CxeArt(u) e^{-k(t-u)} du$$

Solving this equation enables calculation of the blood flow (F) from the second Kety-Schmidt formula, $F = \lambda k$.[270] The image that results is a CBF map which uses a colour scale denoting the blood flow at each pixel on the image (figure 2.7). Numeric flow values (ml/min/100g) can be extracted by placing regions of interest (ROIs) of any shape or size on the image. Numeric values from small ROIs can be inaccurate. Accuracy improves as the ROI size increases until it reaches an acceptable error of ~12% at >1cm^2.[271]

The validity of the XeCT has been confirmed in baboons using a radio labelled microsphere technique[272-274] and a 14C iodoantipyrine technique for CBF measurements < 5ml/min/100g.[275] Primate stroke models have also confirmed that Xe-CT provides a sensitive technique for acquiring serial CBF measurements following transient or permanent occlusion.[276,277] In humans a number of studies have demonstrated good correlations between XeCT and CBF values obtained by SPECT[278,279], CT perfusion[280,281] and MR perfusion studies.[282]

Although Xe is biologically inert administration can lead to side effects. At concentrations of <33% some individuals experience paraesthesiae, drunkenness and dizziness. Such symptoms are transient, but can cause patient motion and movement artefact. At higher concentrations (>71%)[283] Xe has several properties of an ideal anaesthetic; including analgesic and hypnotic abilities, rapid induction and emergence, but negligible cardiovascular effects.[284] Short periods of apnoea can occur in response to any concentration of Xe, probably through a direct effect on the medulla. In one study (1830 subjects) the incidence of apnoea (>10 seconds) was 3.6%, however in no instance was this prolonged enough to require ventilatory assistance.[285] Xe is known to cause cerebral vasodilation (flow activation), which has led to the suggestion that it should not be administered to patients with raised intracranial pressure.[286] However, recent work suggests that flow activation is minimal with current protocols (< 5% increase in flow) and is reversed by mild hyperventilation.[271]

Method

The Enhancer 3000 unit (figure 2.8, Diversified Diagnostic Products XeCT system 2, Houston, USA) was switched on and connected to 100% oxygen (5-10psi, 3L/min) and

an 80% Xe / 20% oxygen cylinder (5-10 psi, 10L/min, Air Products Plc, Crewe, UK). After the Enhancer had warmed up (~6 minutes) the internal re-breathing system was filled with a mix of 28% Xe / 25% oxygen. Meanwhile, the patient was positioned on the CT scanner (GE Medical Systems 'Smalleye' CT scanner, USA), with their head held by a vacuum beanbag (Olympic Medical, Seattle, USA) in order to limit movement artefact. Once they were comfortable a non-contrast CT head was performed to obtain a 'scout' so that XeCT images could be taken through the region of the stroke lesion. After this was complete the breathing interface between the patient and the Enhancer was set up. This consisted of a disposable face mask (Intersurgical Complete Respiratory Systems, Berkshire, UK), breathing circuit (Pennine Ventalink Derby, UK), CO2 sample tube (Vygon UK Ltd, Gloucestershire, UK) and a bacteriological filter (Pennine Ventalink Derby, UK). Since the mask prevented verbal communication patients were given instructions to use hand gestures to answer to any queries during the procedure. The facemask was then securely strapped to the patient's head and the connections were checked for leaks. A good seal was confirmed by checking the expired CO2 concentration on the XeCT computer (XeCT – NT software, Diversified Diagnostic Products, Houston, USA). The dynamic scanning protocol on the CT scanner was then started simultaneously with the Xe administration protocol. For the first 30 seconds the patient breathed in room air whilst the CT scanner took two baseline images at four levels. During the next 4 minutes 30 seconds the Enhancer entered the 'wash-in' phase and the patient was exposed to a mix of 28% Xe / 25% oxygen gas. End tidal Xe concentrations were measured in the expired air by a thermoconductivity analyser. A further 4 CT scans were taken at each level throughout this period, totalling 24 images altogether. A physician and a registered nurse were present in order to reassure the patient and monitor for apnoea. After the study was complete the patient was disconnected from the Enhancer and allowed to recover. A second XeCT scan was

repeated 1 hour after randomisation to treatment. During the intervening period patients were asked to remain on the CT scanner to ensure that the pre and post treatment CBF images came from similar axial planes. Patients that could not tolerate this had a second 'scout' scan to confirm correct positioning prior to the second XeCT.

CBF images were processed and analysed blinded to treatment using the XeCT – NT software. Before calculations were performed images with excessive movement artefact were discarded (where > 15% of the bone pixels moved compared with the 1st baseline image). Those that remained were used to produce CBF maps with a colour scale denoting the blood flow at each pixel (ml/min/100g). Global and hemispheric regions of interest (ROIs) were sited using a rectangular shaped template, the former touching the inside of the skull anteroposteriorly and laterally, and the latter dividing the skull at the midline. Anterior, middle and posterior cerebral artery territory ROIs were placed over the cortex using a template generated by the XeCT software (figure 2.7). An additional pixel based analysis was used to assess the effect of GTN on CBF in the stroke lesion, if visible. A rectangular ROI was placed to cover the whole of the stroke lesion and surrounding brain tissue. A CBF filter was then used to determine the number of pixels within the ROI matching pre-specified CBF values for 'core' <10 ml/min/100g, 'penumbra' 10-19 ml/min/100g, and 'oligaemia' 20-36 ml/min/100g (figure 7.2b).[287-290] Matching ROIs were sited on pre and post treatment scans to ensure consistency and the areas (in pixels) of reduced CBF compared.

2.3 Laboratory Methods

2.3.1 NO measurement

Background

Measurement of NO in biological specimens is difficult because of the small amounts present (nanomolar) and because it reacts readily to produce oxidation products, such as nitrate (NO_3-), nitrite (NO_2-) and NO_2. Most methodologies chemically trap NO with an adduct and then use one of several detection strategies[291]:

i) Measure the adduct; for example, by spectrophotometry with a diazotisation assay[292,293], or by electron paramagnetic resonance with nitroso or haemoglobin traps.[294,295]

ii) Spectrophotometric measurement of the conversion of reduced haemoglobin to met haemoglobin.[292,296]

iii) An amperometric method involving oxidation of NO at the surface of an electrode.[297]

iv) Measure the light emission that occurs when ozone interacts with NO (chemiluminescence).[67,74,298]

Chemiluminescence was the chosen method in this thesis since it is highly sensitive (detects < 1 part per billion of gas phase NO[299]) and allows large numbers of samples to be processed rapidly.[291] In the chemiluminescence assay detection of NO is based on the observation that ozone interacts with NO to form excited state $\bullet NO_2$, which then emits a photon during conversion to its ground state:

$$\bullet NO + O_3 \rightarrow \bullet NO_2^* \text{ (excited state)} + O_2$$

$$\bullet NO_2^* \rightarrow \bullet NO_2 \text{ (ground state)} + \text{photon}$$

The luminecesence that is generated is directly proportional to NO levels and can be detected by a sensitive photomultiplier tube (PMT). If the specimen is gaseous then it is simply mixed with ozone gas in the 'reaction chamber'. Alternatively, if the specimen is liquid the NO has to be driven ('stripped') into the gas phase first. This is achieved by bubbling the solution in a sample chamber (or purge chamber, figure 2.9) with an inert gas under vacuum conditions. In addition, acids or reducing agents are sometimes added to transform previously formed oxidation products back to NO and enhance detection; e.g. vanadium and hydrochloric acid will convert nitrate and nitrite back to NO. However, since these agents also release biologically inactive NO from nitroso compounds, inorganic nitrites and nitrosamines, there is a risk that NO levels will be overestimated.[300] The gaseous NO that is formed by this process is then carried to a separate chemiluminescence reaction chamber. A 'needle valve' is used to regulate the flow and ensure that no foam or bubbles get through. Additional measures to avoid aspiration of bubbles into the reaction chamber include the use of antifoaming agents and de-proteinising samples prior to analysis. Ozone (generated by electrical discharge) is then mixed with the gaseous NO in front of a PMT. The PMT should be red sensitive, as the light emission occurs between 660 and 900 nm, and should be cooled, since this improves the signal / noise ratio.[291,301] The sensitivity threshold for detection of NO with chemiluminescence ranges between 20-50 pmol.[67,302]

Method

The NO assay was based on a method which has previously been published in detail.[436]

Sample preparation

4.5ml citrated blood samples were taken from each patient at baseline and 60 minutes after treatment allocation. The samples were immediately centrifuged (Capricorn Laboratory Equipment, Hants, UK) at 3300 rpm for 12 minutes and the plasma was transferred to 5ml bijou bottles for storage at –80^{o}C. When required, they were allowed to thaw at room temperature and vortexed (Vortex Genie 2, Scientific Industries Inc, New York, USA) to mix the contents. The plasma was then deproteinised by mixing 100μl with 200μl of cold ethanol in microcentrifuge tubes. These were vortexed, allowed to stand at 0^{o}C for 30 minutes and then centrifuged at 13000 rpm (Biofuge Pico, Kendro Laboratory Products, Germany) for a further 15min. The deproteinised supernatant was then removed for NOx analysis. The three-fold dilution of each sample was accounted for in the final NO calculation.

Preparation of the NO assay

Plasma NO was measured by chemiluminescence using a Seivers 280 NO Analyser (Analytix Ltd, County Durham, UK). First the analyser was switched on and allowed to warm up for 20 minutes. The purge chamber (figure 2.9) was filled with 5ml filtered vanadium (111) chloride reducing agent and 100μl of antifoaming agent. The reducing agent was prepared by adding 0.8g of vanadium chloride (VCl_3, Fischer Scientific, Leicester, UK) to a volumetric flask containing 1M hydrochloric acid (Fischer Scientific, Leicester, UK). The flask was inverted several times until the solution turned blue. Then the contents were filtered and stored in a refrigerator. The antifoaming agent (Seivers Instruments, Boulder, Colorado, USA) was prepared by adding 1ml to a volumetric flask containing 29 ml of double deionised water (1:30 dilution). This was also mixed thoroughly and stored in a refrigerator prior to use. For high conversion efficiency the purge chamber had to be heated to 90^{o}C. This was done using a hot water bath that was

connected to a heating jacket surrounding the purge chamber. Hose clamps were used to secure the rubber tubing to the heating jacket and the fittings on the water bath. To minimise damage to the NO analyser from hydrochloric acid vapour the purge vessel outlet was connected to a condenser and a gas bubbler filled with 20ml 1M sodium hydroxide (Fischer Scientific, Leicester, UK). Nitrogen (Air Products Plc, Crewe, UK) at 0.2 – 0.5 psi was then connected to the gas inlet of the purge vessel and the needle valve was adjusted until the 'purging conditions' were considered acceptable (when the 'reaction cell pressure' on the NO analyser was approximately 6 – 7 torr).

Calibration of the NO analyser

85mg of sodium nitrate (Fischer Scientific, Leicester, UK) and 10ml double deionised water were added to a volumetric flask to make 100mM of stock solution. 1:10 progressive serial dilutions were then performed to prepare a series of 1μM to 100μM standard solutions. A calibration curve was constructed by injecting 10μL of the standards into the purge vessel.

Sample analysis

A micro-syringe (Exmire Micro-syringe, Analtix Ltd, Co Durham, UK) was used to draw up 10μL of the sample and 'pumped' several times to remove any air bubbles. A paper towel was then used to wipe the outside of the needle before it was inserted into the purge vessel through an airtight septum. The plunger was then rapidly depressed to transfer the sample into the vessel. Before the next sample was inserted the syringe was removed, wiped and rinsed 2-3 times with de-ionised water.

2.3.2 P-selectin ELISA

A commercially available P-selectin immunoassay kit was used (Parameter, Human soluble P-selectin immunoassay, R&D systems, Oxfordshire, UK). The kit's instruction book was followed.

Sample preparation

7ml clotted blood samples were taken from each patient at baseline and 60 minutes after treatment allocation. The samples were immediately centrifuged (Capricorn Laboratory Equipment, Hants, UK) at 3300 rpm for 12 minutes and the serum was transferred to 5ml bijou bottles for storage at –80^{o}C. When required, these were allowed to thaw at room temperature and vortexed to mix the contents. 15μl from each sample was transferred to appropriately labelled polypropylene tubes and diluted 20-fold with 285μl of Sample Diluent (containing buffered protein base with blue dye and preservative).

Reagent preparation

500ml of wash buffer was prepared by combining 20ml of buffer concentrate (containing buffered surfactant, with preservative) with 480ml of distilled water in a measuring cylinder. The six vials containing p-selectin standards (lypophilised recombinant human p-selectin with blue dye and preservative) were then reconstituted by adding 1.0ml of distilled water and mixed by gentle inversion to give a series of concentrations ranging from 0.0 – 46.0ng/ml. Next, the p-selectin control (lypophilised human serum containing p-selectin) was reconstituted by combining it with 500μl distilled water. This was left at room temperature for 10 minutes before a 20-fold dilution was prepared by combining 15μl with 285μl of Sample Diluent. Finally, 250μl of Conjugate Concentrate (containing sheep polyclonal antibody to recombinant human p-selectin conjugated to horseradish

peroxidase in buffer, with preservative) was transferred into the bottle containing Conjugate Diluent. This was mixed thoroughly by gentle inversion. All reagents were brought to room temperature before use.

Preparation of microtitre plate

A microtitre plate, coated with mouse monoclonal antibody to human p-selectin, was set up with 96 wells. 100μL of the solution from the standards, samples and the p-selectin control was transferred (in duplicate) to each of the wells. 100μL aliquots of the diluted p-selectin conjugate was then added to each well with sufficient force to ensure mixing. Next, the plate was covered with the lid provided and incubated at room temperature. After 1 hour the wells were rinsed three times with wash buffer using a multi-channel pipette. Residual buffer was removed by blotting the plate on tissue. Next, 100μL of substrate (containing tetramethylbenzidine) was transferred to all the wells and the plate was incubated at room temperature on a microtitre plate shaker (Ika Schuttler MTS 4, Germany). The reaction was halted at 15 minutes by the addition of 100μL of stop solution (containing acid) and the optical density was read at 450nm (Dynatech mr5000 Plate Reader, Gurnsey, Channel Islands).

2.4 Statistical Methods

2.4.1 General statistical methods

Various parametric and nonparametric techniques were chosen according to the distribution and type of data to be examined. Microsoft Excel 2001 and SPSS v10.0 computer packages were used to perform the analyses and generate figures. Significance was set at $p<0.05$. Precise details of statistical methods are highlighted in each relevant chapter.

2.4.2 Systematic Review Methods

Background

A systematic review begins with a search for studies that are relevant to the topic of interest. Since only 30-80% of all known published randomised controlled trials can be found on MEDLINE[303] it is important to use more than one search method. Studies that do not meet pre-specified inclusion criteria are then discarded. More than one reviewer at this stage will reduce the possibility that relevant reports are lost.[304]

The statistical methods used to combine the results of the included studies are known as meta-analysis. This is a two-stage process. First a summary statistic is calculated for each study. For continuous data the mean difference (also known as weighted mean difference, WMD) and the standardised mean difference (SMD, also known as the 'effect size') are used. The former is required when the outcome measurements are made on the same scale and the latter is required when different measurement scales are used for the same outcome. Alternatively, for categorical data the risk ratio (RR), odds ratio (OR), risk difference (RD, also known as the absolute risk reduction) and number needed to treat[305] are used as measures of effect.

In the second stage of a meta-analysis a summary (pooled) effect estimate is calculated as a weighted average of the effects in the individual studies. Several statistical methods exist that can be used to pool data. The approach that is chosen is determined, in part, by whether heterogeneity is expected. Heterogeneity is present when the differences in effect between the individual studies are more than can be explained by sampling variation (i.e. chance) alone. Common sources of variability are clinical diversity (differences in participants, interventions and outcomes studied) and methodological diversity (differences in trial design and quality).[305] If heterogeneity is thought to be present then a 'random effects model' is used. Alternatively, if it can be assumed that differences in effect are purely a result of sampling variation, then a 'fixed effect model' is used. This assumption can be checked by performing a χ^2 test on the effect measurements of the included studies. There are four widely used methods of meta-analysis for dichotomous (categorical) data. Three of these use fixed effect models; the Mantel-Haenszel[306], Peto[307] and Inverse variance methods.[308] The other, the DerSimionian and Laird method[309], uses a random effects model. The Peto method can only pool OR whilst the other three methods can combine OR, RR and RD. When continuous measures of effect are pooled with a fixed effect model the inverse variance method is commonly used. By contrast, if a random effect model is needed the DerSimionian and Laird method can be applied. Alternative methods to the above exist, however they require advanced statistical software.

Finally, a meta-analysis is displayed in a standard way known as a 'forest plot' (figure 3.2a, b). The horizontal lines correspond to the 95%CI for each study, with the central box drawn proportional to the relative size ('weight') of the study. The diamond at the bottom of the figure is the summary estimate of all the individual studies combined. A

result to the left or right of the unbroken vertical line represents an overall positive or negative effect. By contrast, if the diamond crosses the line then this means that the systematic review is neutral.

Method

A protocol was designed for each systematic review. This defined the question being addressed, study inclusion / exclusion criteria, quality scales and the subgroups of interest. Studies matching the pre-specified criteria were then identified by me through systematic searches of two or three electronic databases; 'Pubmed', 'EMBASE' and 'Web of Science'. In addition, articles were found from review papers and reference lists. The abstracts from these were used to select relevant studies for a re-examination of the full publication against the review criteria. Final decisions on inclusion or exclusion were taken be me and independently by another researcher to limit bias. Discrepancies were resolved by the project supervisor. Articles included in the NO donor and NOS inhibitor reviews were also assessed for methodological quality on an 8 point scale.[310]

Study data were grouped according to the protocol prior to analysis. Where numerical values were not available for extraction, data were taken directly from enlarged graphs using a ruler. All data extraction was performed independently and in duplicate by me and another researcher. Any discrepancies were resolved by the project supervisor. The Cochrane Collaboration Review Manager (RevMan version 4.1) software was used to analyse the data as forest plots. Dichotomous data were analysed as OR and continuous data were analysed as WMD or SMD with 95% CI. A random effects model was used since statistical heterogeneity, assessed with a χ^2 test, was expected in view of the wide range of study protocols. Subgroup analyses were performed to look at suspected

sources of heterogeneity. Publication bias was assessed using funnel plots and Egger's asymmetry test[311] (Stata function 'metabias').Significance was set at $P<0.05$.

Figure 2.0 Omron HEM-705CP, Omron Corp, Tokyo, Japan

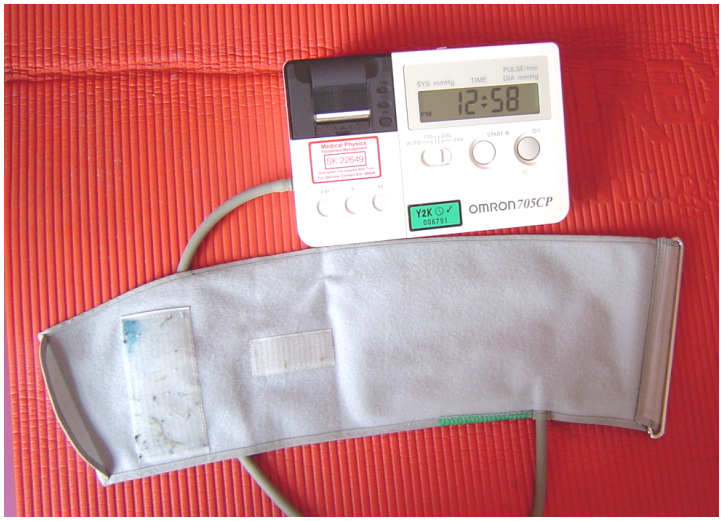


Figure 2.1 Portapres, TNO-TPD Biomedical Instrumentation

Figure 2.2 Finger cuff applied to the middle phalanx of the middle finger

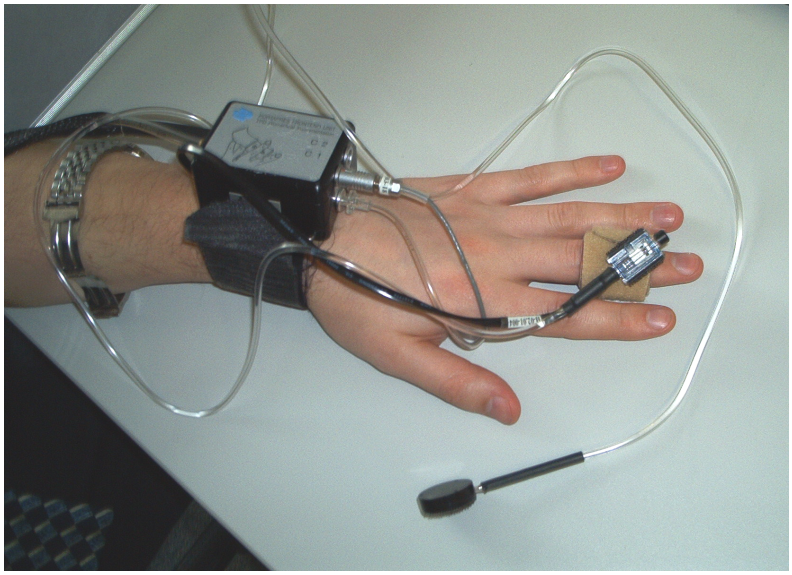


Figure 2.3 Typical waveforms of aortic and brachial pulse

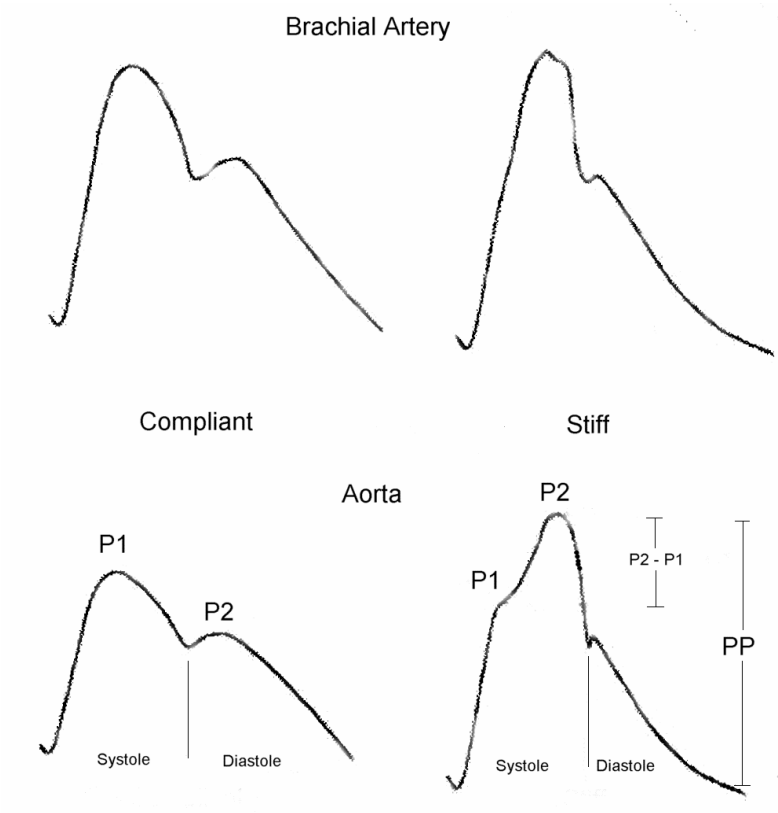


Figure 2.4 Applanation tonometry at the radial pulse

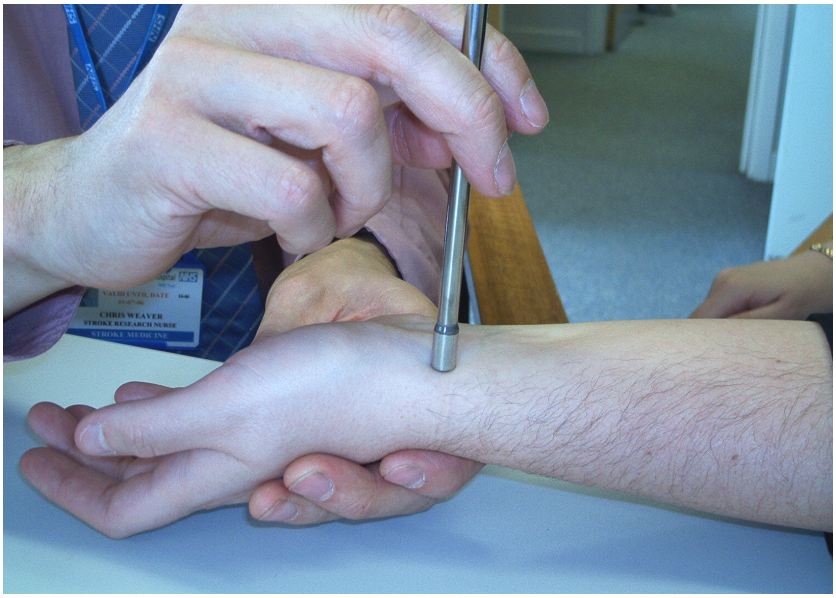


Figure 2.5 Nicolet EME Companion TCD system.

Figure 2.6 A typical MCA waveform

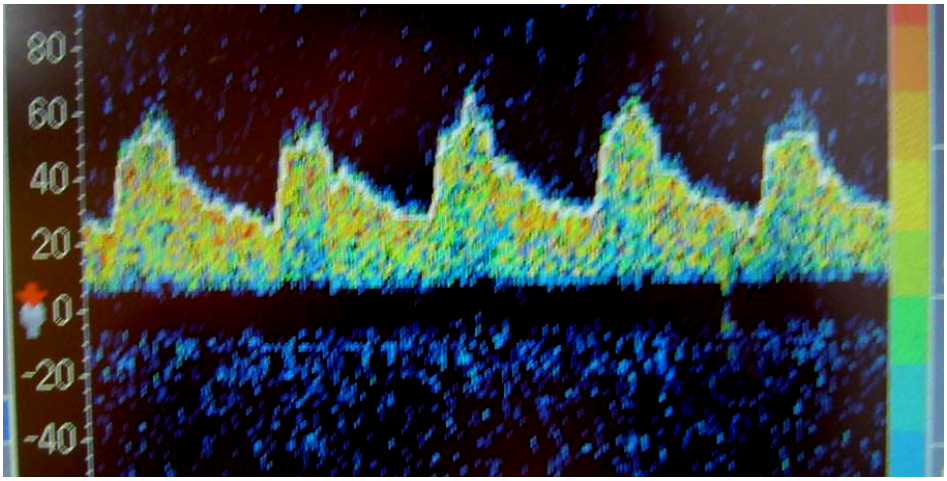


Figure 2.7 XeCT head showing right MCA infarct

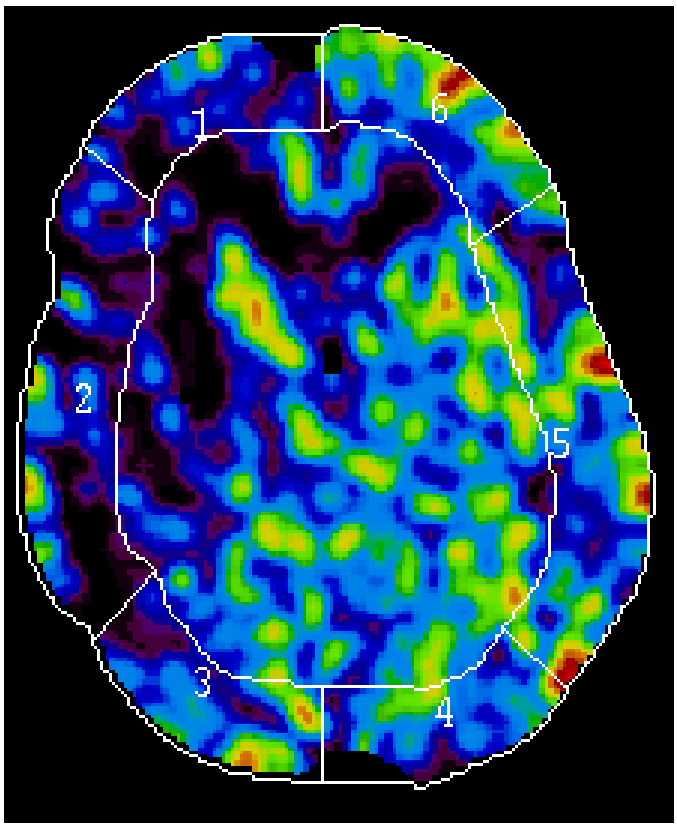


Figure 2.8 Diversified Diagnostic Products XeCT system 2, Houston, USA

Figure 2.9 NO analyser purge chamber

Table 2.1 Typical TCD findings in Elderly Patients

Artery Window	Artery	Direction of blood flow	Depth (mm)	MFV, cm/sec (95%CI)	PI (95%CI)	RI (95%CI)
Transtemporal	MCA	Towards	45-55	58 (55 – 61)	0.97 (0.93 – 1.02)	0.62 (0.60 – 0.64)
	ACA	Away	55-75	51 (48 –54)	0.92 (0.87 – 0.97)	0.59 (0.57 – 0.62)
	PCA	Towards	65-80	42 (40 – 45)	0.97 (0.91 – 1.02)	0.60 (0.58 – 0.62)
Transforaminal	VA	Away	65-85	33 (30 - 36)	0.94 (0.89 – 0.99)	0.59 (0.57 – 0.61)
	BA	Away	>85	35 (31 – 40)	0.95 (0.86 – 1.03)	0.60 (0.56 – 0.64)

Chapter 3.

High BP in acute stroke and subsequent outcome: a systematic review

3.1 Abstract

Background: High BP is common in acute stroke and might be associated with a poor outcome although observational studies have given varying results. Method: In a systematic review articles were sought which reported both admission BP and outcome (death, death or dependency, death or deterioration, stroke recurrence, haematoma expansion) in acute stroke. Data were analysed using the Cochrane Review Manager software and are given as OR or WMD with 95% CI. Result: Altogether, 32 studies were identified involving 10,892 patients. When including all data, death was significantly associated with an elevated mean arterial BP (OR 1.61, 95% CI 1.12, 2.31), and high diastolic BP (OR 1.71, 95% CI 1.33, 2.48). Combined death or dependency was associated with high systolic BP (OR 2.69, 95% CI 1.13, 6.40) and diastolic BP (OR 4.68 95% CI 1.87, 11.70) in PICH. Similarly, high systolic BP (+11.73 mmHg, 95% CI 1.30, 22.16), mean arterial BP (+9.00 mmHg, 95% CI 0.92, 17.08) and diastolic BP (+6.00 mmHg, 95% CI 0.19, 11.81) were associated with death or dependency in IS. Combined death or deterioration was associated with a high systolic BP (OR 5.57, 95% CI 1.42 to 21.86) in patients with PICH. Conclusion: High BP in acute IS or PICH is associated with subsequent death, death or dependency, and death or deterioration. Moderate lowering of BP might improve outcome. Acute BP lowering needs to be tested in one or more large randomised trials.

3.2 Background

High BP (BP >140/90 mmHg, as defined by the WHO) occurs in acute stroke in up to 75% of cases.[116,117] Subsequently, BP settles over a period of about a week although around 40% of patients remain hypertensive. The causes of this pathophysiological response are multifactorial and relate to pre-existing high BP, activation of the neuroendocrine systems (sympathetic nervous system, renin-angiotensin axis, glucocorticoid system), increased CO, and 'white coat hypertension'.[118-122]

It has been suggested that high BP is associated with a poor outcome after acute stroke, although the results of observational studies have given conflicting results. Some authors have even demonstrated better outcome in patients with high initial BP.[144,312] Data from the International Stroke Trial confirmed that the risk of early death and late death or dependency was independently associated with increasing systolic BP in 17,398 patients.[138] This chapter reports a systematic review of observational studies of BP and outcome, and assesses the relationship between the two.

3.3 Methods

Study identification

Published observational studies which reported baseline BP and outcome (death, death or dependency, death or deterioration) or mechanisms for poor outcome (recurrent stroke, haemorrhagic transformation, development of cerebral oedema, haematoma expansion) in acute (<7 days) stroke were sought. Systematic searches of 'EMBASE' and 'Pubmed' were made. The search strategy employed 10 keywords: blood pressure, hypertension, outcome, prognosis, death, mortality, recovery, stroke, cerebr*, acute. Additional studies were found from reference lists of identified articles and review papers.[139,313,314] Disability or dependency were typically measured using the Barthel Index or Rankin Scale;

deterioration was defined as worsening on a stroke neurological impairment scale, e.g. NIH stroke scale, or where an ordinal scale (e.g. 'improved', 'unchanged', 'worse') was used. Publications were excluded if they were a randomised trial (these tend not to enrol consecutive patients and may have a less representative sample), gave insufficient data, used other outcomes, or were duplicate articles. Decisions on inclusion and exclusion of studies were made by two researchers.

Data extraction

Two researchers independently extracted data; discrepancies were resolved by the project supervisor. Studies with dichotomous data were analysed separately from those with continuous data. Within both types, the studies were subdivided by outcome measurement (death, death or dependency, death or deterioration, stroke recurrence, cerebral oedema, haematoma expansion) and by BP measurement (systolic BP, diastolic BP, mean arterial BP). The articles were then arranged by stroke type (PICH, IS, or mixed). Figures 3.2a and 3.2b show typical forest plots and illustrate the layout of studies.

Where articles quoted several BP measurements the earliest readings were used. Outcomes after the longest follow-up period were used where they were present at multiple time points. Some articles gave outcome data for several BP strata (e.g. mortality with systolic BP <140 mmHg, 141–180 mmHg and >180 mmHg).[315] In such instances the data were dichotomised into a high BP and a combined normal / low BP group using a cut nearest to 150 mmHg since outcome may be best at this level.[138] Likewise, publications with diastolic BP or mean arterial BP in several strata were dichotomised as close as possible to 90 mmHg and 110 mmHg respectively. Additionally, some articles gave continuous BP data in more than two groups (e.g. systolic BP for patients with 'improved', 'unchanged' or 'worse' neurological status). These studies were incorporated in the review

after the data were transformed into two groups (e.g. combined 'improved' / 'unchanged' and 'worse'). This was achieved by generating pseudo-random BP data (assuming a normal distribution for BP) and then merging this before recalculating an overall combined mean BP and standard deviation.

Analysis

Data were analysed using the Cochrane Collaboration Review Manager (version 4.1) software. Dichotomous data are given as OR and 95% CI, and WMD with 95% CI for continuous data. These were calculated with a random effects model and statistical heterogeneity was assessed with a χ^2 test. The causes of heterogeneity were examined using sensitivity analyses based on type of stroke. Publication bias was assessed using Egger's asymmetry test[311] (Stata function 'metabias') on dichotomous outcome data. Significance was set at $P<0.05$.

3.4 Results

A flow diagram illustrating the search process is given in figure 3.1. Altogether, 32 studies with a total of 10,892 patients (median size 184) were included. Eleven of these focussed on PICH, five on IS, and the rest included patients with either type of stroke. A further 64 studies were excluded; 35 did not provide BP and/or outcome data in a suitable form, 26 did not assess BP within seven days of onset, and 3 were duplicate publications.

BP was recorded at admission in 18 of the included articles. The method used was given in only nine studies; five used 'clinic' BP measurement, three used both 'clinic' BP measurement and ambulatory BP monitoring (ABPM), and one used ABPM alone. The criteria used to define high BP varied considerably; systolic BP thresholds ranged from150-200 mmHg,[127,131,132] mean arterial BP levels 140-145 mmHg,[129,132] and diastolic

BP between 90-115 mmHg.[132,134,316,317] One article did not specify the criteria used for defining high BP;[318] this study was analysed with the publications that dichotomised according to mean arterial BP. In addition, three studies allocated subjects to the high BP group if either systolic BP or diastolic BP or both exceeded certain threshold values.[124,128,133] These articles were also analysed with publications that dichotomised according to mean arterial BP.

Death was the most commonly reported outcome measure (survival status present in 7242 patients, 66.5%). There were fewer data for combined death or dependency (1290, 11.8%) and combined death or deterioration (1196, 11.0%). These outcomes were assessed using validated stroke scales in 7 articles (Rankin score[130,148,319-321], Scandinavian Neurological Stroke Scale[320,322], Canadian Neurological Score[323], National Institutes of Health Scale[130]). The other studies either used non-validated scales[144] or qualitative assessments such as discharge disposition.[124,127] Patient follow-up varied considerably between 6 days and 6 years, although most articles chose to measure outcome at discharge from hospital. Potential mechanisms of poor outcome were reported in four articles. Three looked at PICH (708, 6.5%) and assessed the relationship between BP and haematoma expansion; the other study investigated early recurrence in IS (1273, 11.7%). No studies assessed cerebral oedema or haemorrhagic transformation.

There was no publication bias (Egger's test p=0.21) in articles reporting systolic BP and mortality data. When assessing stroke as a whole, patients with high systolic BP or diastolic BP were at a 1.5-5.0 fold increased risk of dying or combined death or dependency / deterioration (table 3.2). Similarly, high mean arterial BP was associated with an increased odds for combined death and dependency. In patients with poor outcome, judged as death and death or dependency / deterioration, there was a trend for

higher systolic BP and diastolic BP levels of 5/3 mmHg. Heterogeneity was present in several of these analyses (table 3.2) and so the data were further examined by stroke type.

PICH

Increased odds of death, and death or disability / deterioration were found in patients with high BP (table 3.3). Additionally, mean arterial BP was higher in patients who died after PICH (table 3.4). The odds of haematoma expansion was increased for patients with high systolic BP (table 3.2).

IS

Limited data were available for studies specifically including patients with IS. Systolic BP and diastolic BP were higher by 12/6 mmHg in patients who died or became dependent (table 3.4). Similarly, IS patients had a twofold increase in the risk of stroke recurrence if their diastolic BP was elevated (table 3.2).

Mixed stroke studies

The odds of death were doubled in patients with high diastolic BP. Systolic BP was higher by 6.39 mmHg in patients who subsequently died (table 3.4).

3.5 Discussion

High systolic BP, mean arterial BP and diastolic BP in the acute phase of stroke are associated with a poor outcome, assessed either as death, or combined death or disability, and possibly combined death or early deterioration. This observation was present irrespective of stroke type. The result, based on 32 studies involving 10,892 patients, is similar to that found in IST where a high systolic BP (>140 mmHg) was independently

related to an increased risk of early death, and combined death or dependency in 17,398 patients with acute IS.[138] The individual studies that form the basis of this systematic review were generally either positive or neutral for the association between BP and outcome. The neutral findings in some studies probably reflect their small size, and therefore, limited statistical power. Three articles found improved outcomes for high systolic BP[144,322] and diastolic BP[135]; however, when analysed with other studies there was no evidence of a protective effect for high BP.

This review has found evidence for mechanisms that may link high BP to poor outcome. In IS high diastolic BP was associated with a two-fold increase in the risk of early recurrence. systolic BP was an important determinant of recurrent stroke in IST where an initial systolic BP of 200 mmHg or more conferred a >50% higher risk of recurrence than that for a systolic BP of 130 mmHg.[138] No studies investigating the relationship between BP and haemorrhagic transformation or cerebral oedema in IS fulfilled the inclusion criteria. Nevertheless, evidence that was excluded from the review suggests that an acutely elevated systolic BP is associated with increased fatal cerebral oedema.[138,141] Also, several studies have observed that high BP promotes haemorrhagic transformation in animal models[324-326], although this was not found in IST.[138] As far as PICH is concerned, patients with high systolic BP were almost twice as likely to have haematoma expansion. This could support the concept that high BP in the acute phase of haemorrhagic stroke leads to worse outcome at least in part by promoting continued intracerebral bleeding. However, this relationship may be confounded by timing of inclusion since patients with severe PICH tend to present earlier and are at greater risk of re-bleeding.

Although this review has demonstrated a positive association between high BP and subsequent events in acute stroke, the findings are limited by several factors. First, there

were considerable differences in patient eligibility, case mix (including baseline BP), definition of high BP, measurement and timing of BP, and type and timing of outcome between the studies. This could be considered an advantage since it means the relationships observed are more likely to be generalisable. However it may also have led to statistical heterogeneity. To assess whether stroke type was a potential source of heterogeneity in the review IS and PICH were analysed separately. The relationship between outcome and BP tended to be stronger in patients with PICH (OR 2.26-5.57) as compared with those with IS. Similarly, patients who had a poor outcome had a higher BP if they had PICH (mean arterial BP 11 mmHg) than IS (systolic BP and diastolic BP 12/6 mmHg, equivalent to a mean arterial BP of ~8 mmHg). Nevertheless, these comparisons are indirect and not precise. Other explanations for heterogeneity between the studies are likely but it was impossible to explore these due to the paucity of data.

A second limiting factor is that it was impossible to judge whether the relationships that were observed were independent of other factors such as age, premorbid BP status and drug therapy, stroke severity or timing of BP measurement. A mega-analysis of the studies, based on individual patient data, would be required to investigate this further. This issue is important since some of the relationships might have been different if potential confounding factors had been accounted for. For example, the relationship between BP and re-bleeding in PICH is probably mostly explained by an interaction between timing of BP measurement and severity.[142,327] Likewise, high premorbid BP contributes to elevated BP in acute stroke and could contribute, in part, to poor outcome through previous cerebral vascular damage. Third, the strategy of dichotomising BP, while clinically relevant, may create artificial strata for the analysis. For example, several articles have reported a ‘U’ shaped relationship between BP and outcome, with the least bad outcome at a systolic BP of 140-160 mmHg in IST (judged by the nadir of the ‘U’).[136-138] Hence, studies including a

significant proportion of patients with BP below a cut of 160 mmHg might be expected to miss a relationship between high BP and poor outcome. Unfortunately, since only one article gave sufficient data on low BP in acute stroke it was not possible to examine how it influenced outcome. Last, it is worth noting a consistent weakness in the observational studies themselves, namely the lack of reporting how BP was measured. All studies assessing BP should give information on equipment (manufacturer, model, technique, validation), user (how trained, assessed and re-assessed) and patient (number and site of readings, position).[178]

In summary this chapter assesses data from all available observational studies and shows that there is a positive association between high BP and poor outcome in acute stroke. This relationship may be mediated, at least in part, by early recurrence in patients with IS and, possibly, by early re-bleeding in those with PICH. Although these relationships suggest that high admission BP is directly causal it cannot be ruled out that this relationship was independent of other factors such as stroke severity or timing of measurements. Nevertheless, since lowering a high BP prevents first and recurrent stroke, it can be hypothesised that moderate lowering of a high BP in acute stroke might similarly reduce early death and deterioration, and late death and dependency**.**

Table 3.1 Included studies.

Study	N	Stroke type	BP timing	BP method	BP data	Outcome measure	Outcome timing
Acheson [134]	497	M	Admission	Not given	D	Death	4.6 years (mean)
Allen [144]	148	M	Admission, 24 hours	Not given	C	Death or dependency	2 and 6 months
Armario [319]	49	IS	Admission and daily	Not given	C	Death or dependency	Discharge
Bhalla [320]	72	M	< 1 day, 7 days	Manual, ABPM	C	Death or dependency	1 week
Britton [124]	388	M	Admission	Manual	D	Death; Death or dependency	Discharge
Brott [328]	103	PICH	< 3 hours	Not given	C	Haematoma growth	< 4 hours
Carlberg [315]	916	M	Admission	Manual	D	Death	30 days
Dandapani [132]	87	PICH	Admission	Not given	D	Death; death or dependency	30 days
Dawson [148]	92	IS	< 3 days	Manual, ABPM	C	Death or dependency	30 days
Dunne [127]	40	Cerebellar PICH	Admission	Not given	D	Death or deterioration	6 days
Fogelhol [329]	141	IS (brainstem)	< 24 hours	Not given	C	Death	46.5 months (median)
Fogelholm [330]	282	PICH	< 24 hours	Manual	C	Death	28 days
Fuji [327]	419	PICH	< 24 hours	Not given	D	Haematoma growth	< 2 days
Fullerton [331]	206	M	12- 48 hours	Not given	C	Death; death or dependency	6 months
Harmsen [128]	97	M	Admission	Manual	D	Death	Discharge
Jorgensen [322]	868	M	Admission	Not given	C	Death or deterioration	Day 2, weekly; at discharge
Kazui [142]	186	PICH	< 24 hours	Not given	C	Haematoma growth	< 5 days
Latorre [332]	200	M	Admission	Not given	D	Death	Discharge
Longo-Mbenza [135]	1032	M	Admission	Not given	C	Death	Discharge

Marquarsden [316]	371	M	Admission, day 1, day 7	Not given	D	Death	5 years
Marshall [317]	251	M	Admission	Not given	D	Death	6 years
Mbala-Mukendi [333]	388	M	Admission	Not given	C	Death	Discharge
Panayiotou [126]	55	M	< 24 hours	ABPM	C	Death	2 years
Portenoy [318]	112	PICH	Admission	Not given	D	Death or dependency	At last recorded follow up
Qureshi [129]	182	PICH	Admission	Manual	D	Death	Discharge
Rankin [133]	247	M	< 7 days	Not given	D	Death	Discharge
Robinson [130]	136	M	< 24 hours	Manual, ABPM	C	Death or dependency; death or deterioration	30 days
Sacco [140]	1273	IS	< 7 days	Not given	D	Recurrent stroke	30 days
Tennent [334]	107	PICH	Admission	Not given	D	Death	Discharge
Terayama [335]	1701	PICH	< 24 hours	Not given	C	Death	Discharge
Toni [323]	152	IS	< 5 hours	Not given	C	Death or deterioration	Discharge
Tuhrim [131]	94	PICH	Admission	Not given	D	Death	30 days

PICH: intracerebral haemorrhage; IS: ischaemic stroke; M: mixed; C: continuous; D: dichotomous

Table 3.2 BP in acute stroke by outcome.

Outcome		Dichotomous BP				Continuous BP			
		Studies / subjects	OR (95% CI)	P	Heterogeneity	Studies / subjects	WMD (95% CI)	P	Heterogeneity
Death	SBP	6 / 1211	1.85 (1.17,2.93)	<0.01*	0.03*	5 / 1799	4.81 (-0.98,10.60)	0.10	0.04*
	MABP	6 / 1912	1.61 (1.12,2.31)	0.01*	0.15	2 / 1983	11.40 (8.21,14.58)	<0.01*	0.27
	DBP	6 / 1655	1.71 (1.33,2.18)	<0.01*	0.38	5 / 1799	-0.05 (-2.56,2.47)	1.00	0.23
Death / disability	SBP	1 / 87	2.69 (1.13,6.40)	0.03*		6 / 691	5.11 (-3.00,13.22)	0.20	<0.01*
	MABP	3 / 587	1.92 (0.83,4.44)	0.13	0.05*	1 / 92	9.00 (0.92,17.08)	0.03*	
	DBP	1 / 87	4.68 (1.87,11.70)	<0.01*		6 / 691	2.55 (-1.53,6.62)	0.20	0.04*
Death / deterioration	SBP	2 / 891	1.86 (0.28,12.50)	0.50	<0.01*	3 / 1155	-1.04 (-7.59,5.50)	0.80	0.21
	DBP					3 / 1155	0.59 (-3.55,4.73)	0.80	0.12
Recurrent stroke	SBP	1/ 1273	1.52 (0.80,2.88)	0.20					
	DBP	1 / 1273	2.19 (1.16,4.14)	0.02*					
PICH enlargement	SBP	2 / 600	1.93 (1.22,3.06)	<0.01*	0.43	2/ 284	7.24 (-2.63,17.10)	0.15	0.31
	DBP	1 / 180	1.14 (0.47,2.75)	0.80		2/ 284	0.81 (-4.57,6.19)	0.80	0.76

Table 3.3 Outcome by type of stroke for dichotomous data.

Outcome		PICH			Infarct			Mixed		
		Studies / subjects	OR (95% CI)	P	Studies / subjects	OR (95% CI)	P	Studies / subjects	OR (95% CI)	P
Death	SBP	3 / 244	3.55 (1.80,7.00)	<0.01*				3 / 967	1.40 (0.85,2.30)	0.18
	MABP	3 / 354	2.26 (1.40,3.66)	<0.01*	1 / 831	0.96 (0.60,1.54)	0.90	3 / 727	1.56 (0.98,2.48)	0.06
	DBP	2 / 162	1.74 (0.88,3.46)	0.11				4 / 1493	1.71 (1.24,2.36)	<0.01*
Death / disability	SBP	1 / 87	2.69 (1.13,6.40)	0.03*						
	MABP	2 / 199	2.90 (1.57,5.36)	<0.01*				1 / 388	0.87 (0.40,1.91)	0.70
	DBP	1 / 87	4.68 (1.87,11.70)	<0.01*						
Death / deterioration	SBP	1 / 40	5.57 (1.42,21.86)	0.01*				1 / 851	0.79 (0.59,1.05)	0.11

Table 3.4 Outcome by type of stroke for continuous data.

Outcome		PICH			Infarct			Mixed		
		Studies / subjects	WMD (95% CI)	P	Studies / subjects	WMD (95% CI)	P	Studies / subjects	WMD (95% CI)	P
Death	SBP				1 / 131	-2.04 (-12.76, 8.68)	0.70	4 / 1668	6.39 (0.70, 12.09)	0.03*
	MABP	2 / 1983	11.40 (8.21,14.58)	<0.01*						
	DBP				1 / 131	2.22 (-4.86,9.30)	0.50	4 / 1668	-0.18 (-3.04,2.68)	0.90
Death / disability	SBP				2 / 141	11.73 (1.30,22.16)	0.03*	4 / 550	2.95 (-7.38,13.28)	0.60
	MABP				1 / 92	9.00 (0.92,17.08)	0.03*			
	DBP				2 / 141	6.00 (0.19,11.81)	0.04*	4 / 550	1.32 (-3.91,6.55)	0.60
Death / deterioration	SBP				1 / 152	3.00 (-6.70,12.70)	0.50	2 / 1003	-1.99 (-11.83,7.85)	0.70
	DBP				1 / 152	-0.60 (-5.20,4.00)	0.80	2 / 1003	4.44 (-8.18,17.07)	0.50

Figure 3.1 Search process

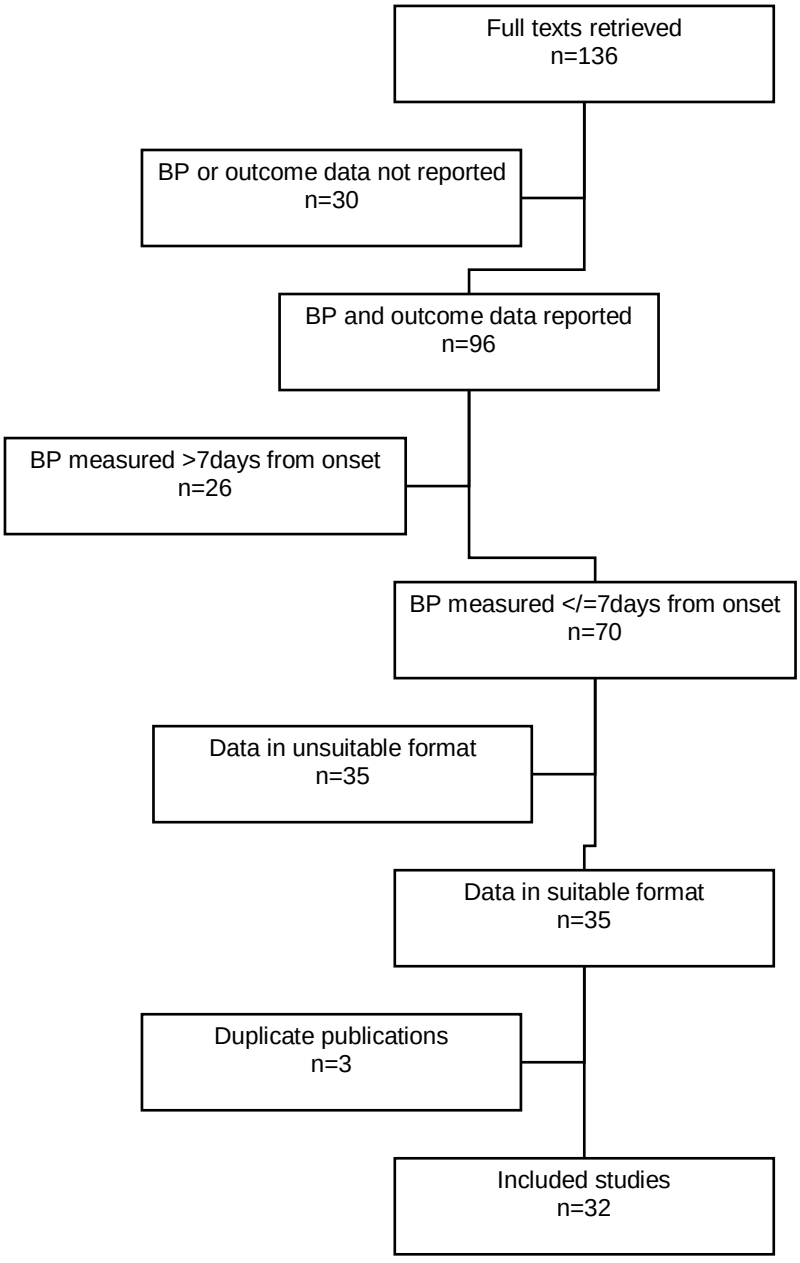


Figure 3.2a Forest plot of OR for death in ‘high’ verses ‘low/normal’ diastolic BP

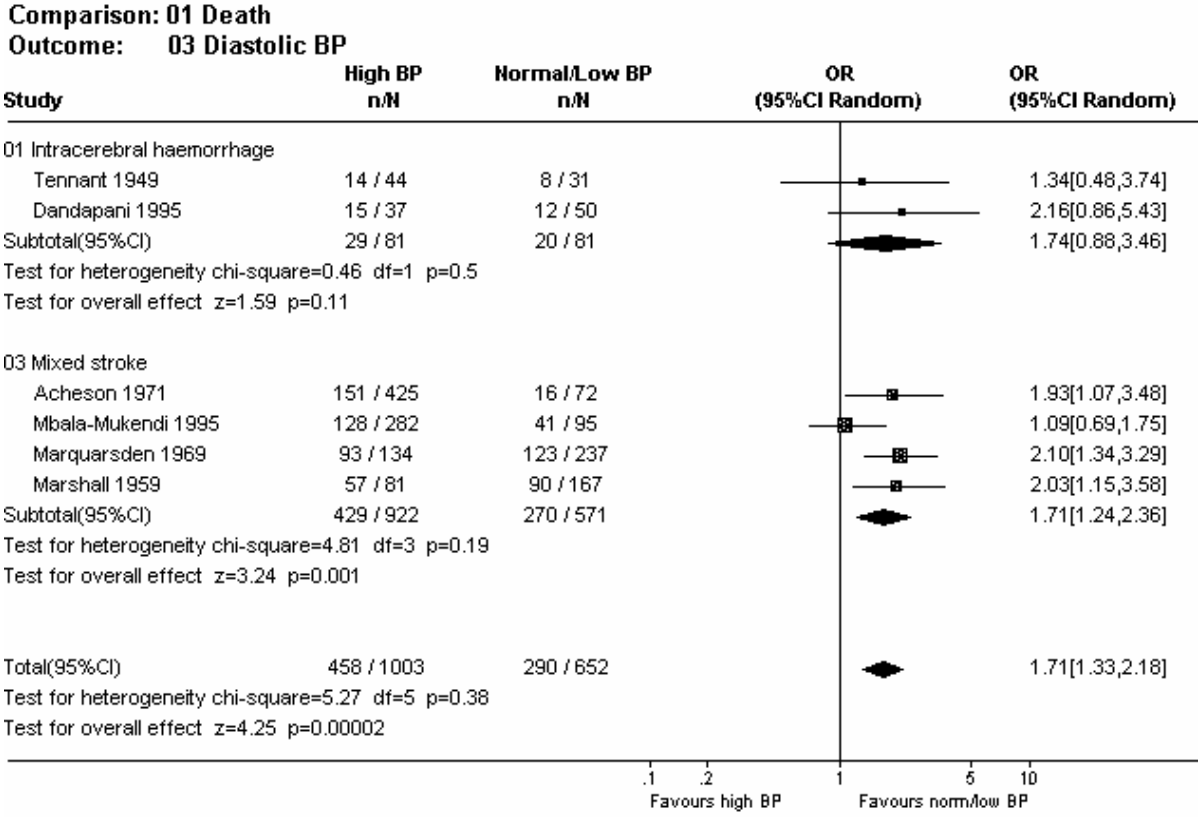
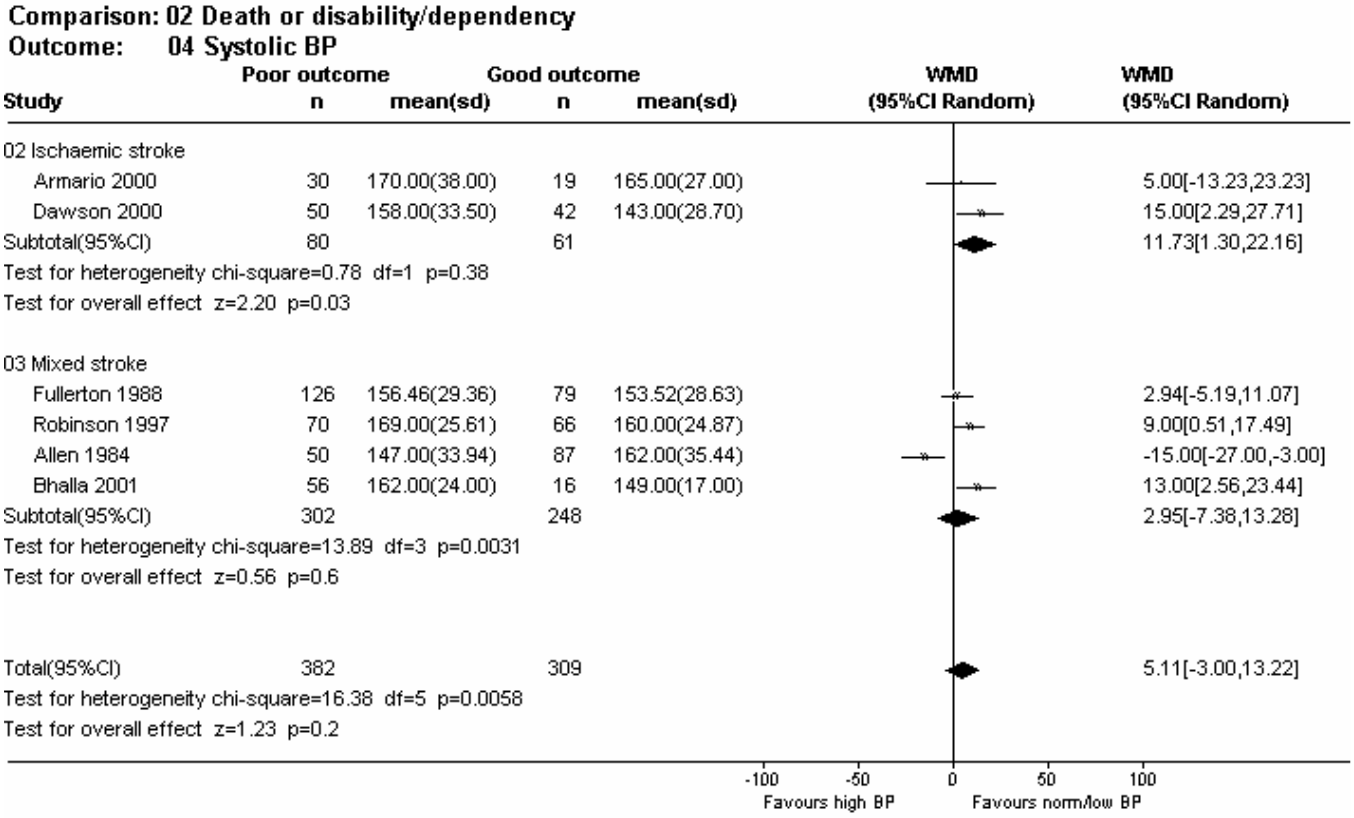


Figure 3.2b Forest plot of WMD (mmHg) for systolic BP in dead or dependent patients compared with good outcome.



Chapter 4.

A systematic review of NO donors and L-arginine in experimental stroke; effects on infarct size and CBF

4.1 Abstract

Background: NO is a candidate treatment for acute IS, however published studies in experimental stroke have given conflicting results. Methods: A systematic review of published controlled studies of L-Arg (the precursor for NO) and NO donors in experimental stroke was performed. Data were analysed using the Cochrane Collaboration Review Manager software. SMD and 95% CI were calculated. Results: Altogether, 25 studies (s) were identified. L-Arg and NO donors reduced total cerebral infarct volume in permanent (SMD -1.21, 95% CI -1.69 to -0.73, p<0.01, s=10) and transient models of ischaemia (SMD –0.78, 95% CI –1.21 to -0.35, p<0.01, s=7). Drug administration increased cortical CBF in permanent (SMD +0.86, 95% CI 0.52 to 1.21, p<0.01, s=8) but not transient models (SMD +0.34, 95% CI –0.02 to 0.70, p=0.07, s=4). Conclusions: Administration of NO in experimental stroke reduces stroke lesion volume in permanent and transient models. This may be mediated, in part, by increased cerebral perfusion in permanent models. These data support clinical trials in stroke patients, although the presence of a narrow therapeutic time window may be a limiting factor.

4.2 Background

NO is a multimodal endogenous mediator that is synthesised from its precursor L-Arg by the action of NOS.[108] It is implicated in the pathophysiology of acute IS, although its precise role remains to be determined.[100,336] In stroke, NO produced by the neuronal and inducible isoforms of NOS (nNOS, iNOS) can be neurotoxic[101,104], partly as a consequence of the formation of peroxynitrite, a free radical, which leads to direct damage to mitochondrial enzymes and DNA.[86,88] In contrast, NO produced by the endothelial isoform of NOS (eNOS) is beneficial in acute stroke.[99] Endothelial-derived NO may limit neuronal damage through effects in vascular beds or within the brain itself.[337] In the intravascular space NO acts as a powerful vasodilator that can modulate blood flow. Also, NO inhibits leukocyte adhesion / migration to endothelium[78,79] and has antiplatelet effects.[75] In the brain NO may be neuroprotective through several mechanisms including: scavenging of reactive oxygen species[338-340], anti-inflammatory effects[78,79] and possibly through attenuation of NMDA receptors.[341] Furthermore, in experimental stroke exogenous NO limits metabolic derangement[211,342], reduces apoptosis[199] and stimulates neurogenesis.[195] Consequently, administration of NO has been considered to be a candidate treatment for acute stroke.

Useful sources of NO for study in animal models are its essential amino acid substrate L-Arg and pharmacological donors. Exogenous L-Arg appears to increase NO levels partly via the NOS pathway, but also by the release of other vasoactive substances and arginase enzyme.[106,107] In contrast, NO donors are drugs that generate NO through mechanisms that are independent of NOS. Commonly used agents are the organic nitrates (e.g. GTN, ISDN), SNP , sydnonimines (e.g. molsidomine, SIN-1), S-nitrosothiols (e.g. s-nitrosoglutathione), NONOates (e.g. SPERMINE-NONOate, DETA-NONOate), and hybrid donors (e.g. nitroaspirins, nicorandil). Pre-clinical studies of these agents have given

variable results for effects on lesion size and CBF in animal models of cerebral ischaemia.[343] The aim of the present investigation was to determine systematically what effect NO donors have on these parameters.

4.3 Methods

Study identification

Experimental studies of L-Arg and NO donors were sought and included if they reported the effect on infarct volume or CBF in IS models (transient or permanent, global or focal). Systematic searches of 'Pubmed', 'EMBASE' and 'Web of Science' were made for articles published from 1980 - 2002. The search strategy employed four primary keywords (nitric oxide, cerebro*, brain, ischaemia) combined with a fifth word chosen from a list of NO donors and L-Arg, and limited to animal studies. Additional articles were identified from previous non-systematic reviews[336] and reference lists by three researchers. On the basis of title and abstract, articles of interest were selected for a review of the full publication. Decisions on inclusion or exclusion were then made by one researcher and the project supervisor. Exclusion criteria were: non-stroke model, NO donor not administered, outcomes other than infarct volume or CBF, no control group, insufficient data given, or duplicate publication.

Data Extraction

Data were extracted on infarct volume and CBF from the selected articles. Infarct volume was recorded in mm^3 or as a % of normal brain. Measurements from the longest period of follow up were used. CBF was recorded as $ml.min^{-1}.g^{-1}$ or % of baseline readings or baseline control. In permanent models, the CBF measurement closest to one hour after onset of occlusion was used. For transient models CBF measurements were used after one hour of reperfusion. Where articles gave infarct volume or CBF readings for different

brain regions the data were classified as sub-cortical, cortical, or total. If the location was not specified then it was assumed to be total brain. When studies used multiple groups of animals to assess dose response relationships or optimal timing of administration, the data from each group were individually extracted for separate analysis. If the number of animals in a specific group was given as a range then the lowest figure quoted was used. Occasionally, numerical data were not available in print and it was necessary to extract data directly from enlarged, photocopied graphs. All discrepancies were resolved by the project supervisor. Methodological quality of studies was assessed according to published recommendations[344] using an 8 point ' STAIR' rating scale.[310] One point was given for written evidence of each of the following factors; (i) presence of randomisation; (ii) monitoring of physiological parameters; (iii) assessment of dose response relationship; (iv) assessment of optimal time window; (v) blinded outcome measurement; (vi) assessment of outcome at day 1-3; (vii) assessment of outcome at day 7-30 and; (viii) combined measurement of infarct volume and functional outcome.

Analysis

Data were analysed using the Cochrane Collaboration Review Manager (RevMan version 4.1) software. Figure 4.1 shows a typical forest plot and illustrates the layout of studies. Published recommendations suggest that candidate neuroprotectants should be effective in multiple brain regions, at different times of administration, and in permanent and transient stroke.[344,345] Hence, infarct volume and CBF data were grouped 'a priori' for analysis in several ways: (i) by experimental model - permanent or transient; (ii) by outcome location - total brain, cortex, sub-cortex; and (iii) by timing of administration – NO administered before (pre-treated), 0 to 1 hour after (early treatment) or >1 hour after (late treatment) onset. Results are given as SMD (which allows data measured on different scales to be merged) with 95% CI. A random effects model was used since heterogeneity

was likely to be present due to the use of different protocols, animal species, drugs and administration timings. Statistical heterogeneity was assessed with a χ^2 test. The likely causes of heterogeneity were explored using subgroup analyses based on drug type (L-Arg, organic nitrates, SNP, sydnonimines, S-nitrosothiols, NONOates, and hybrid donors) and timing of administration. Publication bias was assessed using Egger's asymmetry test[311] (Stata function 'metabias') and visual assessment of a funnel plot. Significance was set at $P<0.05$.

4.4 Results

Design of studies

The literature search identified 440 potential articles, although most of these were excluded (figure 4.2). The characteristics of the remaining 25 studies (median 41 animals) are given in table 4.1. Most of these studied NO administration in permanent / focal ischaemia (15 articles). Transient models utilised both focal (6 articles) and global (4 articles) ischaemia. Rat species (Sprague Dawley, Wistar, Spontaneously Hypertensive, Long Evans) were used in 23 studies (table 1), whereas only 1 chose a rabbit model[346] and 1 used both.[339] Methodological design was variable as far as drug administration was concerned. Several different routes (intra-venous, intra-arterial, intra-peritoneal) were used at timings ranging from 18 hours before to 24 hours after induction of ischaemia. Likewise, dosage regimens differed between studies, e.g. L-Arg 3.0 – 300 mg/kg, SNP 0.1 – 3.0 mg/kg, SIN-1 0.1 – 10.0 mg/kg. In three studies[188,189,337], the hypotensive effect of high doses of SNP and SIN-1 were countered by co-administration of phenylephrine.

Study design was more consistent for outcome measures. Infarct volume was mostly measured in mm^3, apart from two instances when it was given as percentage of total brain[163] or hemisphere.[195] Volumes were assessed by histological staining techniques and

image analysis of sequential coronal brain sections in all studies. Regional CBF was commonly measured as percentage of baseline values in the cerebral cortex using laser Doppler flowmetry.[163,188,189,222,337,339,347-351] In addition, several studies analysed CBF as $ml.min^{-1}.g^{-1}$ using 14C-iodoantipyrine[192,352] or hydrogen clearance[211,342,346] techniques.

The median STAIR rating for the included articles was 2 points (range 1-7 out of 8). Animals were allocated treatment by randomisation in 8 articles.[114,191,195,199,339,346,351,352] In 9 studies[114,163,189,191,195,347,349,350,353] different dosage regimens were tested, but only 4 assessed the optimal timing window for administration.[114,195,337,348] Most studies examined outcome measures between days 1-3; only one did this blinded to treatment[339] whilst two looked at functional outcome as well as infarct volume.[114,195]

Infarct volume and CBF

Overall, NO administration resulted in significant reductions in total, cortical and sub-cortical infarct volume in permanent models of ischaemia (table 4.2). NO administration also reduced total infarct volume in transient models; data were limited to single studies for cortical and sub-cortical stroke. At approximately one hour of permanent occlusion CBF was significantly increased by NO donors in the cerebral cortex (table 4.2). There was no significant effect at 1 hour of reperfusion in transient stroke. Data were lacking for other comparisons. Publication bias was present for studies reporting the effect of NO administration on lesion volume in permanent (Egger's test p=0.02), but not transient (Egger's test p=0.39) models. Heterogeneity was present in several of the analyses and so infarct volume and CBF were further examined by drug type (table 4.3, Figure 4.1). Likewise, the effects of different timings of administration were examined separately in permanent and transient models (Table 4.4).

L-Arg (NO substrate)

L-Arg did not significantly alter lesion volume in both permanent and transient stroke. This was despite evidence of a beneficial effect on cortical CBF in permanent models.

SNP

SNP reduced total infarct volume after permanent and transient stroke, although it did not appear to change cortical CBF.

Organic nitrates

GTN did not alter cortical CBF after permanent ischaemia, although this finding was based on one study. There were no studies examining the effect of GTN on infarct volume.

NONOates

The NONOates significantly reduced total infarct volume in transient, but not permanent, models. There was a non-significant trend to increased cortical CBF following reperfusion in transient models.

Sydnonimines

The sydnonimines were effective in both permanent and transient stroke models. In permanent models they reduced total infarct volume and increased cortical CBF. In transient models they significantly reduced total infarct volume.

Hybrid donors

Total infarct volume was reduced in one study.[199] There were no data available for CBF outcomes.

Timing of treatment

Pre-treatment with NO from any source reduced infarct volume in transient but not permanent models of ischaemia. Early administration of NO from 0 – 1 hour of onset was equally effective in both transient and permanent stroke. Later treatment with NO had no overall beneficial effect on infarct volume in either transient or permanent stroke.

4.5 Discussion

This systematic review has assessed the effect of NO donors on lesion size and CBF in experimental stroke models. With one exception[354] most of the individual studies were positive or neutral; when combined, NO significantly reduced infarct volume and improved CBF after permanent stroke. NO similarly reduced lesion volume in transient stroke. One article noted that NO-induced changes in CBF preceded electroencephalographic brain recovery.[348] Others found concomitant increases in pial vessel diameter.[347,350,355] Taken together these findings suggest that vascular effects of NO are important for limiting neuronal damage, perhaps because they lead to improved collateral blood flow in areas of compromised cerebral perfusion.

Source of NO

In general, the NO donors (SNP, Sydnonimines, NONOates and hybrid donors) were all effective at reducing lesion volume in transient stroke, permanent stroke, or both. An exception was L-Arg, which increased cortical CBF after permanent occlusion but was otherwise ineffective. In addition, L-Arg significantly increased infarct volume in one article.[354] Several factors may account for these findings: L-Arg stimulates hormones such as insulin, glucagon, growth hormone and catecholamines[356] and can be converted to toxic by-products such as polyamines[357] or agmatine.[358] Alternatively, L-Arg may enhance the synthesis of potentially detrimental NO from iNOS since it countered the

neuroprotective effects of iNOS inhibitor in two studies.[222,354] Hence, L-Arg is probably not an agent of first choice for testing in clinical stroke.

Timing of treatment

The timing of drug administration varied considerably from 18 hours prior to 24 hours after onset of ischaemia. Early treatment within 1 hour of ischaemia was effective in both transient and permanent models of stroke whereas later treatment was ineffective. This biphasic response can be explained by NO having neuroprotective activity. Further experimental studies with delayed NO administration are required in order to assess when the optimum time window closes.

Types of models

Reductions in lesion volume were present in both permanent and transient models of stroke. No direct comparisons of efficacy in these different models have been published. However, indirect assessment using the data in this review suggest that NO donors may be less effective after transient ischaemia. Reductions in total lesion volume for transient models were about half those seen for permanent ischaemia. Also, whilst there was evidence of benefit on infarct volume in permanent cortical and sub-cortical stroke there was none in transient stroke. It is difficult to interpret the findings in transient models since focal and global models of transient ischaemia differ neuro-pathologically[359], and it was not possible to examine them separately because of lack of studies. Nevertheless, the most likely explanation is that since transient ischaemia was associated with less cerebral damage than seen with permanent models, there was a smaller lesion for NO donors to modify. Alternatively, the apparent differences may reflect chance, paucity of data and varied protocol design (e.g. timing of drug administration). Additionally, NO might induce

reperfusion injury after transient ischaemia,[214] although NO has been proposed as a treatment for reducing reperfusion injury after thrombolysis.[339]

Limitations

Although this review has demonstrated an association between NO administration and reduction in infarct volume and improved CBF in experimental stroke, the findings are limited by several factors. First, there were considerable variations in animal species, physiological parameters (e.g. BP), drug administration (timing, dosage, route), surgical methodology, and duration of ischaemia between the studies, as highlighted above. Unfortunately, it is not possible to judge if the relationships that were observed are independent of these factors. Inevitably, the variety of protocols will have made the results more heterogeneous although this was accounted for by using a random effects statistical model. Second, since Egger's asymmetry test suggested publication bias was likely, it is possible that the extensive search strategy did not identify all studies. Publication bias could have resulted from both the lack of reporting of some neutral or negative studies, and through commercial pressures not to publish positive studies involving patented drugs in development. Consequently, the benefits of NO on infarct volume and CBF might have been either over or underestimated.

Third, when numerical data were not available the information on volume and blood flow was extracted from published figures. This can be imprecise, although graphs were enlarged and two independent authors extracted the data. Fourth, a few articles administered phenylephrine in order to counter hypotension induced by high doses of SNP and sydnonimines.[189,337,360] These studies are complex to assess, in part because phenylephrine could itself be beneficial after stroke.[361] Fifth, the studies came from a relatively small number of research groups and involved a limited number of animals. Both

of these factors could have influenced the outcome of the analysis. The extraction of multiple pieces of information from a limited number of sources further risks introducing bias into the review. Finally, it is worth noting that the median STAIR rating was only 2 out of a maximum of 8. Several key concerns exist when considering study quality: few studies stated that they used randomisation to treatment; only one study reported using a blinded observer to assess outcome[339]; and functional outcomes were only assessed in two studies.[114,195] Future studies evaluating neuroprotective agents in experimental stroke models should follow published recommendations on preclinical drug development.[344]

In summary, administration of NO in experimental stroke is associated with a reduction in infarct size. This effect may be mediated, at least in part, through beneficial effects on CBF in areas of compromised perfusion. NO administration was also effective in multiple brain regions in permanent and, to a lesser extent, transient stroke. This would support the continued development of NO sources for treatment of human stroke, as has been started in phase II clinical trials.[166,362] However, the potentially narrow time window for efficacy with NO donors might be a limiting factor. The potential of NO administration in acute stroke will remain uncertain until the results of a large ongoing RCT is available.[363]

Table 4.1 Included studies

Drug	Studies	Species	STAIR rating	Total N	Model P/T	Occlusion G/F	1st dose timing (min)	Route	Measures	
									Infarct vol	CBF
L-Arg	Bednar, 1997 [346]	NZR	2	27	P	F	+30	i.v		$ml.min^{-1}.g^{-1}$
	Buisson, 1993 [355]	SDR	2	31	P	F	-30	i.p	mm^3	
	Dalkara, 1994 [348]	SHR	2	28	P	F	+5, +15, +30	i.v		%
	Escott, 1998 [114]	SDR	6	55	T	F	+5	i.p.	mm^3	
	He, 1995 [342]	SHR	2	43	P	F	-20	i.p	mm^3	$ml.min^{-1}.g^{-1}$
	Humphreys, 1998[349]	SDR	2	63	T	G	-30	i.v		%
	Iadecola, 1995 [222]	SHR	2	53	P	F	+1440	i.p	mm^3	
	Morikawa, 1992a[364]	SHR	2	60	P	F	-960	i.p	mm^3	
	Morikawa, 1992b [350]	SHR	2	36	P	F	+5	i.v		%
	Morikawa, 1994 [347]	SHR, SDR	3	53	P	F	+5	i.v	mm^3	%
	Prado, 1996 [352]	SHR	3	31	P	F	-1080	i.p	mm^3	$ml.min^{-1}.g^{-1}$
	Sadoshima, 1997 [211]	SHR	1	34	T	G	+60	i.v		$ml.min^{-1}.g^{-1}$
	Zhang, 1996 [354]	SDR	1	71	T	F	+1440	i.p	mm^3	
	Zhao, 1999 [351]	WR	2	12	T	G	-30	i.p		%
GTN	Bednar, 1997 [346]	NZR	3	27	P	F	+30, +150	i.v		$ml.min^{-1}.g^{-1}$
SNP	Bednar, 1997 [346]	NZR	3	27	P	F	+30, +150	i.v		$ml.min^{-1}.g^{-1}$
	Chi, 1995 [192]	LER	1	28	P	F	+40	i.v		$ml.min^{-1}.g^{-1}$
	Salom, 2000 [163]	WR	3	56	T	F	0	i.v	%	%
	Zhang, 1993 [188]	SDR	2	31	P	F	+3	i.a	mm^3	%
	Zhang, 1994a [189]	SHR, SDR	3	74	P	F	+3	i.a	mm^3	%

Sydnonimines	Chi, 1995 [192]	LER	1	28	P	F	+40	i.v		$ml.min^{-1}.g^{-1}$
	Coert, 1999 [191]	WR	3	92	T	F	-30	i.v	mm^3	
	Coert, 2002 [353]	WR	4	60	T	F	-30	i.v	mm^3	
	Zhang, 1994a [189]	SHR, SDR	3	74	P	F	+3	i.a	mm^3	%
	Zhang, 1994b [337]	SHR	2	39	P	F	+3, 15, 30, 60, 120	i.a	mm^3	%
NONO-ates	Coert, 1999 [191]	WR	3	92	T	F	-30	i.v	mm^3	
	Mason, 2000 [338]	WR	2	12	T	G	+5	i.v	mm^3	
	Pluta, 2001 [339]	SDR, NZR	2	41	T	F	+60	i.a	mm^3	%
	Salom, 2000 [163]	WR	3	56	T	F	0	i.v	%	%
	Zhang, 2001 [195]	WR	7	?	P	F	+1440, 2880	i.p	%	
Hybrid donors	Fredduzzi, 2001 [199]	SHR	2	40	P	F	+10	i.p	mm^3	

Abbreviations: Spontaneously Hypertensive rat (SHR); Sprague-Dawley rat (SDR); Wistar rat (WR); Long-Evans rat (LER); New-Zealand rabbits (NZR); male (M); female (F); permanent (P); transient (T); global (G); focal (F); intra-venous (i.v.); intra-arterial (i.a.); intra-peritoneal (i.p.); infarct volume (infarct vol.); cerebral blood flow (CBF)

Table 4.2 Effect of no donors on lesion volume and CBF by brain region; data are SMD (95% CI)

Outcome	Permanent models			Transient models		
	Total	Cortical	Sub-cortical	Total	Cortical	Sub-cortical
Lesion volume	-1.21* (-1.69, -0.73) S=10, n=235	-1.40* (-1.94, -0.86) S=6, n=181	-0.55* (-0.86, -0.24) S=5, n=131	-0.78* (-1.21, -0.35) S=7, n=228	+2.35* (0.97, 3.72) S=1, n=16	+0.54 (-0.49, 1.58) S=1, n=16
CBF	+0.66 (-0.02, 1.35) S=1, n=24	+0.86* (0.52, 1.21) S=8, n=220	+0.48 (-0.18, 1.14) S=3, n=42	No data	+0.34 (-0.02, 0.70) S=4, n=108	+0.64 (-0.18, 1.47) S=1, n=24

Abbreviations: number of studies (S); number of animals (n); *p<0.05

Table 4.3 Effect of different NO donors infarct volume and CBF; data are SMD (95% CI)

	Permanent		Transient	
	Total infarct volume	Cortical CBF	Total infarct volume	Cortical CBF
L-Arg	-0.59, (-1.33, 0.16) S=5, n=118	+0.82*, (0.42, 1.23) S=4, n=101	+0.96, (-2.15, 4.07) S=2, n=36	+0.52, (-0.16, 1.20) S=2, n=35
SNP	-2.23*, (-3.27, -1.20) S=2, n=41	+0.60, (-0.44, 1.64) S=4, n=45	-1.14*, (-2.08, -0.20) S=1, n=25	-0.04, (-0.76, 0.67) S=1, n=22
GTN	No data	-0.35, (-1.60, 0.91) S=1, n=10	No data	No data
NONOates	-0.42, (-1.24, 0.40) S=1, n=16	No data	-0.79*, (-1.39, -0.20) S=4, n=83	+0.43, (-0.09, 1.28) S=2, n=51
Sydnonimines	-2.06*, (-3.06, -1.07) S=2, n=50	+1.26*, (0.56, 1.95) S=3, n=64	-1.03*, (-1.54, -0.52) S=2, n=84	No data
Hybrid donors	-1.09*, (-2.04, -0.14) S=1, n=20	No data	No data	No data

Abbreviations: number of studies (S); number of animals (n); *p<0.05

Table 4.4 Effect of timing of administration (within 1 hour versus >1 hour) on total infarct volume; data are SMD (95% CI)

	Timing		
	Pre-treatment	Early Treatment	Late Treatment
Permanent models	-0.08 (-1.78, 1.61) S=2, n=35	-1.77* (-2.23, -1.30) S=6, n=158	-0.17 (-0.73, 0.38) S=3, n=46
Transient models	-0.75* (-1.21, -0.29) S=2, n=102	-1.15* (-1.58, -0.73) S=4, n=86	+2.60* (1.15, 4.05) S=1, n=16

Abbreviations: number of studies (S); number of animals (n); *p<0.05

Figure 4.1 Effect on total lesion volume by different no donor classes for permanent models of ischaemia

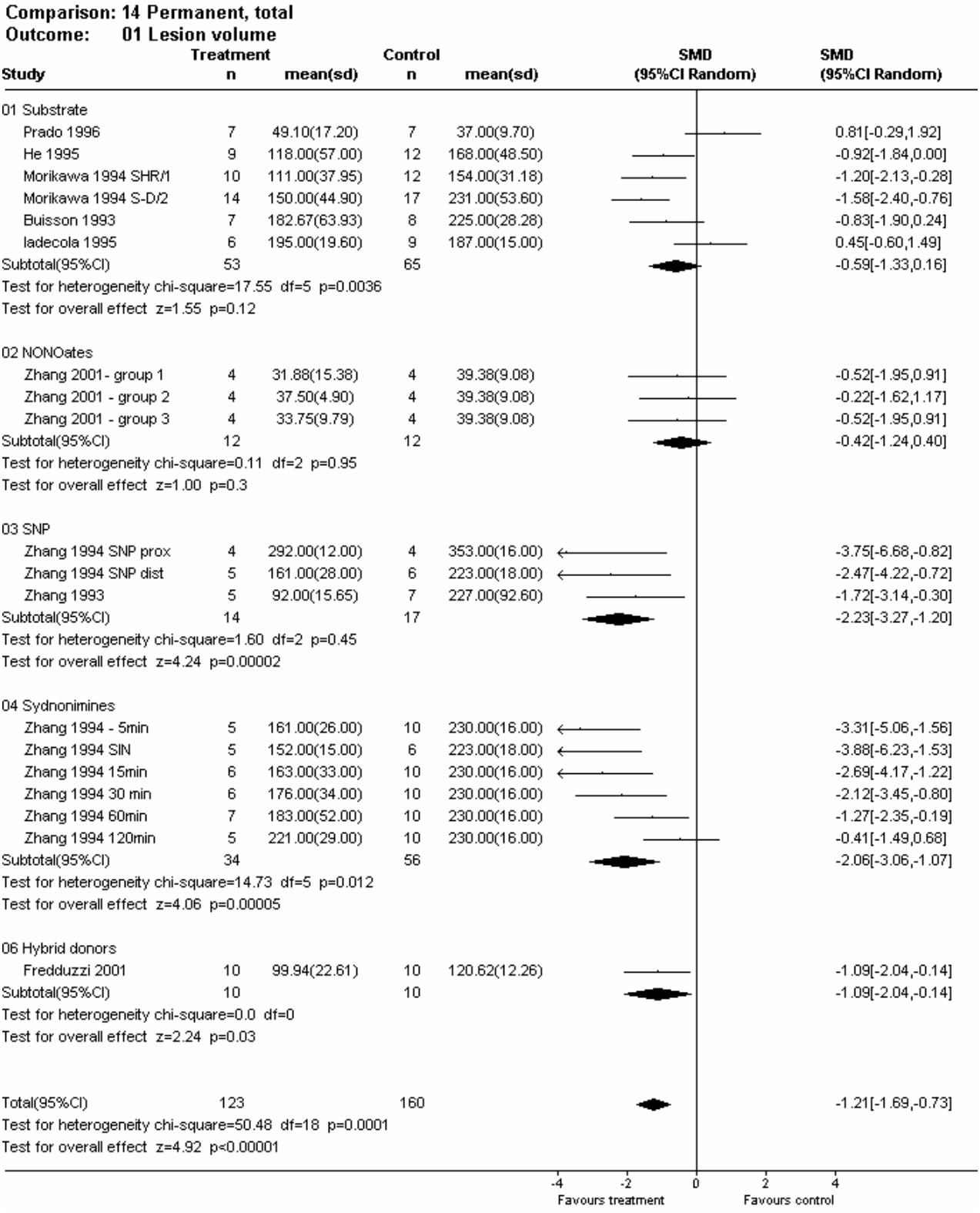
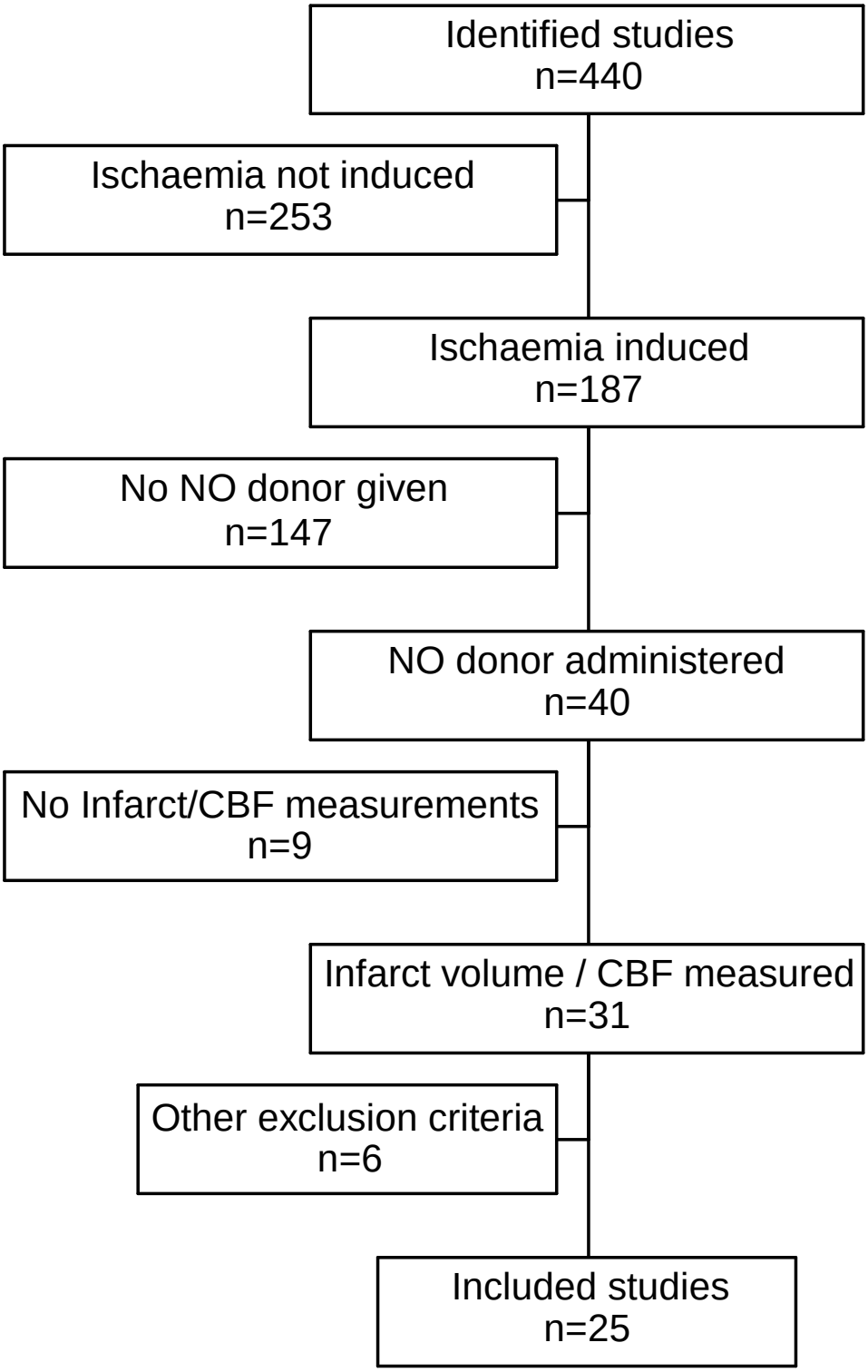


Figure 4.2 Search process showing identified trials



Chapter 5.

NOS inhibitors in experimental IS and their effects on infarct size and CBF; a systematic review

5.1 Abstract

Background: NO produced by the neuronal or inducible isoforms of NOS (nNOS, iNOS) is detrimental in acute IS , whilst that derived from the endothelial isoform (eNOS) is beneficial. However, experimental studies with NOS inhibitors have given conflicting results. Method: Relevant studies were found from searches of EMBASE, PubMed and reference lists; of 456 references found, 73 studies involving 2321 animals were included. Data on the effect of NOS inhibition on lesion volume (mm^3, %) and CBF (%,$ml.min^{-1}.g^{-1}$) were analysed using the Cochrane Review Manager software. Result: NOS inhibitors reduced total infarct volume in models of permanent (SMD -0.56, 95% CI, -0.86, -0.26) and transient (SMD -0.99, 95% CI -1.25, -0.72) ischaemia. Cortical CBF was reduced in models of permanent but not transient ischaemia. When assessed by type of inhibitor, total lesion volume was reduced in permanent models by nNOS and iNOS inhibitors, but not by non-selective inhibitors. All types of NOS inhibitors reduced infarct volume in transient models. Conclusion: NOS inhibition may have negative effects on CBF but further studies are required. Selective nNOS and iNOS inhibitors are candidate treatments for acute IS.

5.2 Introduction

NO is synthesised from its precursor L-Arg by the action of NOS. NO is produced in the brain following the onset of cerebral ischaemia, although it's precise role in the pathophysiology of IS is unclear. Gene knockout studies have determined that NO derived from the eNOS is beneficial in acute IS.[99] This may be due, in part, to antiplatelet effects[75] and preservation of CBF.[336] In contrast, NO produced by nNOS and iNOS can be neurotoxic.[101,104] This probably occurs through NO induced formation of peroxynitrite[365] and toxic free radicals leading to damage by lipid peroxidation.[366] NO further potentiates damage by inhibiting enzymes needed for mitochondrial respiration (cytochrome oxidase), glycolysis (GAPDH) and DNA replication (ribonucleotide reductase).[367-370] Moreover, NO has been reported to stimulate the release of the neurotransmitter glutamate and could contribute to excitotoxicity.[371,372] Consequently, inhibition of NO production has been considered to be a candidate treatment for acute IS.

The first NOS inhibitors were the guanidino aminoacids, many of which act competitively at the NOS active site, e.g, L-NNA, L-NAME (a methyl ester pro-drug that is activated to become L-NNA) and L-NMMA. Both L-NAME and L-NNA exhibit greater in-vitro potency than L-NMMA in inhibiting nNOS and eNOS versus iNOS (table 5.1)[373] However, none of the guanidino aminoacids discriminate sufficiently to enable them to be used to target a single NOS isoform. By contrast, some inhibitors possess higher affinity against one isoform and are commonly referred to as 'selective', although this term is used rather indiscriminately.[108] Agents used to target iNOS include: aminoguanidine, NG-iminoethyl-L-lysine (L-NIL), the bis-isothioureas (PBITU)[109], 1400W (N-[3-(aminomethyl)benzyl]acetamidine), GW273629 and GW274150.[110] Other agents are used to target nNOS and include: 7-NI, tri(fluoromethylphenyl)imidazole (TRIM)[111], ARL 17477, AR-R18512[112], BN 80933[113], S-ethyl and S-methyl thiocitrulline and vinyl L-NIO. Recent

in-vitro studies have suggested that in some cases the distinction between selective iNOS and selective nNOS inhibitor may not be straightforward. For example, aminoguanidine is only mildly selective against iNOS in-vitro (~5 fold) and probably affects other molecular targets.[108] Similarly, 7-NI has been found to be an equipotent inhibitor of all three isoforms of NOS at the isolated enzyme level (table 5.1)[108,114] although it has more selectivity for nNOS in vivo, possibly a consequence of cell specific effects (neuronal verses endothelial).[108]

Studies of NOS inhibitors in IS models have given contradictory results for effects on lesion size and CBF, with many demonstrating beneficial effects[113,204,215,218,226] whilst others report contradictory findings.[188,210,374-377] Hence, the aims of the present investigation were to undertake a systematic review to determine the efficacy of NOS inhibitors to decrease brain injury following cerebral ischaemia, and to assess whether their effects may be influenced by changes in CBF, timing of administration, type of model and animal species.

5.3 Methods

Study identification

Experimental studies assessing the effects of NOS inhibitors on IS lesion volume and CBF in IS models (transient or permanent, global or focal, any species) were identified. Searches were made of 'EMBASE' and 'Pubmed' for articles published from 1980 - 2002. For the EMBASE search four primary keywords (nitric oxide, brain, ischaemia, non-human) were chosen combined with a fifth chosen from a list of NOS inhibitors. Different primary keywords were used in the Pubmed search (nitric oxide, cerebro*, ischaemia) which was then limited to animal studies. Other publications were found from reference lists and review articles. Abstracts were then used to select relevant articles for an

examination of the full publication. Final decisions on inclusion or exclusion were made by two researchers. Pre-specified exclusion criteria were used to minimise the potential for bias, namely; (i) not an IS model; (ii) NOS inhibitor not administered; (iii) No infarct volume or CBF data reported; (iv) no control group; (v) incompatible data (for instance, standard deviations omitted); or (vi) duplicate publication.

Data Extraction

Infarct volume data (mm^3 or % of normal brain) and CBF data ($ml.min^{-1}.g^{-1}$ or % of baseline readings or baseline control) were extracted for analysis. Infarct volume measurements from the longest period of follow-up were used. CBF measurements after one hour of occlusion or reperfusion were used in models of permanent and transient ischaemia respectively. Where possible, regional infarct volume and CBF data were obtained separately for total brain, cortex and sub-cortex. In cases where region was not specified then the measurements were classified as total brain. If an article investigated dose response relationships or optimal timing of administration then data from each individual experimental condition were included separately. In cases where the number of animals in each experiment was given as a range then it was assumed to be the lowest figure. Where numerical values were not available, data were estimated directly using a ruler from graphs that were enlarged twofold. All data extraction was done by two independent researchers; discrepancies were resolved by the project supervisor. Finally, the methodological quality of the included articles was assessed as an 8 point 'STAIR rating'[310] based on published recommendations for investigating new agents in experimental IS[344]; one point was given for written evidence of each of the following factors; (i) randomisation; (ii) monitoring of physiological parameters; (iii) assessment of dose response relationship; (iv) assessment of optimal time window; (v) blinded outcome

measurement; (vi) assessment of outcome at day 1-3; (vii) assessment of outcome at day 7-30 and; (viii) combined measurement of infarct volume and functional outcome.

Analysis

Study data were grouped by protocol prior to analysis: (i) experimental model - permanent or transient; (ii) outcome location - total brain, cortex, sub-cortex; (iii) outcome measure - infarct volume (mm^3, %), and CBF ($ml.min^{-1}.g^{-1}$, %). Data from each of these groups were analysed as forest plots using the Cochrane Collaboration Review Manager (RevMan version 4.1) software. Figures 5.1A and 5.1B illustrate the general layout of the forest plots. Publication bias was assessed using Egger's asymmetry test[311] (Stata function 'metabias'). Results are given as SMD (reported in units of standard deviation), which allows data measured on different scales to be merged, and 95% CI. A random effects model was used since statistical heterogeneity, assessed with a χ^2 test, was expected in view of the wide range of protocols. Sensitivity analyses were performed to look at likely sources of heterogeneity, including study quality, NOS inhibitor type (grouped as non-selective inhibitors, iNOS inhibitors, nNOS inhibitors), animal species and timing of administration. For the latter, articles were divided into those that administered NOS inhibitors before (pre-treated), 0 to 1 hour after (early treatment) or >1 hour after (late treatment) onset. Meta-regression (STATA, version 7.0) was used to analyse the relationship between timing of administration and the effect on total infarct volume (SMD) in order to try and determine the size of the therapeutic window. Studies were weighted by sample size and only those that administered NOS inhibitors after onset of ischaemia were included. Significance was set at $P<0.05$.

5.4 Results

Design of studies

Altogether 456 articles were found in the literature search (figure 5.2). A large proportion did not qualify for the review, leaving 73 studies (s). Data from a total of 2321 experimental subjects were used in the analysis. Table 5.2 summarises the characteristics of the included articles. The most commonly used agents were non-selective inhibitors such as L-NAME (s=38) and L-NNA (s=15). In addition, several compounds were used to target nNOS, including: 7-NI (s=9), AR-R 17477 (s=4), BN 80933, PPBP and TRIM. In contrast, only two types of iNOS inhibitor were represented: aminoguanidine (s=12) and 1400W (s=1). NOS inhibitors were administered to 22 permanent / focal models and to one permanent / global model. More articles assessed the effect of NOS inhibition in transient ischaemia (30 focal, 14 global and 2 using both). In addition, 2 studies were included which compared a combination of permanent and transient models.[220,378] Rats were the animal models used in the majority of studies (24 Sprague Dawley, 21 Wistar, 8 spontaneously hypertensive, 1 Long Evans, 1 Lewis, 1 Fischer). Other studies used diverse species, including; rabbits[379,380], cats[381-383], mice[210,212,214,226,384-387], gerbils[113,388,389], pigs[390-393], and lambs.[394]

Variable methods of drug administration were utilised, e.g. different routes (oral, intra-venous, intra-ventricular, intra-arterial, intra-peritoneal), different timings (1st dose ranging from 6 weeks before to 48 hours after induction of ischaemia) and different dosage regimens (e.g. total administered dose of L-NNA 0.06 – 40.0 mg/kg, L-NAME 0.1mg/kg – 4.2 g/kg, aminoguanidine 100 – 800 mg/kg, ARL-17477 1.0 – 10.0 mg/kg and 7-NI 0.1 – 100 mg/kg). Study design was more consistent for outcome measures. Infarct volumes were assessed by histological staining techniques and image analysis of sequential coronal brain sections in nearly all of the studies. An exception to this were 3 articles that used serial MRI techniques to monitor lesion progression.[395-397] Regional CBF after IS onset was commonly measured as percentage (of baseline or control values) using laser

doppler flowmetry.[188,214,220,222,226,349,351,376,392,398-404] In addition, one article indirectly assessed total CBF using an ultrasonic flow transducer.[394] Alternatively, several studies analysed CBF as ml.min-1.g-1 using 14C-iodoantipyrine[204,209,405], hydrogen clearance[211,393,406,407], radio labelled microsphere[381-383,390,391] or umbelliferone fluorescence[379,380] techniques.

The median STAIR rating for the included articles was 3 points (range 1-6/8). Treatment was allocated by randomisation in only 17 articles whilst 26 studies assessed dose response; just 14 studies assessed the optimal timing of administration. Most studies examined outcome measures between days 1-3; only 9 did this blinded to treatment whilst 11 looked at functional outcome as well as infarct volume. Classifying studies by STAIR score accounted for a significant part of the between group heterogeneity (Figure 5.3). There was no evidence of a beneficial effect on infarct volume in studies with a STAIR rating of 1 point.

Infarct volume and CBF

Collectively, NOS inhibitors caused a significant reduction in total, cortical and sub-cortical infarct volume of magnitude 0.5 to 1.0 standard deviations (table 5.3, figure 5.1A). Paradoxically, detrimental effects on CBF were observed in the cerebral cortex of permanent models. Most CBF data came from studies administering non-selective inhibitors. There was no evidence of publication bias for articles reporting the effect of NOS inhibition on total lesion volume in permanent (Egger's test p=0.33), or transient (Egger's test p=0.65) models. Since heterogeneity was observed in several of the analyses, infarct volume and CBF were further examined by type of NOS inhibitor (figure 5.4), in different animal models and by different timings of administration (figure 5.5).

Type of NOS inhibitor

Non-selective inhibitors

In permanent models non-selective inhibitors did not significantly alter infarct volume but did reduce cortical CBF (figures 5.1A, 5.4A and 5.4B). By contrast, after transient ischaemia non-selective inhibitors reduced total infarct volume and did not affect CBF (figures 5.1B, 5.4C and 5.4D).

iNOS inhibitors

Aminoguanidine and 1400W significantly reduced infarct volume in both permanent and transient models. No studies of selective iNOS inhibitors on CBF were included.

nNOS inhibitors

nNOS inhibitors significantly reduced infarct volume in both permanent and transient models (figures 4A and 4C). At 1 hour of reperfusion there was no overall effect on cortical CBF in transient models. However, reduced cortical CBF was seen in one small (n=8) study involving a model of permanent ischaemia.

Timing of treatment

Treatment before IS onset was effective at reducing infarct volume in transient models (figure 5B) whilst early administration of NOS inhibitors (within 1 hour of onset) was effective in permanent ischaemia (figure 5A). Later treatment after 1 hour of onset had a beneficial effect on infarct volume in both types of IS model. Meta-regression analysis found no evidence of a relationship between total infarct volume (SMD) and timing of administration in permanent ($p=0.08$) or transient ($p=0.18$) models (figure 6).

Animal Model

Overall, NOS inhibitors appeared to reduce total brain lesion size in agyrencephalic species (figure 5C and D) but not in cats. Surprisingly, NOS inhibition in Wistar rat IS models increased infarct volume after permanent ischaemia and decreased infarct volume after transient ischaemia. Limited data were available on the effects of NOS inhibitors in higher animals.

5.5 Discussion

This chapter has examined systematically the effects of NOS-inhibitors on infarct size in experimental IS models. Apart from 7 studies[188,208,210,374,375,377,408], most of the individual articles were either positive or neutral for this outcome. However, when considered together there was an overall beneficial effect, such that NOS-inhibitors significantly decreased lesion size by about 0.5 to 1.0 standard deviations. Several mechanisms are likely to be involved, including; reduced formation of peroxynitrite and reactive oxygen species[409-412], inhibition of brain oedema[395,413], reduced vascular damage[214,386], and inhibition of apoptosis and necrosis.[413,414] Furthermore, NOS inhibitors increase hippocampal neuronal survival in experimental IS[113,388,415-420] and improve functional outcome.[113,214,224,225,386]

The findings are limited by several factors. First, there were differences between study protocols in terms of animal species, physiological parameters (e.g. BP), drug administration (dosage, route), surgical methodology, and duration of ischaemia. Unfortunately, it is not possible to judge whether the relationships that were observed were independent of these factors. In addition, protocol variations can lead to statistical heterogeneity and make the analysis less reliable. To take account of this a random effects model was used and sensitivity analyses were performed to identify sources of

heterogeneity. Second, some relevant articles may not have been identified for inclusion in the review. Publication bias can contribute to this, either through of lack of reporting of neutral or negative studies or through suppression of positive studies for commercial reasons, e.g. intellectual property rights. This could mean the benefits of NO on infarct volume and CBF have been either over or underestimated. Statistical assessment using Egger's asymmetry test did not suggest the presence of publication bias but the possibility that some relevant data was omitted cannot be ruled out. Third, there were several instances when numerical data were not readily available and it had to be derived directly from published figures. This can be imprecise, although graphs were enlarged and two authors extracted data. Fourth, some NOS inhibitors work though additional neuroprotective mechanisms that do not involve inhibition of NO synthesis. Moreover, some of the 'selective' inhibitors only discriminate moderately between iNOS and nNOS.[108] Hence, it is not possible to ascertain whether the relationships observed in each sub-group are independent of either of these factors. Fifth, the technique of extracting multiple pieces of information from single publications has a potential to introduce bias into the review since the results would have been generated by the same investigators and laboratories. Sixth, female animals may be resistant to the damaging effects of iNOS derived NO, perhaps because of modulation by hormones like progesterone.[421] Since the findings in this review are based almost entirely on male animals it is impossible to say whether iNOS inhibitors would work as therapeutic agents in females. Further studies using female animals are required. Finally, since the median STAIR rating of the studies was only 3 out of a total of 8, there are likely to be methodological weaknesses in the included studies. Two key areas of concern are that studies are not reporting that they randomised animals to active and control treatment or performing blinded outcome assessments. All studies evaluating agents in experimental IS models should follow published recommendations on preclinical drug development.[344]

Despite the limitations, the present investigation has shown that NOS inhibitors can decrease brain injury following cerebral ischaemia. Not all published experimental studies agree with this finding, possibly because of differences in drug pharmacology, dosage, route of administration, timing of treatment, animal model and type of ischaemia. Hence, the impact of some of these factors was examined further.

Type of experimental model

Indirect assessment of the pooled data in table 5.3 suggests that NOS inhibitors were equally effective in models of transient and permanent ischaemia. However, the sub-group analysis in figure 5.4 revealed that non-selective inhibitors did not work in permanent ischaemia but did in transient ischaemia models. This discrepancy could be attributed to the presence of additional beneficial effects after transient ischaemia, such as limitation of reperfusion injury caused by eNOS-derived NO.[214] Alternatively, the beneficial effects of non-selective inhibitors may have been limited in models of permanent ischaemia because they inhibit eNOS to a similar degree as nNOS or iNOS. Consequently, they may aggravate brain ischaemia by increasing platelet aggregation and white cell activity, raising BP, and by restricting penumbral blood supply. Evidence of reduced CBF after administration of non-selective inhibitors to permanent models is consistent with this hypothesis. Hence, the non-selective inhibitors are not agents of first choice for testing in clinical IS.

Timing of treatment

The administration of 1st dose varied considerably from 6 weeks prior to 24 hours after onset of ischaemia. Treatment with NOS-inhibitors was effective prior to onset of transient ischaemia, within 1 hour of permanent ischaemia, and even beyond 1 hour of onset in

both transient and permanent ischaemia models. The neutral findings seen with pre-treatment of permanent models and early treatment of transient models probably represents the use of non-selective agents and paucity of data rather than lack of response to NOS inhibitors. More important is the confirmation that these agents reduce infarct volume even when administered beyond 1 hour after IS onset. Beneficial activity several hours after IS suggests that the NOS inhibitors might be useful in clinical IS. Meta-regression analysis did not provide evidence of a therapeutic time window. More experimental studies with delayed administration are required to assess when the optimum window of opportunity closes.

Animal model

Significant infarct reductions after either transient or permanent ischaemia were seen in almost all rat strains. Surprisingly, in Wistar rats (WR), the NOS inhibitors decreased infarct volume after transient ischaemia, but they increased infarct volume in models of permanent ischaemia. This probably reflects the use of non-selective agents in permanent models, rather than being evidence of a different response to NOS inhibition by WR. Only spontaneously hypertensive rats (SHR) responded to NOS inhibitors in both models of transient and permanent ischaemia. This could arise partly because normotensive strains (SDR, WR, FR) suffer smaller and more variable infarcts than SHR.[359] In mice, NOS inhibitors worked in permanent but not transient ischaemia models. This inconsistency is most likely due to lack of data, although these studies are complex to assess because different transgenic mouse strains were used. Unfortunately, paucity of data prevents any definitive statements about the role of NOS inhibitors in rabbits and cats. More studies need to be performed in lissencephalic and/or gyrencephalic species before clinical trials are commenced, as per the STAIR recommendations.[344]

Overall, this chapter brings together data from all published studies and confirms that NOS inhibitors, especially if ‘selective’ to nNOS or iNOS, reduce infarct volume in experimental IS. The data support the development of selective NOS inhibitors as treatments for clinical IS, assuming that side effects are tolerable. Unfortunately, many compounds that provide effective neuroprotection in animal models have progressed to full human studies, and yet none is clinically efficacious. Systematic reviews of animal data can help in the transition to clinical trials and are increasingly recognised as an important prerequisite to human studies.[422-425]

Table 5.1 In-vitro potency (IC50, μM) of commonly recognised inhibitors against NOS isoforms (based on published data[108,110-112,373])

Inhibitor	*iNOS*	*nNOS*	*eNOS*
1400W	0.2	7.3	1000.0
7-NI	6.9-9.7	1.1-8.3	2.1-14.8
Aminoguanidine	27.2-31.0	20.3-170.0	330.0
ARR18512	5.5	0.1	24.0
ARL17477	0.3-5.0	0.04-0.1	1.6-3.5
GW273629	8.0	630.0	1000.0
GW274150	1.4	145.0	466.0
L-NAME	13.5	0.1	1.0
L-NIL	1.6	37.0	49.0
L-NMMA	3.5-6.6	0.3-4.9	1.0-3.5
L-NNA	3.1-6.0	0.02-0.3	0.09-0.6
TRIM	27.0	28.2	1057.5

Abbreviations: 50% inhibition (IC50)

Table 5.2 Included studies

Drug	Studies	Species	STAIR rating	Total N	Model P/T	Occlusion G/F	1st dose timing (min)	route	Measures	
									infarct vol	CBF
Non specific inhibitors										
L-NAME	Anderson, 1996 [380]	R	3	42	T	F	-20	i.v.		$ml.min^{-1}.g^{-1}$
	Anderson, 2000 [379]	R	3	15	P	F	-30	i.v.	%	$ml.min^{-1}.g^{-1}$
	Ashwal, 1993 [206]	SHR	2	14	T	F	-60	i.v.	mm^3	
	Ashwal, 1994 [204]	SHR	3	?	T	F	+0, +120, +150	i.v.	mm^3, %	$ml.min^{-1}.g^{-1}$, %
	Ashwal, 1995 [205]	SHR	3	14	T	F	-60	i.p.	% , mm^3	
	Batteur-Parmentier, 2000 [376]	SDR	3	110	T	F	+5	i.p.	mm^3	%
	Buisson, 1992 [426]	SDR	2	?	P	F	+5	i.p.	mm^3	
	Buisson, 1993 [355]	SDR	2	50	P	F	-30	i.p.	mm^3	
	Charriaut-Marlangue, 1996 [414]	SDR	1	42	T	F	+5	i.p.	mm^3, %	
	Clavier, 1994 [381]	C	3	24	T	G	+180	i.v.		$ml.min^{-1}.g^{-1}$
	Coert, 1999 [191]	WR	3	92	T	F	-30	i.v.	mm^3	
	Dawson, 1992 [427]	SDR	3	18	P	F	-30	s.c	mm^3	
	Dawson, 1994 [378]	SDR	3	67	P, T	F	-30	i.p.	mm^3	
	Ding-Zhou, 2002 [386]	M	4	?	T	F	+180	i.p.	mm^3	
	Greenberg, 1995 [392]	P	4	20	T	G	-60	i.v.		$ml.min^{-1}.g^{-1}$
	Hamada, 1995 [408]	WR	4	77	P	F	-20	i.c.v.	mm^3	
	Hiramatsu, 1996 [390]	P	2	40	T	G	-?	i.v.		$ml.min^{-1}.g^{-1}$
	Humphreys, 1998 [349]	SDR	3	63	T	G	-30	i.v.		%
	Iuliano, 1995 [207]	WR	4	78	T	F	-30	i.v.	mm^3	
	Kamii, 1996 [210]	M	2	?	T	F	+5	i.p.	mm^3	
	Kuluz, 1993 [208]	WR	4	24	T	F	-15	i.v.	mm^3	
	Margaill, 1997 [203]	SDR	4	?	T	F	+5, 180, 360,	i.p.	mm^3	

							540,720			
	Nishikawa, 1993 [382]	C	4	20	P	F	-30	i.v.	% , mm^3	$ml.min^{-1}.g^{-1}$
	Nishikawa, 1994 [383]	C	2	49	T	F	-60, +45	i.v.	%	$ml.min^{-1}.g^{-1}$
	Prado, 1993 [404]	WR	2	14	T	G	-5	i.v.		%
	Puisieux, 2000 [428]	WR	2	52	T	F	-4320	i.p.	mm^3	
	Quast, 1995 [395]	SDR	2	36	T	F	-1	i.v.	mm^3	
	Schleien, 1998 [391]	P	3	18	T	G	-?	i.v.		$ml.min^{-1}.g^{-1}$
	Segawa, 1998 [393]	P	4	12	T	G	+90	i.v.		$ml.min^{-1}.g^{-1}$
	Sercombe, 2001 [429]	SDR	1	37	P	F	-20160, -60480	p.o.	mm^3	
	Stagliano, 1997 [209]	WR	3	29	P	F	+5	i.v.		$ml.min^{-1}.g^{-1}$
	Sugimura, 1998 [400]	WR	3	18	T	G	-?	?		%
	Uetsuka, 2002 [406]	WR	3	40	T	G	-120	i.v.		$ml.min^{-1}.g^{-1}$
	Wei, 1994 [405]	LER	2	28	P	F	+15	i.v.		$ml.min^{-1}.g^{-1}$
	Wei, 1998 [396]	SDR	2	22	T	F	-1	i.p.	mm^3	
	Zhang, 1993 [188]	SDR	2	31	P	F	+3	i.a.	mm^3	%
	Zhang, 1995 [430]	SHR	4	37	P	F	+5, 120, 180, 360	i.a.	mm^3	
	Zhao, 1999 [351]	WR	2	36	T	G	-30	i.p.		%
L-NNA	Buchan, 1994 [220]	WR, SHR	6	?	T, P	G, F	-30 , +5	i.p.	mm^3	%
	Carreau, 1994 [212]	M	3	?	P	F	+5	i.p.	mm^3, %	
	Dorrepaal, 1997 [394]	L	3	18	T	G	+35	i.v.		%
	Gursoy-Ozdemir, 2000 [214]	M	4	65	T	F	+105	i.p.	mm^3	
	Hashimoto, 1999 [402]	WR	2	?	T	F	-10	i.p., i.a..	%	%
	Matsui, 1997 [407]	SDR	4	148	T	F	-5, +5	i.p.	mm^3	$ml.min^{-1}.g^{-1}$
	Nakashima, 1999 [374]	WR, FR	1	?	T	F	+120	i.p., i.v.	mm^3	
	Nowicki, 1991 [384]	M	1	31	P	F	+5	i.p.	mm^3	
	Sadoshima, 1997 [211]	SHR	2	34	T	G	+60	i.v.		$ml.min^{-1}.g^{-1}$, %
	Santizo, 2000 [398]	SDR	2	26	T	G	-45	i.v.		%
	Spatz, 1995 [389]	MG	2	?	T	G	-240	i.p.		%
	Spinnewyn, 1999 [215]	SDR	3	88	T	F	+240	i.v.	mm^3	
	Xu, 2000 [377]	SDR	1	24	P	F	-30	i.p.	mm^3	

	Yamamoto, 1992 [375]	WR	2	37	P	F	+5	i.v.	mm^3	
	Zhang, 1996 [218]	WR	3	73	T	F	+120	i.v.	%	%
L-NMMA	Sakashita, 1994 [401]	WR	1	30	T	G	-5	i.p.		%
nNOS inhibitors										
7NI	Coert, 1999 [191]	WR	3	92	T	F	-60	i.p.	mm^3	
	Escott, 1998 [114]	SDR	6	55	T	F	+5, +90	i.p.	%, mm^3	
	Goyagi, 2001 [226]	WR, M	5	115	T	F	-30	i.p.	%	
	Gursoy-Ozdemir, 2000 [214]	M	4	65	T	F	-30, +90	i.p.	mm^3	
	Humphreys, 1998 [349]	SDR	3	63	T	G	-30	i.v.		%
	Jiang, 1999 [399]	WR	3	18	T	G	-20	i.p.		%
	Kamii, 1996 [210]	M	2	?	T	F	+5	i.p.	mm^3	
	Uetsuka, 2002 [406]	WR	3	40	T	G	-60	i.p.		$ml.min^{-1}.g^{-1}$
	Yoshida, 1994 [227]	SDR	3	55	P	F	+5	i.p.	mm^3	
AR-R 17477	Harukuni, 1999 [431]	WR	5	53	P	F	-30, +60	i.v.	mm^3	
	O'Neill, 2000 [388]	MG, WR	4	?	T	F, G	0, +30, +120	i.v.	mm^3	
	Santizo, 2000 [398]	SDR	2	26	P	F	-45	i.v.		%
	Zhang, 1996 [218]	WR	3	48	T	F	+120	i.v.	%	%
BN 80933	Chabrier, 1999 [113]	SDR, MG	6	?	T	F, G	+5, 240, 360, 480, 1440	i.v.	mm^3	
PPBP	Goyagi, 2001 [226]	WR, M	5	115	T	F	-30	i.p, i.v.	%	
TRIM	Escott, 1998 [114]	SDR	6	55	T	F	+5 or 90	i.p.	%, mm^3	
iNOS inhibitors										

1400W	Parmentier, 1999 [225]	SDR	3	?	T	F	+1080	s.c.	mm^3
Aminoguanidine	Cash, 2001 [397]	SDR	3	?	T	F	+360	i.p.	mm^3
	Cockroft, 1996 [223]	LR	4	?	P	F	+15, 60, 120, 180	i.p.	%
	Han, 2002 [432]	SDR	3	?	T	F	+0	i.p.	%
	Iadecola, 1995 [222]	SHR	2	53	P	F	+1440	i.p.	mm^3 %
	Iadecola, 1996 [403]	SDR	2	79	T	F	+360	i.p.	mm^3
	Nagayama, 1998 [224]	SHR	4	60	P	F	+1440	i.p.	mm^3, %
	Sugimoto, 2002 [385]	M	3	58	P	F	+1080	i.p.	mm^3
	Tsuji, 2000 [433]	WR	3	31	P	G	-60	i.p.	%
	Xu, 2000 [377]	SDR	1	24	P	F	-30	i.p.	mm^3
	Zhang, 1996 [354]	SDR	1	71	T	F	+1440	i.p.	mm^3, %
	Zhang, 1998 [434]	SHR	3	47	P	F	+0	i.p.	mm^3
	Zhu, 2002 [387]	M	2	75	T	F	+360	i.p.	%

Abbreviations: Mice (M); Mongolian Gerbil (MG); Piglets (P); Lambs (L); Cats (C); Spontaneously Hypertensive rat (SHR); Sprague-Dawley rat (SDR); Wistar rat (WR); Long-Evans rat (LER); Lewis Rats (LR); Fischer Rats (FR); Rabbits (R); male (M); female (F); permanent (P); transient (T); global (G); focal (F); intra-venous (i.v.); intra-arterial (i.a.); intra-peritoneal (i.p.); infarct volume (infarct vol.); cerebral blood flow (CBF); value not reported (?)

Table 5.3 Effect of NOS inhibitors on lesion volume and CBF (SMD, 95% CI) by brain region in models of permanent and transient ischaemia

Outcome		Permanent			Transient	
	Total	Cortical	Sub-cortical	Total	Cortical	Sub-cortical
Lesion volume	-0.56* (-0.86, -0.26) S=18, n=554	-0.89* (-1.31, -0.47) S=16, n=409	-0.47* (-0.78, -0.16) S=13, n=361	-0.99* (-1.25, -0.72) S=25, n=927	-0.71* (-0.95, -0.46) S=13, n=543	-0.45* (-0.76, -0.14) S=12, n=418
CBF	No data	-0.80* (-1.34, -0.27) S=6, n=87	-0.73 (-1.83, 0.36) S=1, n=14	-0.57 (-1.26, 0.11) S=8, n=120	-0.22 (-0.52, 0.07) S=15, n=320	-0.38 (-1.03, 0.26) S=3, n=66

Abbreviations: Standardised mean difference (SMD); cerebral blood flow (CBF); 95% confidence intervals (95% CI); number of studies (S); number of animals (n); *p<0.05

Figure 5.1a Total lesion volume by different NOS inhibitor types for permanent models of ischaemia

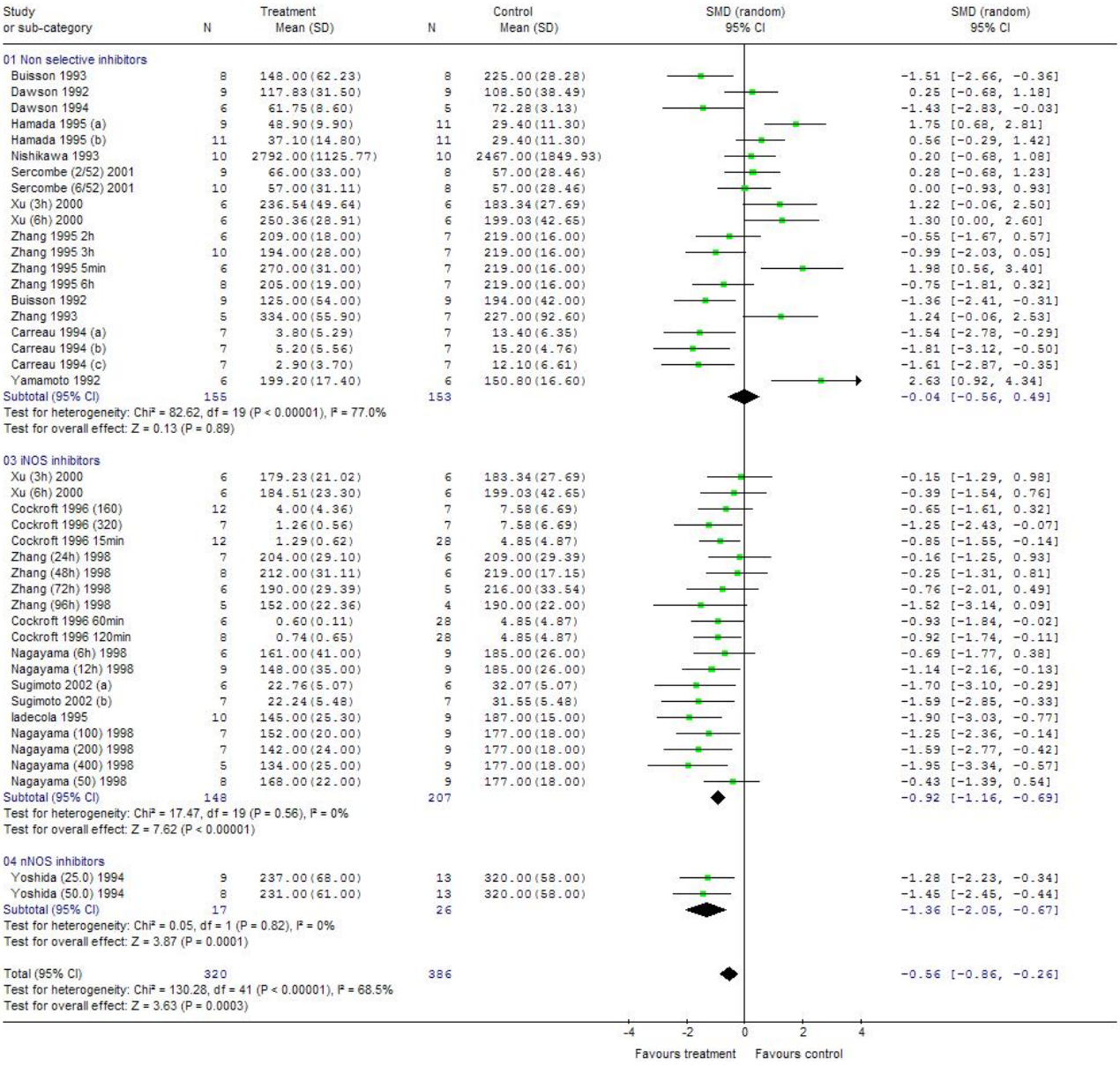


Figure 5.1b Cortical CBF by different NOS inhibitor types for transient models of ischaemia

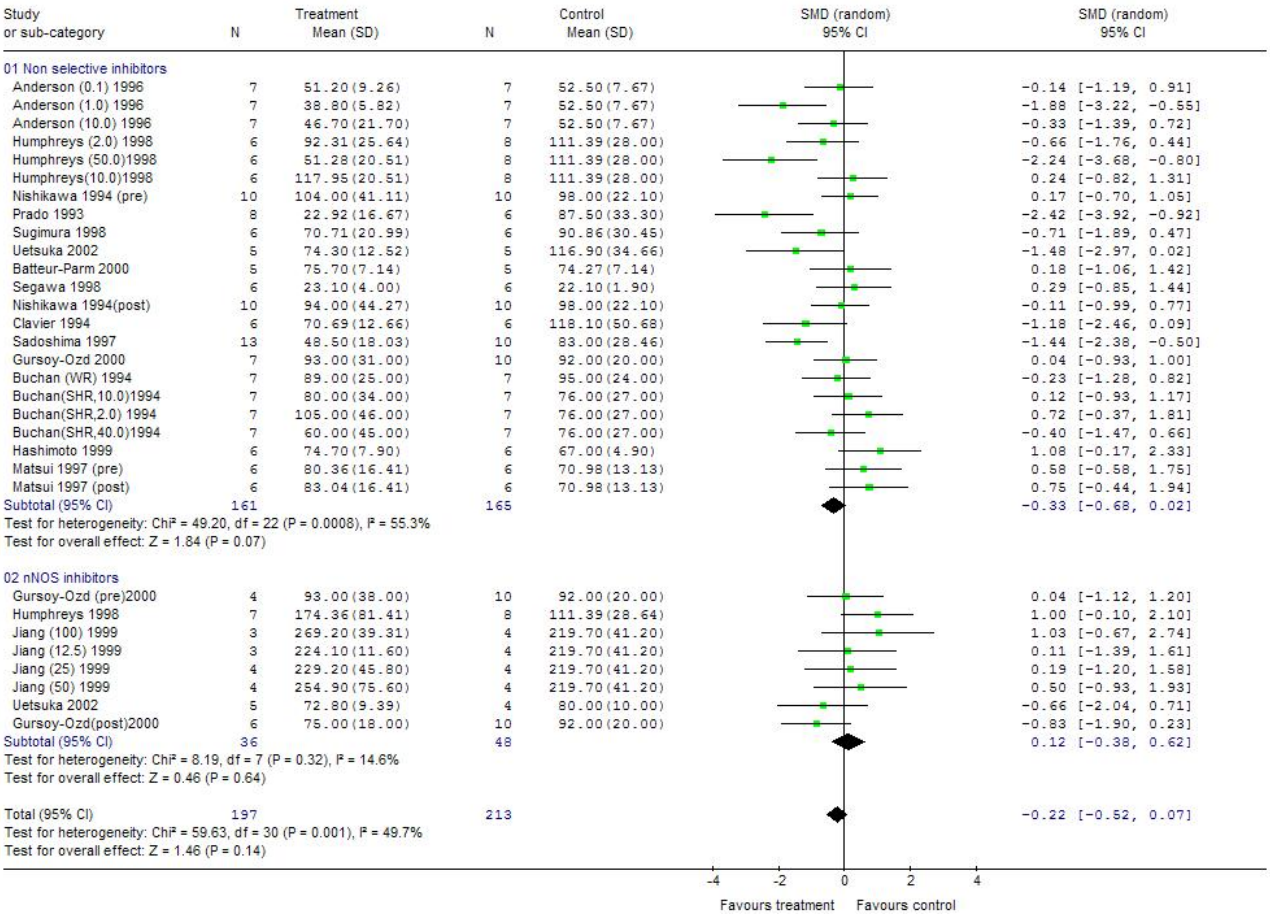


Figure 5.2 Search process showing reasons for exclusion of studies

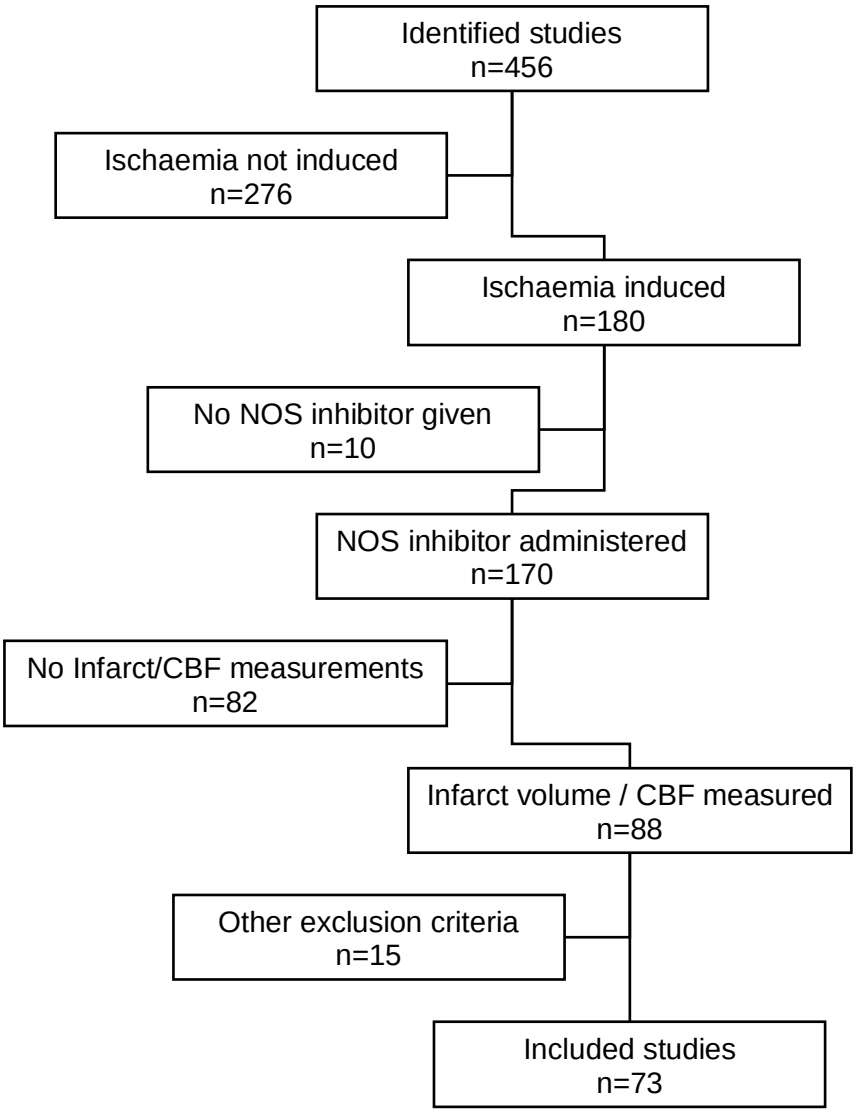


Figure 5.3 SMD and 95%CI by reported STAIR score for (A.) Permanent models; (B.) Transient models

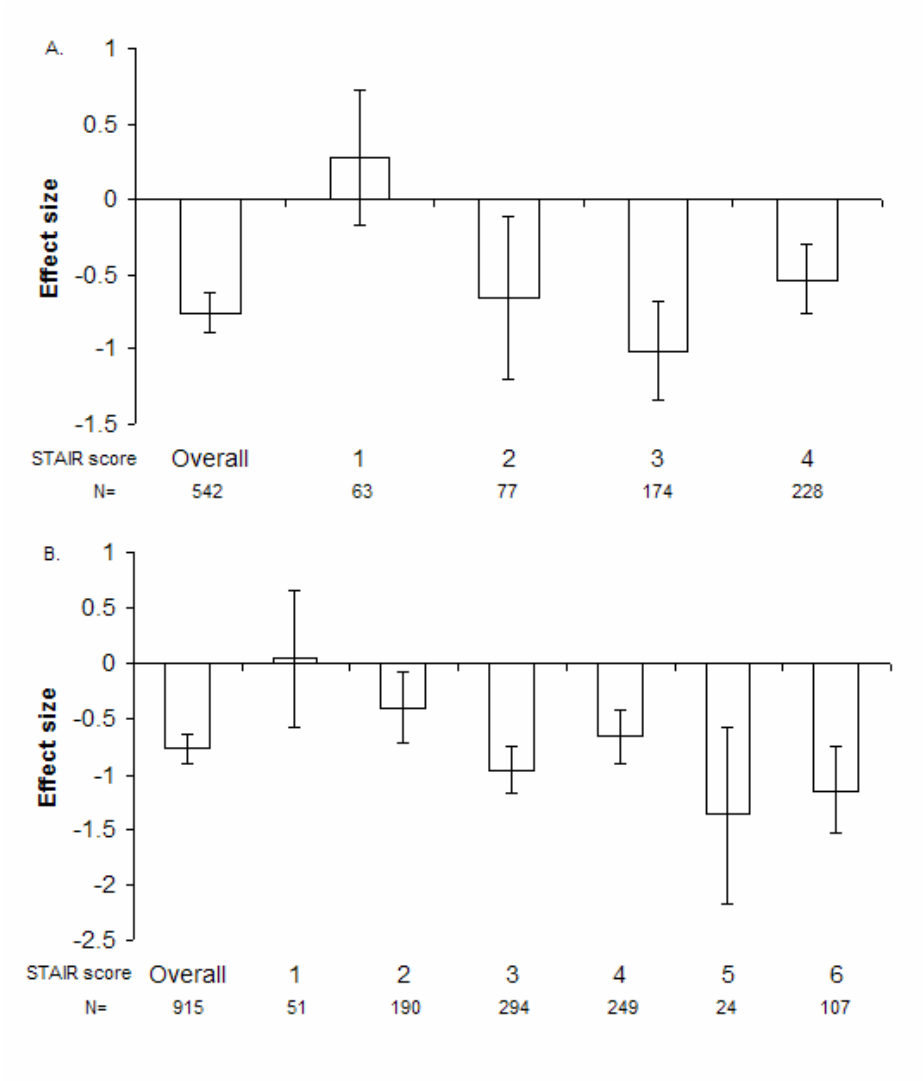


Figure 5.4 SMD and 95%CI by type of NOS inhibitor for (A.) Total lesion volume in permanent models; (B.) Cortical CBF in permanent models; (C.) Total lesion volume in transient models; (D.) Cortical CBF in transient models.

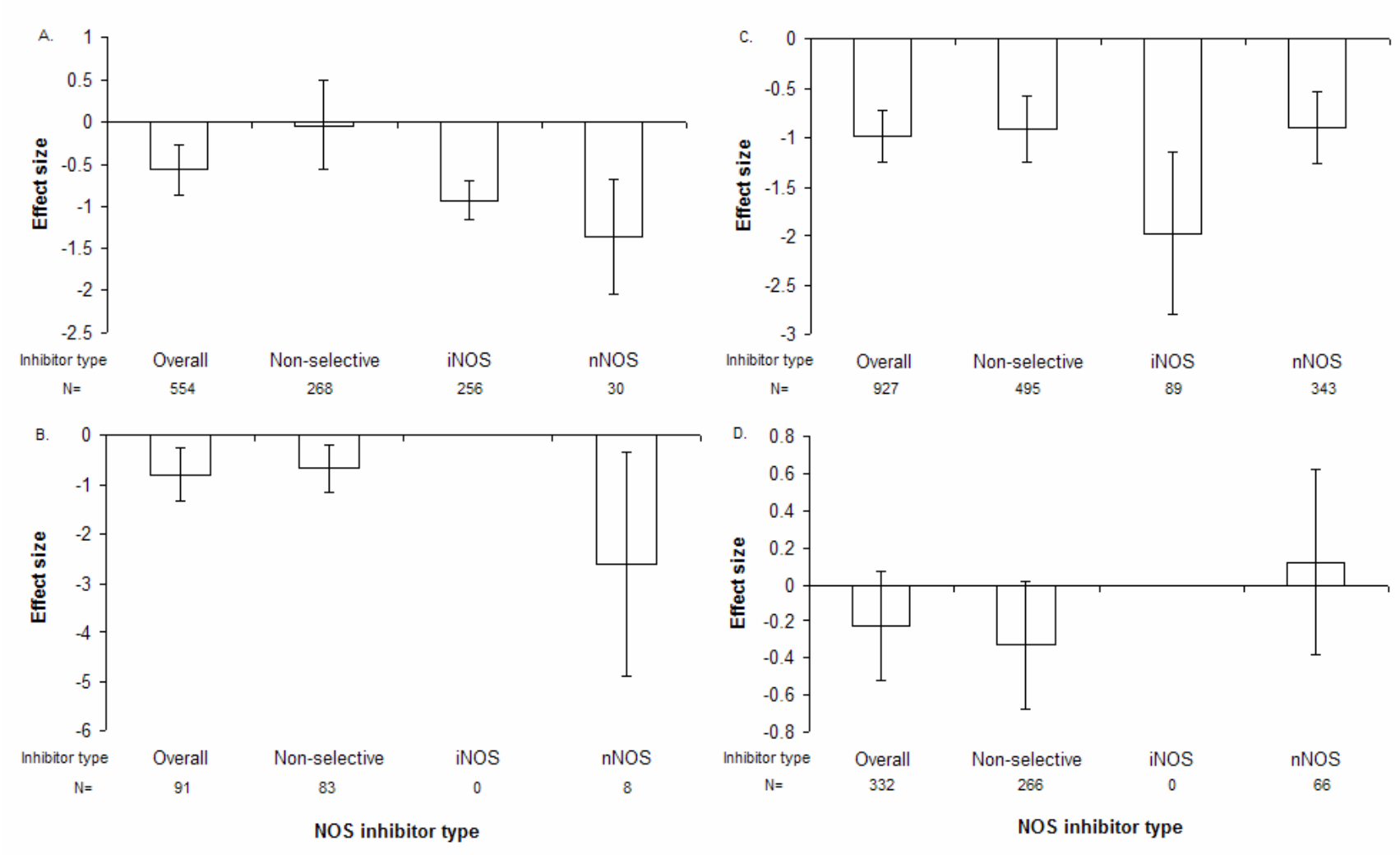


Figure 5.5 SMD and 95%CI by timing of treatment (A.) in permanent models; (B.) in transient models; and by animal species (C.) in permanent models; (D.) in transient models.

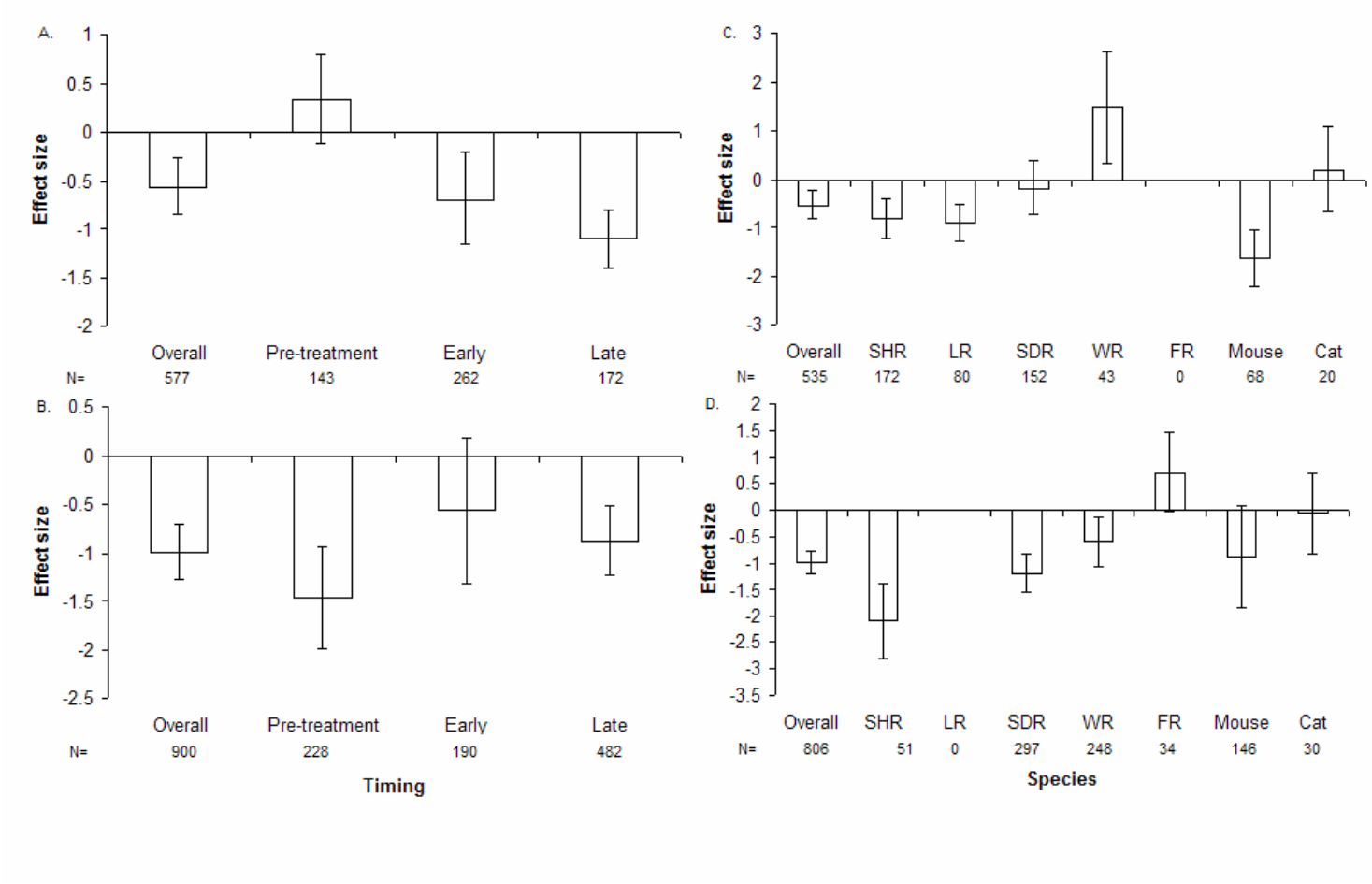
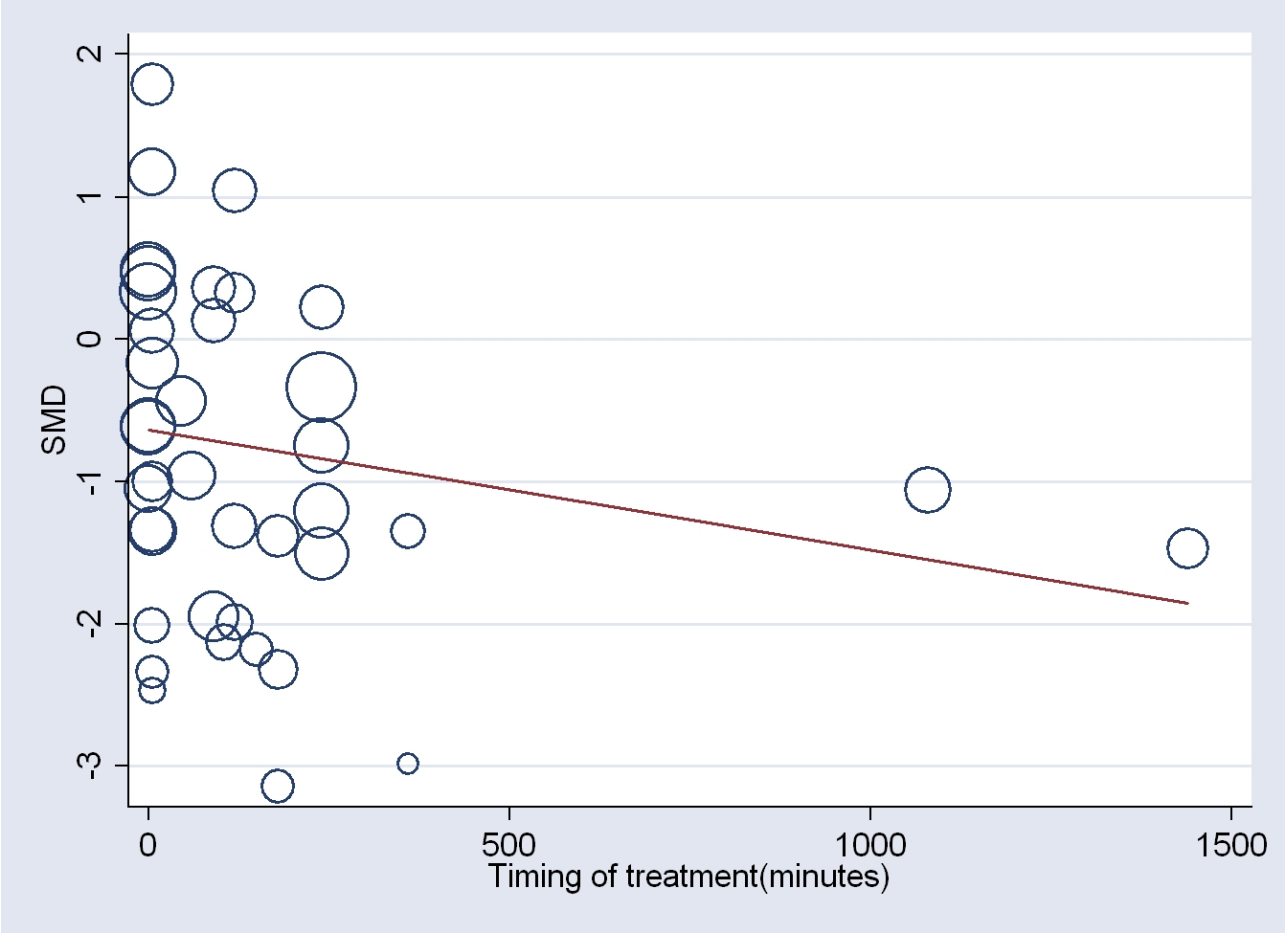


Figure 5.6 Effect of delay until first dose (minutes) on total infarct volume (SMD) in transient ischaemia. Size of circle proportional to size of study.



Chapter 6.

Haemodynamic and plasma effects of transdermal GTN, a NO donor, given <48 hours after stroke

6.1 Abstract

Background: High BP is a potential therapeutic target in acute stroke because it is independently associated with a poor outcome. Hence, the effect of GTN on haemodynamics and platelet activation was investigated in patients with acute (< 48 hours) stroke. Methods: 42 patients with ischaemic or haemorrhagic stroke were randomised within 48 hours to transdermal GTN (5 mg) or control. Peripheral BP measurement (Omron 705CP), pulse wave analysis (Sphygmocor), non-invasive CO monitoring (Portepres), TCD (Nicolet EME Companion TCD system), NO analysis (Seivers 280 NO Analyser) and P-selectin ELISA (Parameter, Human soluble P-selectin immunoassay) were performed before and 1hour after treatment. Daily GTN was continued for 7 days. The effect of GTN on the measurements were adjusted for baseline (ANCOVA); mean and 95% CI are given. Results: GTN lowered peripheral and central systolic BP by -15.1 (95% CI -26.6, -3.5, p=0.01) and -15.6 (95% CI -24.2, -7.0, p<0.01) mmHg respectively. GTN also lowered PP, mean arterial BP (central and peripheral) and AI. Heart rate, diastolic BP, MCA velocity, PI, BI, plasma NO metabolites and p-selectin were unaffected. Conclusions: It is feasible to lower BP <48 hours from stroke onset with transdermal GTN. The absence of any detrimental effect on MCA velocity and PI suggests that cerebral perfusion was not compromised, although further studies are required. Neutral effects on platelet activation imply that GTN patches can continue to be investigated in haemorrhagic stroke.

6.2 Background

BP is raised in three quarters of IS patients and is associated with poor outcome.[138] Therefore, it may be possible to improve outcome by lowering BP as long as CBF is not compromised. NO , given as GTN, is a potent endogenous vasodilator that lowers BP and increases CBF in normal subjects.[435] Moreover, in animal models NO is neuroprotective, possibly due to scavenging of reactive oxygen species[338-340], inhibition of platelets and leucocytes[78,79], and attenuation of NMDA receptors.[341] Two trials have previously studied the effects of GTN in patients with recent stroke on BP. The first demonstrated a significant (~8%) reduction in 24 hour ambulatory systolic BP on day 1. However there was evidence of tolerance at day 8, a known property of organic nitrates.[166] The second found that GTN lowered 24 hour ambulatory mean arterial BP by 5 - 7% on day 1 in a dose dependent manner.[167] Tolerance was overcome by increasing the GTN on day 4. In addition, GTN had no effect on cerebral blood velocity in the affected hemisphere, suggesting that CBF did not change.

The optimum therapeutic time window for GTN administration is not known, however it is likely that the sooner it is given the better. For instance, the NO donor SIN-1 was effective at reducing infarct volume up to, but not beyond 1 hour in one study, suggesting that neuroprotective effects of NO are short-lived.[337] The median time to treatment was 4.4 days in the first GTN trial[166] and 51 hours in the second.[167] Hence, there is a rationale for initiating treatment with GTN earlier than was accomplished in the previous trials. Therefore, the aim of the present study was to undertake a fuller assessment of the effect of GTN on BP when administered <48 hours after onset of ischaemic or haemorrhagic stroke. Effects on plasma NO, platelet adhesion systemic and cerebral haemodynamics were also studied.

6.3 Methods

Design

A single centre sub-study forming part of an ongoing, prospective, international, multicentre, randomised, parallel-group, double-blind, placebo-controlled, trial was performed. The Efficacy of Nitric Oxide in Stroke (ENOS) trial is designed to test the safety and efficacy of NO, given as transdermal GTN, and of continuing or stopping prior anti-hypertensive medication, in 5000 patients with acute ischaemic or haemorrhagic stroke.[363] The ENOS trial database was not interrogated for the purposes of the sub-study; instead data were collected from the ENOS case report forms and prescription charts. Ethical approval was obtained from the local ethics committee (EC01/53) and patients gave written informed consent to participate. The study conformed to the Declaration of Helsinki and International Conference on Harmonisation guidelines. Enrolment took place between September 2001 and September 2004.

Subjects

Previously independent (Rankin score < 3) adult patients with a clinical stroke syndrome, including limb weakness (defined as Scandinavian Neurological Stroke Scale, SNSS Arm and /or leg<6), were recruited within 48 hours of ictus. All subjects had elevated systolic BP (140–220mmHg) on at least one of three consecutive measurements at baseline. Subjects with a requirement for, or a contraindication to nitrate therapy, were excluded. Similarly, those who had a definite need for prior antihypertensive therapy or vasoactive drugs were disqualified.

Treatment

Subjects were randomised to receive either a 5mg GTN patch (Transiderm-Nitro 5, Novartis Pharmaceuticals UK Limited, West Sussex, UK) or nothing once daily for 7 days.

In contrast to a previous study[166] no manufacturer was willing to provide placebo patches, hence a large dressing was used to conceal treatment status from patients ('single blind') and enable blinded outcome assessment. GTN patches were changed at 08:00 every morning by a ward nurse and kept on for a full 24 hours. Subjects taking prior antihypertensive medication were also randomised 1:1 to either stop or continue treatment.

Systemic haemodynamics

Peripheral BP was measured in the non-hemiparetic arm with a validated digital readout oscillometric device (Omron HEM-705CP, Omron Corp, Tokyo, Japan).[235] Average systolic and diastolic BP was determined at baseline, 1 hour after treatment allocation and on day 4 and day 7 of the treatment period. CO and total peripheral resistance (TPR) were assessed using Portapres model-2 (TNO-TPD Biomedical Instrumentation). Measurements were taken for 5 minutes at baseline and at 1 hour after allocation to treatment using the normal hand. BeatScope 2 software (TNO-TPD Biomedical Instrumentation) was used to calculate CO by the Modelflow method.[240] Central systolic BP, diastolic BP and AI were assessed by applanation tonometry at the radial pulse (Sphygmocor Pulse Wave Analysis System, Sydney, Australia). PWV was not determined because measurements from the carotid artery could be dangerous in the presence of un-diagnosed carotid stenosis. Measurements were taken from the non-hemiparetic wrist until two satisfied the quality control system built in to the software. Mean values were then determined at baseline and 1 hour after treatment allocation.

Cerebral haemodynamics

Bilateral MCA blood velocity and PI were measured using a Nicolet EME Companion TCD system connected to a 2 Mhz probe. The highest attainable velocities from the M1 segment of the MCA were recorded at baseline and 1hour after treatment allocation.

Laboratory analyses

Plasma and serum was collected at baseline and 1hour after randomisation to treatment. Samples were analysed in batches blinded to treatment. Plasma NO was measured by chemiluminescence using a Seivers 280 NO Analyser (Analytix Ltd, County Durham, UK). Detailed methods have been published previously.[436] In addition, a commercially available enzyme immunoassay kit was used to assess the effect of GTN on soluble p-selectin (Parameter, Human soluble P-selectin immunoassay, R&D systems, Oxfordshire, UK).

Functional outcome

Data on modified Rankin are collected centrally for ENOS patients and were not available for this study.

Statistical methods

Patients were randomised with minimisation on age, stroke type, stroke severity (SNSS), history of stroke, history of hypertension, and history of nitrate use within previous two days. Data were entered and analysed by received treatment using SPSS (Macintosh version 10.0, SPSS Inc, Chicago, IL). Mean (standard deviation [SD]), median (interquartile range [IQR]) or frequencies (%) are given. All comparisons of BP and haemodynamic data between the treatment groups were analysed with adjustment for baseline values using analysis of covariance (ANCOVA). Data from subjects who were randomised to continue prior antihypertensive medication were excluded from the analysis. Significance was set at $p<0.05$.

6.4 Results

Sample characteristics

Consent or assent was obtained for 51 subjects; 9 of these were randomised to continue prior antihypertensive drugs and hence were excluded from the analysis (figure 6.1). The patients in the control and GTN groups were well matched for age, gender, time to randomisation, previous strokes, smoking, hypertension and antihypertensive usage (table 6.1). However, baseline central (not peripheral) systolic BP, was significantly lower in the GTN group. The sample included a representative mixture of both ischaemic (n=35, 83%) and haemorrhagic strokes (n=7, 17%), however cortical strokes were under-represented (n=14, 33%). The mean delay from stroke onset to randomisation was 32 hours.

BP and pulse

Systolic BP tended to fall over the course of the study in both treatment arms (figure 6.2a). Compared with baseline, significant reductions in systolic BP were observed in the GTN group at 1 hour (-12mmHg, 95%CI -19, -5) and at day 7 (-11mmHg, 95%CI -20, -2). By contrast, there were no significant changes in systolic BP for the control group. After adjustment for baseline values, peripheral and central systolic BP were lower at 1 hour in the GTN group than in the control group by 15 mmHg (9%) and 16 mmHg (10%) respectively ($p=0.01$, $p<0.01$, table 6.2). Similarly, peripheral and central mean arterial BP were lower than in the control group by 10mmHg ($p=0.03$) and 9mmHg ($p<0.01$) respectively. Diastolic BP tended to remain lower than at baseline in the GTN group and rise in the control group over the course of the study (figure 6.2b). In the GTN group significant reductions in diastolic BP compared with baseline were observed at 1 hour (mean change -10mmHg, 95%CI -16, -5) and at day 7 (mean change -5mmHg, 95%CI -10, -0). By contrast, there were no significant changes in diastolic BP for the control group.

Also, after adjustment for baseline values GTN did not significantly lower peripheral (7mmHg, p=0.10) or central diastolic BP (5mmHg, P=0.07) compared with the control group. Finally, GTN had no effect on heart rate.

Other systemic haemodynamic measures

AI at 1 hour was significantly lower by -20% ($p<0.01$) in those who received GTN as compared with the control subjects (table 6.2). GTN had no effect on CO, TPR or BI.

Cerebral haemodynamic measures

MCA velocity and PI did not change with GTN in either the affected or unaffected hemispheres at 1 hour.

Laboratory measures

GTN had no significant effect on 1 hour plasma NOx levels. Similarly, GTN did not affect expression of soluble p-selectin.

6.5 Discussion

This chapter has shown that transdermal GTN lowers systolic and mean arterial BP in acute ischaemic or haemorrhagic stroke. This finding is compatible with 2 previous studies which demonstrated a similar (~5-8%) reduction in 24 ambulatory systolic[166] or mean arterial BP.[167] The effect on BP was modest and beneath the maximum limit suggested by published guidelines, which recommend that BP should not be lowered by more than 20% in acute stroke.[153,437] Importantly, the study extends the information available and demonstrates the feasibility of administering GTN < 48 hours from onset.

Effects on plasma NO metabolites and central haemodynamics were examined to gain insight into the mechanisms by which GTN lowers BP. Interestingly, GTN did not alter plasma nitrate / nitrite (NOx) levels, the metabolic product of NO. This corresponds with previous studies involving stroke patients[167] and normal humans.[438] Possible explanations include; confounding by the effects of recent food intake,[439] contamination with inorganic nitrites and nitrosamines[300] or inadequate sample size. In contrast, GTN did lower peripheral and central systolic BP, mean arterial BP and PP. Significant effects on central haemodynamics are of importance because they may better indicate the effect of GTN on intracranial haemodynamics than peripheral measurements. BP might have been influenced, in part, by the increase in aortic compliance that was observed. Reduced AI following GTN is consistent with findings in normal subjects[440,441] and in sub-acute stroke.[167] Alternatively, GTN did not appear to influence BP through effects on TPR, which remained unchanged at 1 hour. Previous studies have also failed to detect changes in TPR, suggesting that any effect of GTN on peripheral arterioles is relatively small.[442,443] Similarly, GTN had no detrimental effect on CO at 1 hour, which is reassuring because the brain consumes 15-20% of total CO to maintain resting CBF.[8] Effects of GTN on CO have been variable in published data with some studies reporting neutral results[167,444], and others reporting negative results.[445,446] Presumably, inconsistencies have arisen through differences in the route of administration, dosage, and cardiac status of the study subjects. Finally, GTN did not alter heart rate at 1 hour, contradicting data from several studies where reflex activation of the sympathetic nervous system was thought to be responsible for increases in heart rate.[447,448] This inconsistency is also likely to reflect differences in drug formulation and dosage since 5mg transdermal GTN patches did not effect heart rate in either of the previous stroke trials.[166,167]

NO is known to have anti-platelet properties, in part through suppression of p-selectin expression on the surface of platelets and in the plasma.[449] However, transdermal GTN did not alter plasma soluble p-selectin, suggesting that there was no alteration in platelet activation. Interestingly, this finding corresponds with data from two earlier studies using GTN patches,[166,450] but it conflicts with studies using other NO donors, such as SNP.[162,451,452] GTN does not have antiplatelet properties because platelets are unable to release NO from organic nitrates.[79] The lack of observable effects on platelet function mean that GTN patches can continue to be studied in haemorrhagic as well as IS.

Finally, GTN had no effect on MCA velocity and PI (an index of vascular resistance), implying that CBF was unaffected. This is reassuring since there are concerns that lowering BP might worsen outcome in acute stroke through reducing CBF.[313] However, it is impossible to entirely rule out a detrimental effect on cerebral perfusion because the relationship between blood velocity and CBF remains unclear.[261,453] Further studies involving quantitative CBF measurements with SPECT or XeCT are required. The findings are consistent with previous data involving GTN patches,[362] however they disagree with studies using sublingual or intravenous GTN where velocities decreased significantly.[161,454,455] The inconsistency probably arises because transdermal GTN produces a lower peak plasma concentration than other GTN formulations.[456]

A number of methodological weaknesses may have influenced the findings. First, there was no placebo control due to lack of commercial availability of placebo patches. Consequently, attempts were made to limit bias by the using a gauze dressing to enable blinded outcome assessment. Second, central systolic BP was lower in the GTN group at baseline and therefore the treatment effect could have been overestimated. However, the analysis of covariance (ANCOVA) will have tended to correct for this because it has the

advantage of being unaffected by baseline imbalances. Third, the number of milder, subcortical strokes included in the study was larger than expected, hence the findings may not be generalisable. Future studies should aim to recruit more severe subjects with cortical strokes. Finally, the study was small and the CI were wide. Therefore the possibility of a type 2 error for the neutral measurements cannot be excluded, e.g. plasma NOx, CO, TPR and MCA velocity. Reassuringly, the estimate of difference between control and GTN for all these measurements was close to zero.

In summary, this chapter has demonstrated that it is feasible to lower BP <48 hours from stroke onset with transdermal GTN. BP was probably influenced, in part, by an improvement in aortic compliance but not by changes in TPR or CO. Also, GTN did not appear to compromise indirect measures of cerebral perfusion, although further studies are required. Early administration of GTN is desirable in order to make the most of its potentially neuroprotective and vasculoprotective properties. The optimum time window remains unclear, however this question will be addressed following completion of the ongoing 'Efficacy of Nitric Oxide in Stroke' (ENOS) trial.[363]

Table 6.1 Demographic characteristics and haemodynamic measurements at baseline. Mean (SD), median * (IQR), or frequency (%).

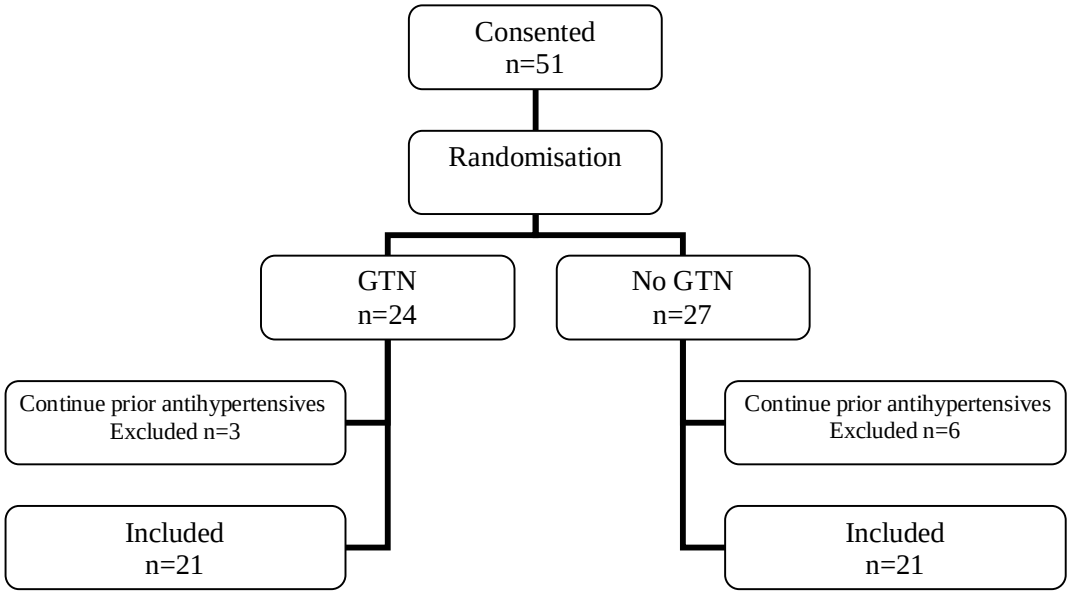
	Control	GTN
Subjects	21	21
Age (years) †	74 (9)	68 (11)
Gender, male (%) †	13 (62)	11 (52)
Previous hypertension (%)	10 (48)	7 (33)
Previous antihypertensive therapy (%)	6 (29)	5 (24)
Previous stroke (%)	4 (19)	2 (10)
Smoker (current, %)	5 (24)	7 (33)
Stroke to randomisation (hours) *†	29 (16)	31 (18)
Scandinavian Stroke Scale (/58) *†	39 (29)	43 (20)
Stroke type, ischaemic (%)	17 (81)	19 (91)
Cortical (TACS, PACS) (%)	8 (38)	6 (29)
Systolic BP (mmHg) †	176 (28)	163 (20)
Diastolic BP (mmHg)	90 (16)	95 (13)
Heart rate (bpm)	75 (13)	69 (12)
Central systolic BP (mmHg)	165 (29)	147 (20)
Central diastolic BP (mmHg)	93 (16)	91 (14)

† Minimisation variable

Table 6.2 Effect of transdermal GTN on measurements at 1 hour. Mean (SD), 95% CI, comparison by ANCOVA with adjustment for baseline.

Measurement		Control, subjects	GTN, subjects	Control	GTN	Difference, 95% CI	2p
Peripheral	Systolic BP (mmHg)	21	21	174.9 (27.9)	151.3 (18.1)	-15.1 (-26.6, -3.5)	0.01*
	Diastolic BP (mmHg)	21	21	89.0 (14.8)	84.7 (13.7)	-6.5 (-14.4, 1.3)	0.10
	Mean arterial BP (mmHg)	21	21	117.6 (18.6)	106.9 (14.3)	-10.0 (-18.9, -1.1)	0.03*
	PP (mmHg)	21	21	85.9 (16.9)	66.6 (11.3)	-9.9 (-18.4, -1.4)	0.02*
	Heart rate (beats/min)	21	21	75.3 (12.1)	72.6 (16.4)	+3.2 (-2.4, +8.7)	0.25
Systemic	Central systolic BP (mmHg)	21	20	160.0 (24.5)	133.7 (17.5)	-15.6 (-24.2, -7.0)	<0.01*
	Central diastolic BP (mmHg)	21	20	92.3 (13.2)	86.5 (13.3)	-5.4 (-11.2, +0.4)	0.07
	Central mean arterial BP (mmHg)	21	20	114.8 (16.2)	102.2 (13.8)	-8.7 (-14.8, -2.6)	<0.01*
	Central PP (mmHg)	21	20	67.7 (15.7)	47.2 (11.8)	-11.5 (-18.2, +4.8)	<0.01*
	AI (%)	21	20	144.2 (22.4)	122.0 (22.0)	-20.4 (-31.3, -9.6)	<0.01*
	BI (%)	20	20	150.5 (45.2)	179.3 (53.6)	+4.1 (-15.3, +23.6)	0.67
	CO (L/min)	13	14	7.2 (3.4)	6.3 (1.9)	+1.1 (-0.2, +2.4)	0.81
	TPR (LogN, m-units)	13	14	0.9 (0.5)	0.9 (0.3)	-0.1 (-0.5, +0.2)	0.44
Cerebral	Ipsilateral MCA velocity (cm/s)	15	17	27.2 (14.3)	26.0 (10.8)	-2.2 (-8.7, 4.3)	0.50
	Ipsilateral MCA PI (LogN)	15	17	1.9 (0.8)	1.4 (0.8)	-0.2 (-6.8, +0.2)	0.33
	Contralateral MCA velocity (cm/s)	18	17	28.9 (16.5)	31.9 (12.5)	-0.7 (-7.7, +6.3)	0.84
	Contralateral MCA PI (LogN)	18	17	1.8 (0.9)	1.4 (0.5)	-0.0 (-0.4, 0.3)	0.86
Laboratory	Plasma NO ($mmol.l^{-1}$)	16	18	25.8 (11.2)	38.2 (19.3)	+2.1 (-3.0, 7.2)	0.42
	Soluble p-selectin ($ng.ml^{-1}$)	19	17	93.1 (48.1)	112.1 (51.3)	-3.7 (-34.4, +27.0)	0.81

Figure 6.1 Trial flow profile



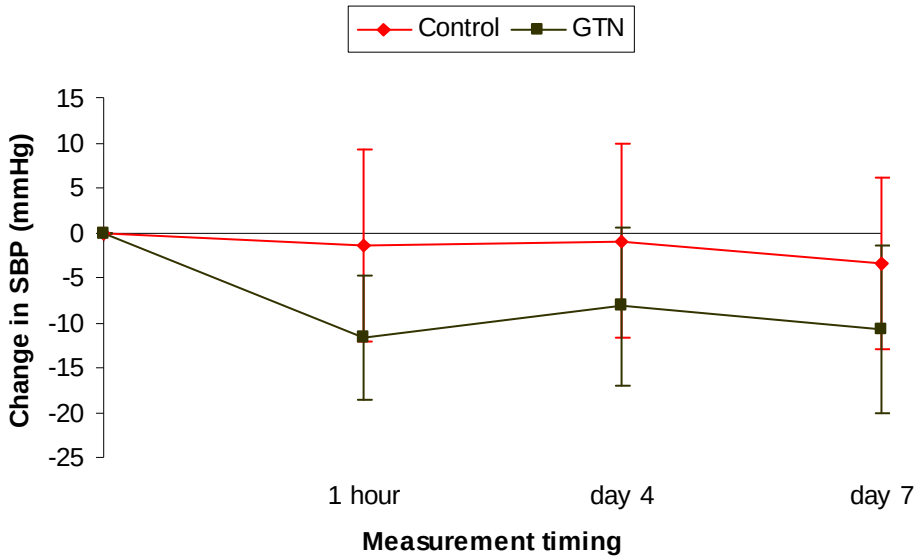


Figure 6.2a Systolic BP change from baseline by treatment group

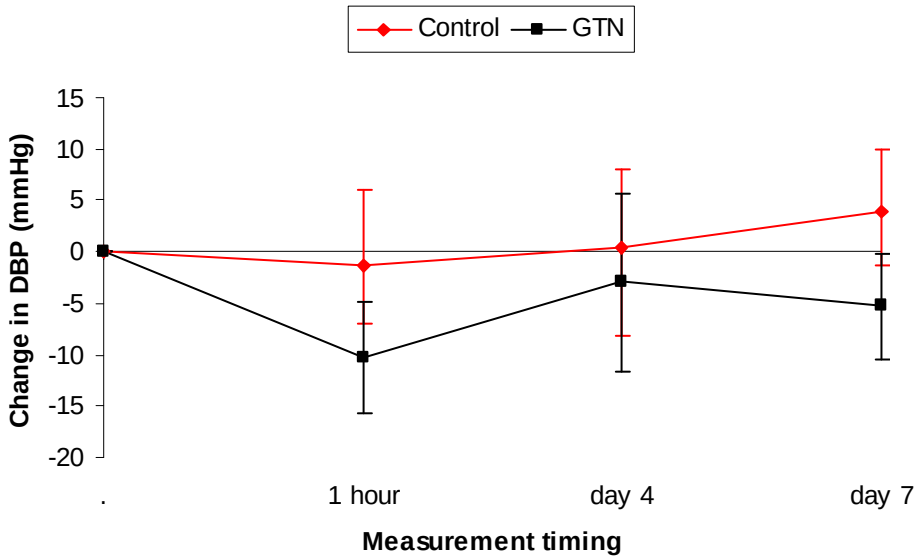


Figure 6.2b Diastolic BP change from baseline by treatment group

Chapter 7.

Transdermal GTN maintains CBF and perfusion pressure whilst lowering blood pressure in patients with acute stroke

7.1 Abstract

Background: High BP is common in acute stroke and independently associated with a poor outcome. Lowering BP might improve outcome if it did not adversely affect CBF or cerebral perfusion pressure. Hence, the effect of GTN on quantitative CBF, BP and cerebral perfusion pressure in patients with recent stroke was investigated. Methods: 18 patients with recent (<5 days) ischaemic (n=16) or haemorrhagic (n=2) stroke were randomised (2:1) to transdermal GTN (5 mg) or control. CBF (global, hemispheric, arterial territory, lesion, using XeCT) and BP (peripheral and central) were measured before and 1 hour after treatment with GTN. The effect of GTN on CBF and BP were adjusted for baseline measurements (ANCOVA). Result: GTN lowered peripheral systolic BP by (mean) 23 mmHg (95% CI 2, 45, p=0.03), and central systolic BP by 22 mmHg (95% CI 0, 44, p=0.048). In contrast, GTN did not alter CBF (mls/min/100 g): global -1.2 (95% CI –6.5, +4.2, p=0.66), ipsilateral hemisphere –1.4 (-7.6, +4.9, p=0.65); or area of stroke oligaemia, penumbra or core (as defined by critical CBF limits). Contralateral CBF did not change: hemisphere 0 (95% CI –7, +6, p=0.96). GTN did not alter cerebral perfusion pressure or zero filling pressure. Conclusions: Significant reductions in BP following transdermal GTN are not associated with changes in CBF or cerebral perfusion pressure, or cerebral steal in patients with recent stroke. Trials need to assess the effect of lowering BP on functional outcome.

7.2 Background

High BP is present in more than three-quarters of patients at presentation with acute IS and is independently associated with a poor outcome and early recurrence.[138,457] Similar findings have been reported for patients with PICH.[132] These observational data raise the possibility that lowering BP might improve functional outcome providing both CBF and cerebral perfusion pressure (CPP) do not fall.

Drugs which lower BP in patients with recent IS vary in their effect on CBF. Calcium channel blockers (CCB) reduced cerebral perfusion in parallel with their effect on BP.[458,459] In contrast, angiotensin modifying drugs such as captopril and perindopril (angiotensin converting enzyme inhibitors, ACE-I), and losartan (angiotensin receptor antagonist, ARA) did not appear to alter CBF or MCA blood velocity.[175,177,460] Hence, the effect of altering BP on CBF may be drug or class specific.

CPP is the difference between upstream (mean arterial pressure) and downstream pressure, the latter being determined by intracranial pressure (ICP) and central venous pressure (CVP). Cerebral vasodilators may increase or decrease CPP depending on their relative effects on these three measures, e.g. venodilators may both increase cerebral blood volume and reduce CVP thereby maintaining ICP. Previous work has shown that vasodilators, including nitrous oxide,[461] can increase CPP by reducing zero flow pressure, a measure of cerebral downstream (venous) pressure.

In a previous trial transdermal GTN lowered both peripheral and central BP in a dose-dependent manner, improved aortic compliance, and did not alter platelet function or activation.[166,362] Indirect evidence also suggested that nitrates lowered BP without attenuating CBF, confirming preclinical data.[163,192,343] SNP did not alter CBF, assessed

qualitatively using SPECT.[162] Similarly, GTN did not change MCA blood velocity and PI, these being indirect measures of CBF.[362] The aim of the present study was to assess simultaneously the effects of GTN on BP, CBF and CPP.

7.3 Methods

Design

A prospective, single-centre, patient and measurement-blinded randomised controlled trial was performed. Approval was obtained from the local Research Ethics Committee (EC01/69) and Medicines Control Agency (DDX MF8000/13187). Patients gave written informed consent to participate; assent was obtained from the next-of-kin if the patient was unable to give consent. Enrolment into the study was made prior to neuroimaging or estimation of CBF. The study conformed to the Declaration of Helsinki and International Conference on Harmonisation guidelines. Enrolment took place between September 2002 and September 2004.

Subjects

18 previously independent (modified Rankin Scale, mRS 0-2) adult patients with a clinical stroke syndrome and limb weakness (Scandinavian Stroke Scale, SSS Arm and/or leg <6), were recruited within 5 days of ictus (figure 7.1). All subjects had an elevated systolic BP (140–220 mmHg) at enrolment. Subjects were excluded if they had a requirement for, or a contraindication to, nitrate therapy; had a definite need for prior antihypertensive therapy or vasoactive drugs; or could not cooperate with scanning.

Treatment

Subjects were randomised using computerised minimisation (on age, gender, baseline systolic BP, baseline SSS, hours from onset and the presence of a visible stroke lesion on

CT) to receive either a 5 mg GTN patch ('Transiderm-Nitro 5', Novartis Pharmaceuticals) or control in a ratio of 2:1. Treatment was given once daily for 7 days. Patients and the assessor who performed haemodynamic and XeCT measurements were blinded to treatment by placement of a large gauze dressing over the patch or empty skin to conceal treatment status. GTN patches were changed at 08:00 hours each day and kept on for a full 24 hours. Any prior antihypertensive medication was discontinued at the time of admission, as is routine at Nottingham City Hospital.

CBF

Quantitative regional CBF was measured using the stable XeCT method (Diversified Diagnostic Products XeCT system 2, Houston, USA).[271] A baseline CT head scan was performed to confirm the diagnosis of stroke (ischaemia, haemorrhage) and to obtain a 'scout' image; subsequent scans comprised four adjacent 10 mm thick slices chosen to encompass the maximum axial dimensions of the stroke lesion. If no lesion was visible, slices included the basal ganglia and internal capsule. The patient was connected to the XeCT system via a facemask and breathed room air for 30 seconds while two baseline sets of images were recorded. Subsequently, the patient breathed a mix of Xe (28%) and oxygen (25%) with monitoring of end tidal Xe concentration. Further sets of images were then collected over five minutes. A second XeCT scan series was performed one hour after administration of GTN or control; careful positioning of the patient ensured that near-identical brain slices as determined by neuroradiological landmarks were imaged in pre and post-GTN scans.

CBF was calculated on a PC using XeCT software in an identical manner for pre and post-GTN scans. Images with excessive movement artefact or where an old stroke lesion was present were discarded. Analyses were blinded to treatment and concentrated on the slice

with the largest visible area of stroke lesion. If no lesion was visible, an appropriate level was selected according to the clinical presentation. Global and hemispheric regions of interest (ROIs) were sited using a rectangular shaped template, the former touching the inside of the skull anteroposteriorly and laterally, and the latter dividing the skull at the midline. Anterior, middle and posterior cerebral artery territory ROIs were placed over the cortex using a template generated by the XeCT software (figure 7.2a).

An additional pixel based analysis was used to assess the effect of GTN on CBF in the stroke lesion, if visible. A rectangular ROI was placed to cover the whole of the stroke lesion and surrounding brain tissue (figure 7.2b). A CBF filter was then used to determine the number of pixels within the ROI matching pre-specified CBF values for 'core' <10 ml/min/100g, 'penumbra' 10-19 ml/min/100g, and 'oligaemia' 20-36 ml/min/100g.[287-290] Matching ROIs were sited on pre and post treatment scans to ensure consistency and the areas (in pixels) of reduced CBF compared.

TCD

MCA blood velocity was determined bilaterally by TCD (Nicolet EME Companion, Kleimoftheim, Germany) with the trans-temporal window accessed at depths from 30 to 60 mm.[462] Duplicate mean, systolic and diastolic velocities and PI were measured separately for affected and unaffected hemispheres.[166,362]

BP

BP was measured immediately before the baseline XeCT scan and immediately after the post-treatment scan. Duplicate measurements of peripheral systolic and diastolic BP was measured in the non-hemiparetic arm with a validated digital readout oscillometric device (Omron HEM-705CP, Omron Corp, Tokyo, Japan).[235] Central BP was assessed by

applanation tonometry of the left radial artery and using the Pulse Wave Analysis (PWA) system (Sphygmocor, Sydney, Australia).[362] Duplicate recordings were taken, comprising a screen of data satisfying the quality control criteria of the PWA system (pulse height and diastolic variability ≤ 10%). The recorded radial artery pressure wave was transformed to the corresponding central wave using a validated transfer function and central BP derived automatically.

CPP

CPP and zero ZFP were estimated from measures of MCA blood flow velocity (FV), assessed using TCD, and peripheral BP using the method of Mahajan and colleagues:[461,463]

$$CPP = (\text{mean FV} / (\text{mean FV} - \text{diastolic FV})) \times (\text{mean BP} - \text{diastolic BP})$$

$$ZFP = \text{mean BP} - CPP$$

Statistical methods

The study assumed that a between group difference in CBF of 10 ml/min/100g (standard deviation 6) would be of clinical relevance. Assuming significance 0.05, power 0.80, 2:1 randomisation, the sample size was estimated at 18. Data were entered and analysed by intention to treat using SPSS (Apple Mac version 11.0.2, SPSS Inc, Chicago, IL). Mean (standard deviation [SD]), median (interquartile range [IQR]) or frequencies (%) are given. All comparisons of haemodynamic and BP data between the treatment groups were analysed with adjustment for baseline values using analysis of covariance (ANCOVA). Linear regression was used to assess the relationship between on treatment CBF and BP with adjustment for baseline values. Significance was set at $p<0.05$.

7.4 Results

Subject characteristics were balanced between the two groups except that more patients randomised to GTN were female or had a cortical-based stroke on CT scan (table 7.1). Peripheral BP was elevated at baseline and higher than central BP. Although the mean areas of oligaemia, penumbra and core were similar in size between the two groups, baseline measures of global, hemispheric and regional CBF, both ipsilateral and contralateral to the lesion, and zero filling pressure were non-significantly higher in patients randomised to GTN (table 7.2). Patients randomised to control tended to have higher cerebral perfusion pressure.

GTN lowered peripheral and central systolic BP by 23 mmHg (14%) and 22 mmHg (13%) respectively (p=0.034, p=0.048). Non-significant reductions in peripheral and central diastolic BP were present, 4 mmHg (3%) for each (p=0.47, 0.55). GTN did not alter heart rate. In contrast to BP, GTN did not alter any measure of global, hemispheric or regional CBF whether on the side ipsilateral or contralateral to the lesion (table 7.3, figures 7.2a, 7.3, 7.4). Nevertheless, the CI were wide and GTN might have reduced ipsilateral hemispheric CBF by 7.6 ml/min/100g or increased it by 4.9 ml/min/100g. When defined by blood flow levels, GTN had no effect on the size of presumptive core, penumbra or oligaemic areas (figure 7.2b). Similarly, GTN did not significantly alter estimates of cerebral perfusion pressure and zero flow pressure (table 7.3).

There was no association between on-treatment measures of ipsilateral CBF and systolic BP (p=0.83, with adjustment for baseline CBF and systolic BP) in patients randomised to GTN.

All patients completed 7 days of treatment. One serious adverse event occurred during the treatment phase; a patient receiving GTN had a non-hypotension related fall leading to trauma of the affected arm. Headache occurred in one GTN patient. There were no deaths and the modified Rankin Scale (telephone assessment blinded to treatment) did not differ between the groups at 90 days, median (interquartile range) GTN 2 (1), control 2 (1) (Mann-Whitney U test,[464] 2p=0.89).

7.5 Discussion

This chapter has shown that transdermal GTN lowers BP modestly (by 14/3%) without having any detrimental effect on CBF or CPP in patients with recent stroke. The neutral effect on CBF was present whether global CBF, ipsilateral and contralateral hemispheric CBF, or ipsilateral and contralateral arterial regions of interest were studied. Similarly, GTN did not alter the size of lesion core, penumbral or oligaemic areas, as defined by pre-specified CBF levels, and did not appear to cause a 'cerebral steal' effect.

Examination of the results for individual patients (figure 7.3a) suggest that GTN may, in some cases, increase CBF both within and around the hypodense tissue, and both superficially and deep within the brain. Similarly, an increase in peri-lesional CBF was seen in a patient with a PICH (figure 7.3b).

Acute stroke, whether of ischaemic or haemorrhagic type, is associated with dysfunctional cerebral autoregulation so that, in the extreme, cerebral perfusion becomes dependent on systemic BP. The mechanism by which GTN can lower BP whilst maintaining CBF was not addressed in this study. However, GTN forms NO which is a potent modulator of cerebrovascular reactivity, especially in collateral vessels such as pial arteries.[347,350] Vascular NO levels are low in stroke[94] so collateral vessels may not be maximally dilated.

Hence, CBF might be held constant with GTN if moderate reductions in systemic BP were counter-balanced by increases in collateral blood supply, which would be potentially beneficial in acute stroke.

It is commonly held that cerebral vasodilators will reduce cerebral perfusion pressure through increasing cerebral blood volume and ICP. However, this hypothesis neglects the effect that such drugs will have on zero flow pressure, a measure of downstream pressure. Both CPP and ZFP were estimate in this study and GTN did not alter either significantly. The observation that GTN did not alter CPP is new and contrary to the expectation that cerebral vasodilators will increase ICP and therefore reduce CPP; presumably venodilation increases blood flow out of the cranium thereby maintaining CPP (and ICP).

Several caveats should be placed on the study. First, it was small and the CI for the haemodynamic effects of GTN were wide such that GTN could have moderately reduced or increased CBF and CPP. Nevertheless, the point estimates for differences in a variety of CBF measurements (and lesion areas) for GTN and control treated patients all lay on or close to zero. Second, the effect of GTN on CBF and CPP in the acute and subacute phases of stroke was assessed (median time to randomisation 78 hours) and hence the effect that GTN has on CBF during the hyperacute period cannot be commented on. Third, patients were included irrespective of stroke type since the CT diagnosis of stroke type was only made at the time of study to reduce radiation doses; since only two patients with PICH were included (one in each group), the effect of GTN on CBF in PICH cannot be specifically addressed, although blood flow was seen to increase adjacent to the haemorrhage in one patient. Fourth, few patients with cortical syndromes were included reflecting the problems of intensive studying of such patients. Finally, there was no direct

measure of ICP but rather an indirect estimate of cerebral perfusion pressure[461,463] to avoid the need for measuring inserting invasive pressure transducers.

In summary it is possible to lower BP with transdermal GTN without reducing CBF or CPP, or inducing cerebral steal, in patients with acute stroke. These data support testing the effect of lowering BP in patients with acute stroke and high BP with the aim of improving functional outcome and reducing stroke recurrence.

Table 7.1 Demographic characteristics of patients. Mean (SD), median * (IQR), or frequency (%).

	Control	GTN
Subjects	6	12
Age (years) †	70.3 (10.8)	69.0 (5.6)
Gender, male (%) †	3 (50)	2 (17)
Previous hypertension (%)	4 (67)	3 (25)
Previous antihypertensive therapy (%)	3 (50)	3 (25)
Previous stroke (%)	1 (17)	2 (17)
Smoker (current, %)	3 (50)	8 (67)
Stroke to randomisation (hours) *†	77 (54)	79 (38)
Scandinavian Stroke Scale (/58) *†	47 (18)	42 (11)
Stroke type, ischaemic (%)	5 (83)	11 (92)
Cortical syndrome (%)	1 (17)	5 (42)

† Minimisation variable

Table 7.2 Haemodynamic measurements at baseline. Mean (SD), median * (IQR), or frequency (%).

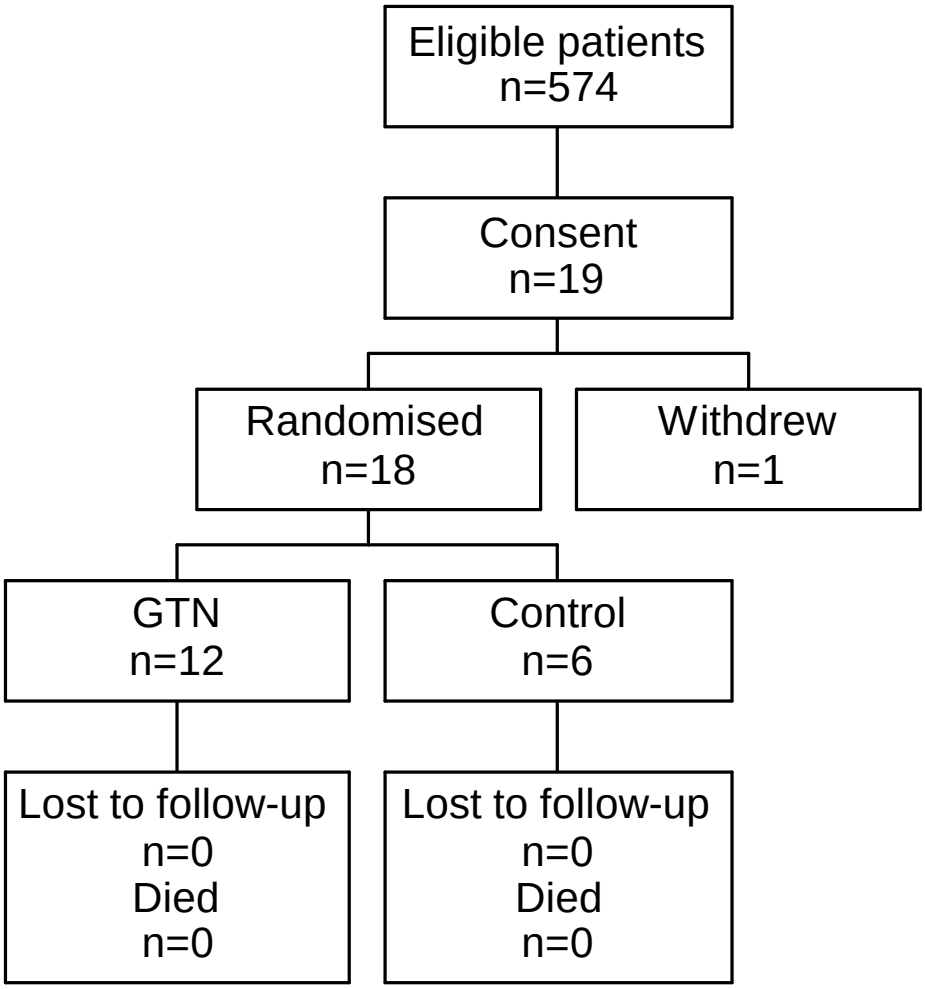
	Control N=6	GTN N=12
BP (mmHg)		
Peripheral		
Systolic †	166 (18)	162 (16)
Diastolic	87 (10)	92 (17)
Central		
Systolic	151 (17)	152 (13)
Diastolic	91 (15)	95 (16)
Heart rate (bpm)	66 (15)	69 (14)
CBF (ml/min/100g)		
Global	31.1 (6.0)	38.4 (10.6)
Ipsilateral		
Hemisphere	30.4 (4.9)	37.8 (12.8)
ACA territory	32.3 (14.7)	38.4 (12.4)
MCA territory	32.1 (5.7)	42.1 (15.0)
PCA territory	30.0 (9.2)	40.6 (17.5)
Contralateral		
Hemisphere	31.9 (7.8)	39.4 (8.9)
ACA territory	34.0 (9.9)	40.4 (11.5)
MCA territory	38.2 (10.0)	45.8 (10.3)
PCA territory	29.8 (12.4)	38.9 (12.1)
Lesion area (pixels)		
Oligaemia	364 (221)	351 (140)
Penumbra	258 (196)	262 (197)
Core	207 (273)	235 (288)
Cerebral perfusion pressure (mmHg)	59.7 (12.0)	43.4 (18.0)
Zero filling pressure (mmHg)	59.5 (14.8)	72.5 (26.6)

† Minimisation variable

Table 7.3 Effect of transdermal GTN on CBF and estimates of cerebral perfusion pressure. Mean (SD), 95% confidence interval, comparison by ANCOVA with adjustment for baseline values.

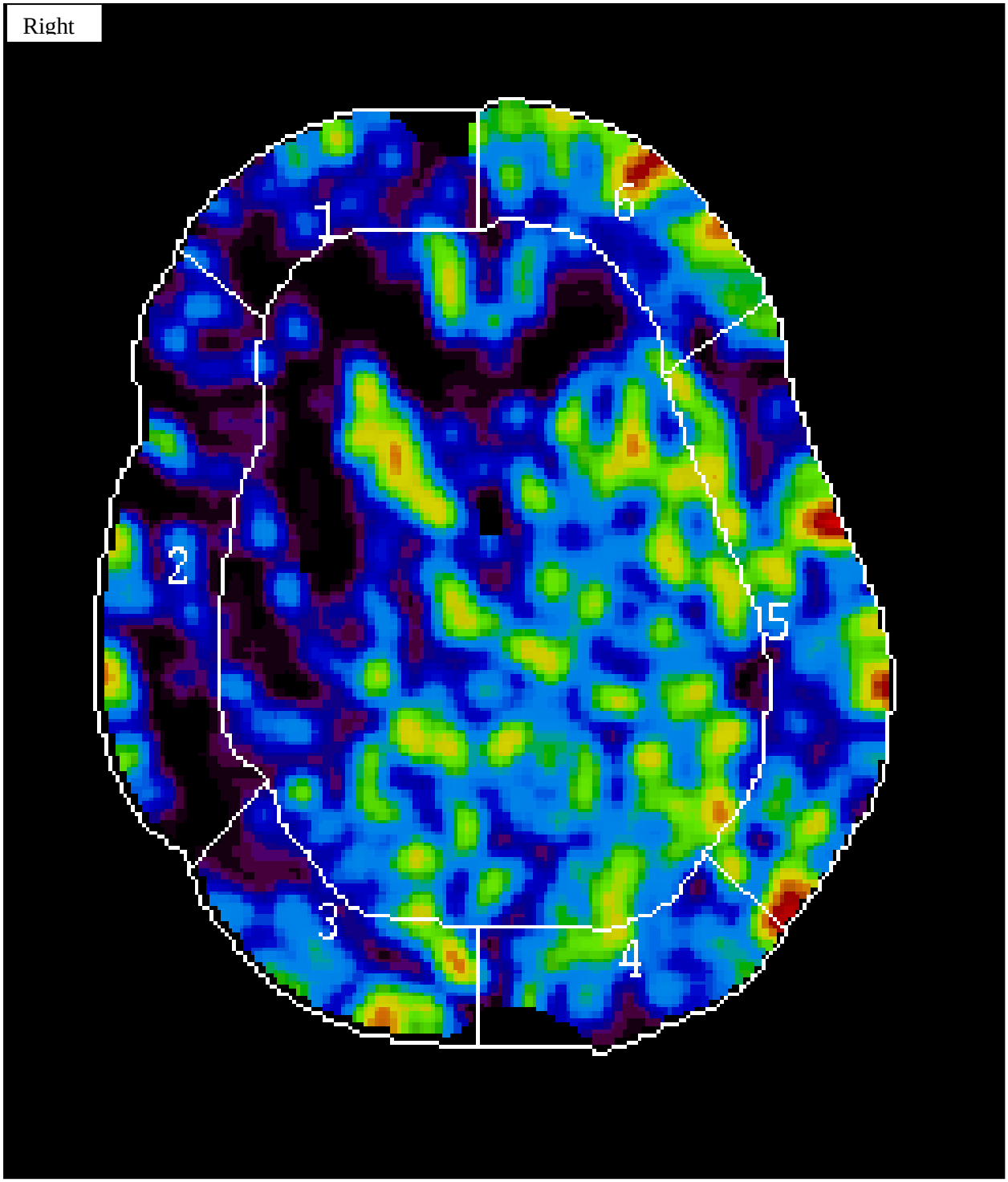
	Control, subjects	GTN, subjects	Control	GTN	Difference, 95% CI	2p
CBF (ml/min/100g)	6	12	32.8 (7.3)	37.8 (9.7)	-1.2 (-6.5, +4.2)	0.66
Global	6	12	32.8 (7.3)	37.8 (9.7)	-1.2 (-6.5, +4.2)	0.66
Ipsilateral						
Hemisphere	6	12	33.4 (8.3)	37.2 (9.8)	-1.4 (-7.6, +4.9)	0.65
ACA territory	6	12	30.3 (8.0)	39.5 (14.6)	+6.3 (-6.5, +19.0)	0.31
MCA territory	6	12	37.0 (10.8)	43.5 (10.8)	+0.6 (-8.4, +9.6)	0.89
PCA territory	6	12	35.3 (10.2)	39.1 (11.8)	-0.7 (-11.4, +10.1)	0.90
Contralateral						
Hemisphere	6	12	32.2 (6.3)	38.4 (10.0)	0 (-7, +6)	0.96
ACA territory	6	12	33.3 (8.1)	41.1 (16.8)	+5.7 (-10.6, +21.9)	0.47
MCA territory	6	12	35.3 (9.6)	44.2 (10.8)	+4.6 (-5.5, +14.7)	0.35
PCA territory	6	12	32.7 (7.3)	42.0 (15.1)	+2.6 (-8.6, +13.9)	0.63
Lesion area (pixels)						
Oligaemia	4	9	329 (146)	355 (167)	+35 (-120, +191)	0.63
Penumbra	4	9	222 (166)	247 (141)	+23 (-76, +123)	0.62
Core	4	9	243 (318)	266 (290)	-2 (-210, +206)	0.98
Cerebral perfusion pressure (mmHg)	3	10	59.1 (32.4)	50.9 (15.8)	-4.4 (-37.4, +28.6)	0.77
Zero flow pressure (mmHg)	3	10	73.4 (47.2)	60.8 (10.5)	-13.6 (-48.8, +21.5)	0.41

Figure 7.1 Trial flow profile



Withdrawal due to intolerance of XeCT procedure

Figure 7.2 A) Example of template for anterior cerebral artery (areas 1, 6), MCA (2, 5) and posterior cerebral artery (3, 4) cortical regions of interest in a patient with a large right hemisphere infarct.



B) Measurement of CBF in a region of interest encompassing an area of 'oligaemia' (20-36 ml/min/100g) in a patient with a left hemisphere infarct. Similar CBF filters were used to identify lesion 'core' (CBF <10 ml/min/100g) and 'penumbra' (CBF 10-20 ml/min/100g).

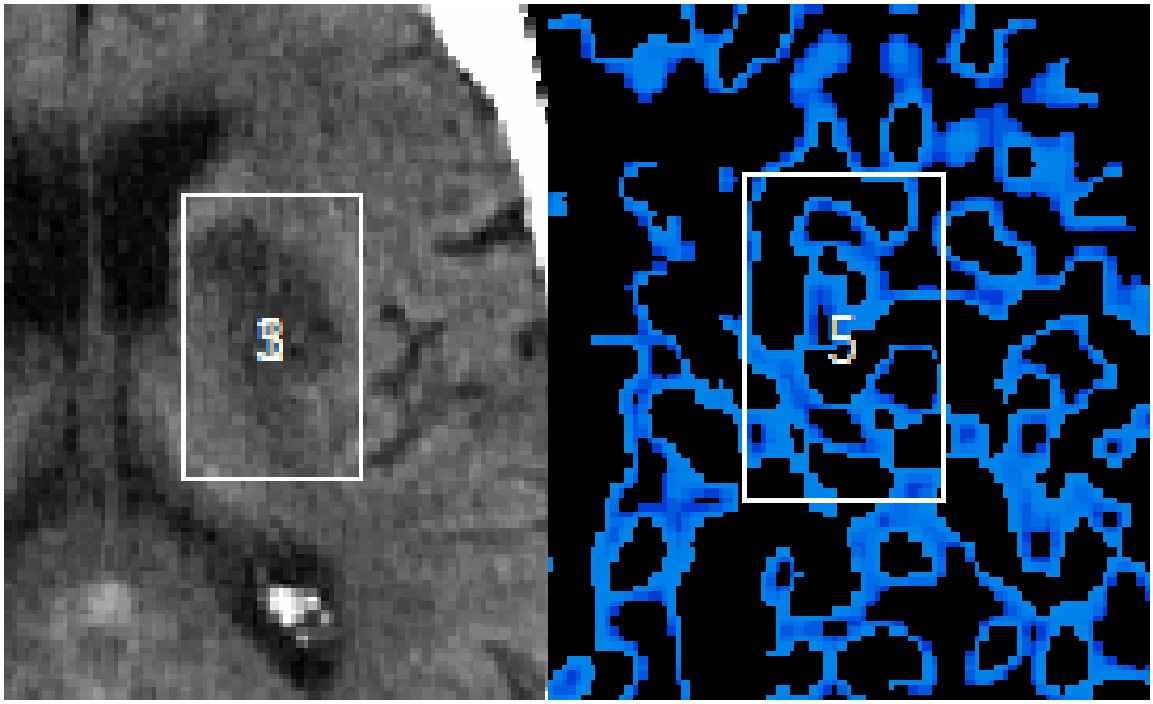
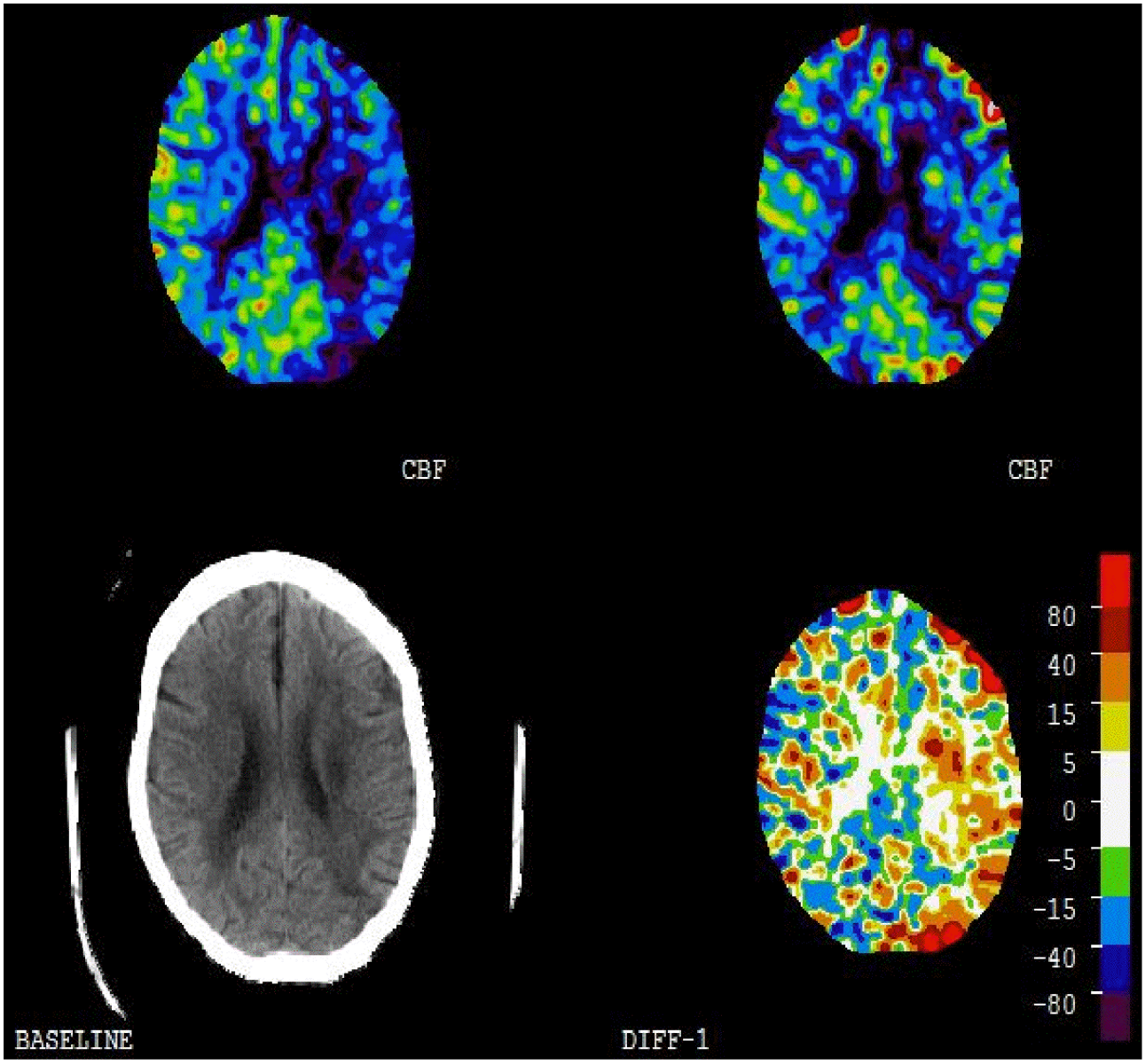


Figure 7.3 A) Patient with a left hemisphere borderzone infarct (bottom left); CBF before (top left) and 1 hour after GTN (top right); CBF has increased with GTN in and around the hypodense lesion and no 'cerebral steal' is present, as shown by the difference between pre and post scans (bottom right)



B) Patient with a left intracranial haemorrhage (bottom left); CBF before (top left) and 1 hour after GTN (top right); CBF has increased with GTN around the bleed and no 'cerebral steal' is present, as shown by the difference between pre and post scans (bottom right)

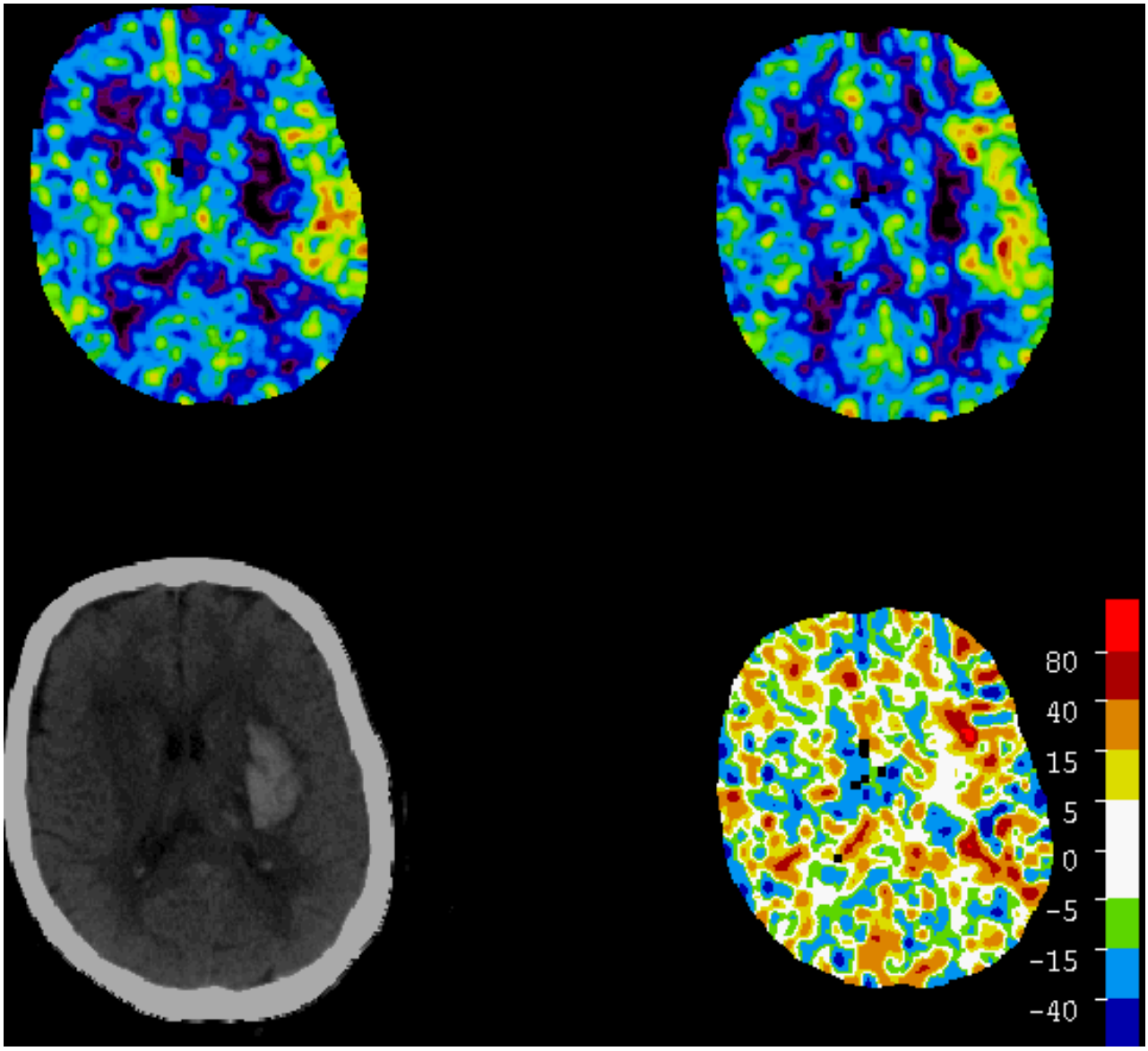
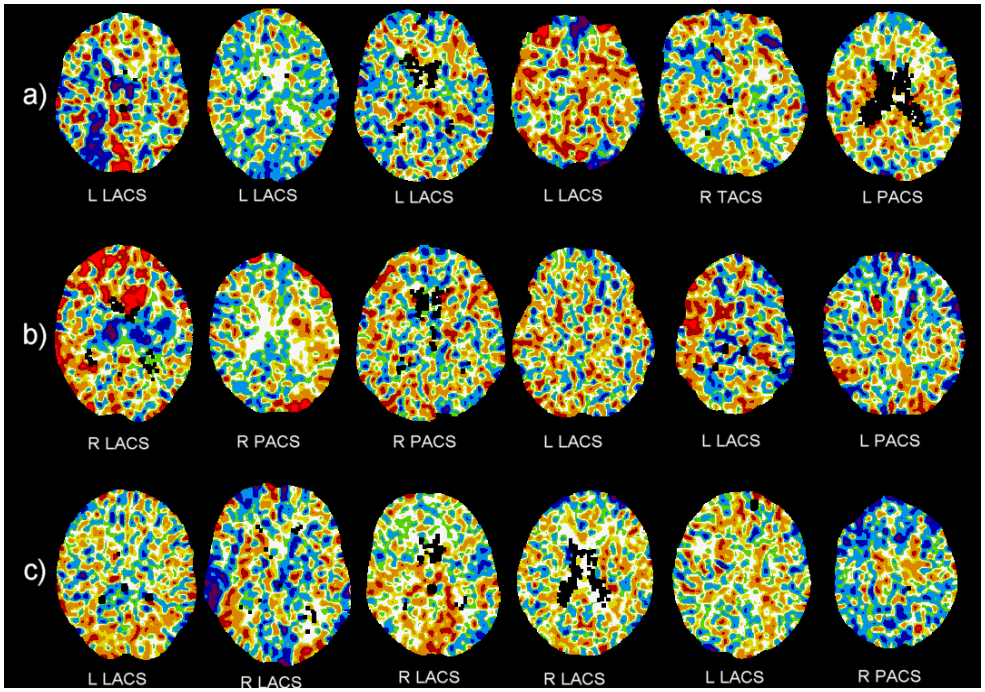


Figure 7.4 CBF difference scans (post GTN – baseline) for all patients. Patients receiving active treatment: rows a) and b); control: row c). Axial slices are shown at the level of the largest lesion diameter, or an appropriate level for the clinical presentation if no lesion was visible on the CT scan. The side of lesion (R: right; L: left) and Oxfordshire Community Stroke Project classification (LACS: lacunar syndrome; PACS: partial anterior circulation syndrome; TACS: total anterior circulation syndrome) is given for each scan.



Chapter 8.

General discussion and conclusions

8.1 Discussion

Physiological disturbances like hypertension, hyperglycaemia and hyperthermia are common following stroke and appear to be related to outcome. Consequently, there has been interest in whether optimising physiological parameters could lead to improved outcome. This thesis has investigated the potential for NO therapeutics for lowering BP in acute stroke. More specifically, it has addressed a number of questions and issues about this treatment strategy, namely;

- There are inconsistencies in the relationship between high BP and outcome in published observational studies.
- NO related therapeutics have demonstrated contradictory findings in animal stroke models.
- There is paucity of clinical data on the effects of BP lowering with NO less than 48 hours from stroke onset.
- Acute BP lowering with NO may not improve outcome because it risks compromising cerebral perfusion.

Chapter 3 dealt with the first of these issues by attempting to clarify the relationship between high initial BP and outcome. This was necessary because previous observational studies have given conflicting data. In addition, some have demonstrated a more positive outcome in patients with high BP suggesting that BP lowering would be harmful. Hence, a systematic review was performed of all published observational studies reporting BP and

outcome in patients with acute stroke. Data from 10,892 patients were pooled using the Cochrane Review Manager software.

The main findings included the confirmation of associations between death and elevated mean arterial BP and diastolic BP. Furthermore in PICH, combined death or dependency was associated with high systolic BP and diastolic BP, and in IS high systolic BP, mean arterial BP and diastolic BP were associated with death or dependency. The relationship between outcome and BP tended to be stronger in patients with PICH as compared with those with IS. Unfortunately, without access to individual patient data it was not possible to judge if the findings were independent of confounding factors such as stroke severity or timing of measurements. Nevertheless the data corroborated with a post hoc analysis of the International Stroke Trial where a high systolic BP (>140 mmHg) was independently related to an increased risk of early death, and combined death or dependency.[138] Hence, the findings from chapter 3 support the contention that high BP is a therapeutic target in acute stroke because it is linked to worse outcome.

Chapter 3 also provided evidence for mechanisms that link high BP to worse outcome; In IS high BP was linked to early stroke recurrence and in PICH high BP was linked to early re-bleeding. Since antihypertensive agents prevent recurrent vascular events in the chronic phase after stoke [52] and limit haematoma expansion, [465] it is conceivable that they might interfere with the same processes in acute stroke and improve outcome. Unfortunately, due to paucity of data the relationship between low BP and outcome was not

examined. Previous studies have raised concerns that outcome worsens when the initial systolic BP is below 150mmHg.[138] Consequently, until the results from further studies are available it would be prudent to avoid BP lowering in stroke patients with low or normal BP at onset.

Having established that high initial BP is a therapeutic target, chapters 4 and 5 went on to examine inconsistencies in the evidence base for NO related therapeutics in animal stroke models. NO might be a useful treatment for lowering BP in acute stroke because it is a key participant in the regulation of vascular tone and contributes to BP control through relaxation of vascular smooth muscle cells.[74] Furthermore, gene knockout studies confirm that eNOS derived NO has additional neuroprotective, anti-thrombotic and anti-inflammatory properties.[99] By contrast, NO produced by iNOS and nNOS was found to be detrimental in gene knockout studies.[365-370] Therefore, many pre-clinical studies have assessed the effect of administering NO or inhibiting NOS on stroke in animal models. Unfortunately, the data from individual studies has been contradictory, and this has raised questions about whether NO related therapeutics would work in humans. Thus it was decided to determine systematically what effect NO donors and NOS inhibitors have on experimental infarct volume and CBF. The impact of differences in drug pharmacology, dosage, route of administration, timing of treatment, animal model and type of ischaemia were examined. Unfortunately it was not possible to look at the impact on BP since physiological parameters are kept tightly controlled in the majority of animal stroke studies.

First, in chapter 4 studies of NO sources (L-Arg and NO donors) in experimental IS were identified. Data were analysed using the Cochrane Collaboration Review Manager software and SMD calculated. Altogether 25 eligible studies were identified. When analysed together, NO sources reduced total cerebral infarct volume in both permanent and transient models. Sub-group analysis revealed that NO was less effective when the source was L-Arg or when it was given >1hour after onset and to transient models. Drug administration increased cortical CBF in permanent but not transient models, suggesting that exogenous NO might be beneficial partly though increases in cerebral perfusion.

In general, chapter 4 supports the continued development of NO sources, such as GTN, for treatment of human stroke. However, there are a couple of caveats. First, there are no studies specifically addressing the effect of BP lowering with NO on experimental infarct volume. Second, lack of benefit on infarct volume after 1 hour from stroke onset suggests that NO may have limited therapeutic window for neuroprotection. However, beneficial effects on CBF were observed at 1 hour in permanent stroke models (and beyond 1 hour in individual studies[337,346]), suggesting that vasculoprotective properties may be present for longer and in a more suitable time frame for use in human stroke.

In chapter 5 studies of NOS inhibitors in experimental stroke were identified in a second systematic review. Of 456 references found, 73 studies involving 2321 animals were included. NOS inhibitors reduced total infarct volume in

both permanent and transient ischaemia. Selective inhibitors (iNOS and nNOS) were better than the non-selective inhibitors, which were ineffective in permanent ischaemia. Cortical CBF was reduced in permanent models, mainly after administration of non-selective inhibitors.

The data in chapter 5 support the development of selective NOS inhibitors as treatments for clinical IS, assuming that side effects are tolerable. Importantly, the NOS inhibitors were observed to be neuroprotective several hours after IS suggesting that they are practical to give in clinical IS. The future may see the development of combined therapeutic strategies in order to get even greater beneficial effects, e.g. combining a NO donor with an iNOS inhibitor. Such an approach has already been adopted in the case of the 'dual action' compounds like the 'nitroaspirins'[197] and the antioxidant / nNOS inhibitor BN80933.[466]

Having established that high BP is a therapeutic target and that animal data support the use of NO sources in acute stroke the next step was to undertake an assessment of BP lowering with a candidate NO donor. A 5mg GTN patch was selected as it is easy to administer, can be given to dysphagic patients, compliance can be monitored and the effects can be reversed by removing the patch. Moreover, previous studies have demonstrated an approximately 7-8% reduction in 24hour ambulatory BP when 5mg GTN patches are given 3-4 days after onset. As demonstrated in chapter 4, early administration of NO is desirable in order to maximise its potentially neuroprotective and vasculoprotective properties. Hence, a clinical trial was designed to test the

haemodynamic effects and assess feasibility of administration of GTN when given <48 hours after onset.

A single centre sub-study forming part of an ongoing, prospective, international, multicentre, randomised, controlled, trial was performed. 51 patients with ischaemic or haemorrhagic stroke were randomised within 48 hours to transdermal GTN (5 mg) or control. When administered at a median 31 hours after onset, GTN significantly lowered peripheral and central systolic BP by ~15mmHg compared with the control group. At 1 hour peripheral systolic BP was approximately 7% lower than at baseline, which is beneath the maximum limit of <20% suggested by published guidelines.[153,437] The fall in BP was mediated by an effect on the central arterial tree (increased aortic compliance), rather than through effects on the heart (CO) or peripheral arterioles (TPR). Furthermore, GTN reduced BP without compromising indirect measures of cerebral perfusion, suggesting that it may be safe to give when autoregulation is impaired. Overall, Chapter 6 successfully demonstrates the feasibility of lowering BP with GTN patches and extends the information available at an earlier time interval than any previous studies.

Unfortunately, MCA velocity may not be a good measure of regional cerebral perfusion since it is affected by vessel diameter as well as blood flow.[261,453] Since GTN is a vasodilator the possibility that blood velocity was maintained in chapter 6 despite a reduction in CBF cannot be excluded. Hence it was felt necessary to undertake a fuller assessment of the effects of GTN on quantitative measures of CBF to dispel concerns that it can compromise

cerebral perfusion. In chapter 7, 18 patients with recent ischaemic or haemorrhagic stroke (<5 days) were randomised (2:1) to transdermal GTN (5 mg) or control. Global, hemispheric, arterial territory and lesion CBF were measured using XeCT before and 1 hour after treatment with GTN. In addition the effects on CPP and ZFP were estimated from measures of MCA velocity, assessed using TCD, and peripheral BP

When GTN was administered at a median 79 hours after onset it lowered BP modestly by 23 mmHg (14%) without having any detrimental effect on CBF. The neutral effect on CBF was present whether global CBF, ipsilateral and contralateral hemispheric CBF, or ipsilateral and contralateral arterial regions of interest were studied. Similarly, GTN did not alter the size of lesion core, penumbral or oligaemic areas, as defined by pre-specified CBF levels, and did not appear to cause a 'cerebral steal' effect. Finally GTN did not alter CPP or ZFP countering concerns that vasodilators will increase intracranial pressure and therefore reduce CPP.

An unanswered question is how GTN can lower BP without compromising cerebral perfusion? The thesis does not address this question directly, although it is possible to speculate based on the findings from individual chapters. First, in chapter 6 transdermal GTN was found to reduce BP by a modest 7%. This is much less than the >20% BP drop seen with high dose i.v. nimodipine that was associated with neurological worsening in one trial.[156] Hence the dose may have been insufficient to alter CBF. Second, the effect of moderate reductions in BP may be counter-balanced by NO induced

increases in collateral blood supply. This theory is indirectly supported by evidence from chapter 4 where increased CBF was observed following administration of NO in experimental stroke. Furthermore, in chapter 7 GTN appeared to increase CBF in some individuals (figure 7.3a, figure 7.3b).

8.2 Conclusion

Overall this thesis has addressed several questions concerning the investigation of GTN as a BP lowering agent in acute stroke. It has demonstrated that high BP is linked to poor outcome and is a therapeutic target. It has shown that experimental data support the use of NO sources in acute stroke. It has shown that 5mg GTN patches lower BP modestly within a narrow time frame, and it has found that there is no detrimental effect on CBF. However, at the present time the data in this thesis cannot be used to justify the routine treatment of high BP following stroke. Until data on the effect of GTN on functional outcome arrives the long standing controversy of how to best manage BP immediately after stroke will remain unresolved.

9. Appendices

9.1 Appendix I – Acknowledgments

The work in this thesis was funded by the Hypertension Trust (UK) and by the Stroke Association. I am grateful to my wife, Vicky who helped with data input. Chris Weaver, Wim Clarke (Division of Stroke Medicine [DOSM], Nottingham University) and Andrew Ghadami (Department of Radiology, City Hospital, Nottingham) provided technical help with haemodynamic and laboratory measurements. Statistical advice came from Dr Jo leonardi-Bee and Laura Gray (DOSM, Nottingham University). Telephone follow-up interviews were performed by Beverley Whysall (DOSM, Nottingham University). Professor Sean Murphy and Dr Claire Gibson (Institute of Cell Signalling, Nottingham University) gave advice on experimental work with nitric oxide and stroke models. Professor Joanna Wardlaw (Division of Clinical Neurosciences, Edinburgh University) provided neuroradiological input and helped with CBF analysis. Finally, I am grateful to my supervisor, Professor Philip Bath (Stroke Association Professor of Stroke Medicine, DOSM, Nottingham University), who provided the inspiration for the project and gave invaluable guidance throughout.

9.2 Appendix II - Abbreviations

(?) Value not reported
(7-NI) 7-nitroindinazole
(AAMI) Association for the Advancement of Medical Instrumentation
(ABPM) Ambulatory blood pressure monitor
(ACA) Anterior cerebral artery
(ACE) Angiotensin converting enzyme
(ADL) Activities of daily living
(ADP) Adenosine diphosphate
(AF) Atrial fibrillation
(AI) Augmentation index
(ANCOVA) Analysis of covariance
(ARA) Angiotensin receptor antagonist
(ARB) Angiotensin receptor blockers
(ATP) Adenosine triphosphate
(BH4) Tetrahydrobiopterin
(BHS) British Hypertension Society
(BI) Buckberg index
(BP) Blood pressure
(bpm) Beats per minute
(C) Cats
(CBF) Cerebral blood flow
(cGMP) Cyclic guanidine monophosphate
(CI) Confidence interval
(CO) Cardiac output
(CPP) Cerebral perfusion pressure
(CSF) Cerebrospinal fluid
(CVP) Central venous pressure
(EAFT) European atrial fibrillation trial
(EDRF) Endothelium derived relaxing factor
(EDV) End diastolic velocity
(ENOS) Efficacy of nitric oxide in stroke trial
(eNOS) Endothelial nitric oxide synthase
(FAD) Flavin adenine dinucleotide
(FMN) Flavin mononucleide
(FR) Fischer Rats
(FV) Flow velocity
(GTN) Glyceryl trinitrate
(HR) Heart rate
(i.a.) Intra-arterial
(i.p.) Intra-peritoneal
(i.v.) Intra-venous
(IC 50) 50% Inhibition
(ICA) Internal carotid artery
(ICP) Intracranial pressure
(infarct vol.) Infarct volume
(iNOS) Inducible nitric oxide synthase
(IQR) Interquartile range
(IS) Ischaemic stroke

(ISDN) Isosorbide di-nitrate
(L) Lambs
(LACS) Lacunar stroke
(L-Arg) L-arginine
(LER) Long-Evans rat
(L-NAME) NG-nitro-L-arginine methyl ester
(L-NIL) NG-iminoethyl-L-lysine
(L-NIO) NG-iminoethyl-L-ornithine
(L-NMMA) NG-monomethyl-L-arginine
(L-NNA) NG-nitro-L-arginine
(LR) Lewis Rats
(M) Mice
(MCA) Middle cerebral artery
(MFV) Mean flow velocity
(MG) Mongolian Gerbil
(mmHg) Millimetres mercury
(mRs) Modified Rankin scale
(n) Number of subjects
(NADP) Nicotinamide adenine dinucleotide phosphate
(NANC) Non-adrenergic, non-cholinergic
(NF-κB) Nuclear transcription factor - κB
(nNOS) Neuronal nitric oxide synthase
(NO) Nitric oxide
(NO2-) Nitrite
(NO3-) Nitrate
(NOS) Nitric oxide synthase
(NOx) Nitrate and nitrite
(O2.-) Superoxide anion radical
(OR) Odds ratio
(OT) Occupational therapy
(P) Piglets
(PACS) Partial anterior circulation stroke
(PCA) Posterior cerebral artery
(PET) Positron emission tonometry
(PI) Pulsatility index
(PICH) Primary intracerebral haemorrhage
(PMT) Photomultiplier tube
(POCS) Posterior circulation stroke
(PP) Pulse pressure
(PSV) Peak systolic velocity
(PWA) Pulse wave analysis
(PWV) Pulse wave velocity
(R) Rabbits
(RCT) Randomised controlled trial
(RD) Risk difference
(RI) Resistance index
(ROIs) Regions of interest
(RR) Risk ratio
(rTPA) Recombinant tissue plasminogen activator
(S) Number of studies

(SAH) Subarachnoid haemorrhage
(SD) Standard deviation
(SDR) Sprague-Dawley rat
(SEVR) Subendocardial viability ratio
(SGC) Soluble guanylate cyclase
(SHR) Spontaneously hypertensive rats
(SMD) Standardised mean difference
(SNP) Sodium nitroprusside
(SNSS) Scandinavian neurological stroke scale
(SPECT) Single photon emission tonometry
(STAIR) Stroke Therapy Academic Industry Roundtable
(SV) Stroke volume
(TACS) Total anterior circulation stroke
(TCD) Transcranial Doppler
(TNFα) Tumour necrosis factor
(TPR) Total peripheral resistance
(TRIM) Tri(fluoromethylphenyl)imidazole
(WHO) World Health Organisation
(WMD) Weighted mean difference
(WR) Wistar rats
(Xe) Xenon
(XeCT) Xenon CT
(ZFP) Zero flow pressure

9.3 Appendix III – Publications arising from this thesis

9.3.1 Full Publications

Willmot M, Ghadami A, Whysall B, Clarke W, Wardlaw J, Bath P. Transdermal glyceryl trinitrate lowers blood pressure and maintains cerebral blood flow in recent stroke. *Hypertension*. 2006 Jun;47(6):1209-15.

Willmot M, Gibson C, Gray L, Murphy S, Bath P. Nitric oxide synthase inhibitors in experimental ischemic stroke and their effects on infarct size and cerebral blood flow: a systematic review. *Free Radic Biol Med*. 2005 Aug 1;39(3):412-25.

Willmot M, Gray L, Gibson C, Murphy S, Bath PM. A systematic review of nitric oxide donors and L-arginine in experimental stroke; effects on infarct size and cerebral blood flow. *Nitric Oxide*. 2005 May;12(3):141-9.

Willmot M, Leonardi-Bee J, Bath P. High blood pressure in acute stroke and subsequent outcome: a systematic review. *Hypertension.* 2004;43(1):18-24.

9.3.2 Posters

Willmot M, Gibson C, Murphy S, Bath P. Systematic review of nitric oxide synthase inhibitors in experimental stroke: effects on infarct size and cerebral blood flow (5th World stroke congress, Vancouver 2004).

Willmot M, Gibson C, Murphy S, Bath P. Systematic review of nitric oxide synthase inhibitors in experimental stroke: effects on infarct size and cerebral blood flow (British Association of Stroke Physicians Annual Conference, Cambridge 2004)

Willmot M, Leonardi-Bee J, Bath P High blood pressure in acute stroke and subsequent outcome: a systematic review. (British Association of Stroke Physicians Annual Conference, Keele 2003)

10. References

1. *Stroke. Towards better management*. 1 ed. London: Royal College of Physicians of London; 1989.
2. Wolfe CD. The impact of stroke. *Br Med Bull*. 2000;56:275-86.
3. Davenport R, Dennis M. Acute Stroke. In: Hughes R, ed. *Neurological Emergencies*. 3rd ed. London: BMJ Publishing Group; 2000:63-92.
4. *National Health Service Executive. National Service Framework for Older People*. London: Department of Health; 2001.
5. Bath PMW, Staff CPsBD. *Stroke therapy: advances and commercial opportunities*. Oxford: Connect Pharma Ltd; 1995.
6. Bonita R, 1401. Epidemiology of stroke. *Lancet*. 1992;339:342-347.
7. Balarajan R. Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England and Wales. *BMJ*. 1991;302:560-564.
8. Warlow CP, Dennis MS, van Gijn J, Hankey GJ, Sandercock PAG, Bamford JM, Wardlaw J. *Stroke. A practical guide to management*. Oxford: Blackwell Science; 1996.
9. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J. Blood pressure, stroke and coronary heart disease. Part I: effects of prolonged differences in blood pressure - evidence from nine prospective observational studies corrected for the regression dilution bias. *Lancet*. 1990;335:765-774.
10. Shinton R, Beevers G, 1948. Meta-analysis of relation between cigarette smoking and stroke. *British Medical Journal*. 1989;298:789-794.

11. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *Jama*. 1979;241:2035-8.
12. Gill JS, Zezulka AV, Shipley MJ, Gill SK, Beevers DG. Stroke and alcohol consumption. *N Engl J Med*. 1986;315:1041-6.
13. Perry IJ, Beevers GG. Salt intake and stroke: a possible direct effect. *Journal of Human Hypertension*. 1992;6:23-25.
14. Welin L, Svardsudd K, Wilhelmsen L, Larsson B, Tibblin G. Analysis of risk factors for stroke in a cohort of men born in 1913. *N Engl J Med*. 1987;317:521-6.
15. Brattstrom L, Lindgren A, Israelsson B, Malinow MR, Norrving B, Upson B, Hamfelt A. Hyperhomocysteinaemia in stroke: prevalence, cause, and relationships to type of stroke and stroke risk factors. *European Journal of Clinical Investigation*. 1992;22:214-221.
16. Prentice RL, Szatrowski TP, Kato H, Mason MW, 1588. Leukocyte counts and cerebrovascular disease. *Journal of Chronic Disease*. 1982;35:703-714.
17. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. *New England Journal of Medicine*. 1984;311:501-505.
18. Kannel WB, Gordon T, Wolf PA, 1587. Hemoglobin and the risk of cerebral infarction: the Framingham Study. *Stroke*. 1972;3:409.
19. Warlow CP. Epidemiology of stroke. *Lancet*. 1998;352 Suppl 3:SIII1-4.
20. Bath P, Lees KR. ABC of arterial and venous disease - Acute Stroke. *BMJ*. 2000;320:920-923.

21. Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE, Investigators TT. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. *Stroke*. 1993;24:35-41.

22. Tuhrim S. Management of stroke and transient ischemic attack. *Mt Sinai J Med*. 2002;69:121-30.

23. Hatano S. Experience from a multicentre stroke register: a preliminary report. *Bulletin of the World Health Organisation*. 1976;54:541-553.

24. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet*. 1991;337:1521-1526.

25. Anderson CS, Taylor BV, Hankey GJ, Stewart-Wynne EG, Jamrozik KD. Validation of a clinical classification for subtypes of acute cerebral infarction. *J Neurol Neurosurg Psychiatry*. 1994;57:1173-9.

26. Bamford J, Sandercock PAG, Dennis MS, Warlow CP. A prospective study of acute cerebrovascular disease in the community: the Oxford Community Stroke Project 1981-1986. 2. Incidence, case fatality rates and overall outcome at one year of cerebral infarction, primary intracerebral and subarachnoid haemorrhage. *Journal of Neurology, Neurosurgery & Psychiatry*. 1990;53:16-22.

27. Ross RK. The pathogenesis of atherosclerosis - an update. *N Engl J Med*. 1986;314:488-500.

28. Heiss WD, Hayakawa T, Waltz AG. Cortical neuronal function during ischemia. Effects of occlusion of one middle cerebral artery on single-unit activity in cats. *Arch Neurol*. 1976;33:813-20.

29. Heiss WD. Experimental evidence of ischemic thresholds and functional recovery. *Stroke*. 1992;23:1668-72.

30. Pulsinelli W, 1668. Pathophysiology of acute ischaemic stroke. *Lancet*. 1992;339:533-536.

31. Steiner T, Hacke W. Combination therapy with neuroprotectants and thrombolytics in acute ischaemic stroke. *Eur Neurol*. 1998;40:1-8.

32. Marchal G, Beaudouin V, Rioux P, de la Sayette V, Le Doze F, Viader F, Derlon JM, Baron JC. Prolonged persistence of substantial volumes of potentially viable brain tissue after stroke: a correlative PET-CT study with voxel-based data analysis. *Stroke*. 1996;27:599-606.

33. Mendelow AD. Mechanisms of ischemic brain damage with intracerebral hemorrhage. *Stroke*. 1993;24:I115-7; discussion I118-9.

34. Chen ZM, Hui JM, Liu LS, Liu ZM, Peto R, Sandercock P, Wang WQ, Wang YX, Wang ZB, Xie JX, You GX, Zhang FL, Zhang HQ, Zhao ZY. CAST: Randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke. *Lancet*. 1997;349:1641-1649.

35. International Stroke Trial Collaborative Group. The International Stroke Trial (IST); a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. *Lancet*. 1997;349:1569-1581.

36. Wardlaw JM, Zoppo G, Yamaguchi T, Berge E. Thrombolysis for acute ischaemic stroke. *Cochrane Database Syst Rev*. 2003:CD000213.

37. Hand P, Lindley R, Wardlaw J, Sandercock P. The third international stroke trial (IST-3). *Cerebrovascular diseases*. 2001;11 (suppl 4):35.

38. Kidwell CS, Liebeskind DS, Starkman S, Saver JL. Trends in acute ischemic stroke trials through the 20th century. *Stroke*. 2001;32:1349-59.

39. Mayer SA, Brun NC, Broderick J, Davis S, Diringer MN, Skolnick BE, Steiner T. Safety and feasibility of recombinant factor VIIa for acute intracerebral hemorrhage. *Stroke*. 2005;36:74-9.

40. Hankey GJ, Hon C. Surgery for primary intracerebral hemorrhage: is it safe and effective? A systematic review of case series and randomized trials. *Stroke*. 1997;28:2126-32.

41. Mendelow AD, Gregson BA, Fernandes HM, Murray GD, Teasdale GM, Hope DT, Karimi A, Shaw MD, Barer DH. Early surgery versus initial conservative treatment in patients with spontaneous supratentorial intracerebral haematomas in the International Surgical Trial in Intracerebral Haemorrhage (STICH): a randomised trial. *Lancet*. 2005;365:387-97.

42. Lees KR, Bath PMW, Naylor AR. ABC of arterial and venous disease - Secondary prevention of transient ischaemic attack and stroke. *BMJ*. 2000;320:991-994.

43. Antithrombotic Trialists Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ*. 2002;324:71-86.

44. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet*. 1996;348:1329-1339.

45. Forbes CD, For the ESPS-2 Collaborators. European stroke prevention study 2: dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. *International Journal of Clinical Practice*. 1997;51 (4):205-208.

46. ESPS Group. European Stroke Prevention Study. *Stroke*. 1990;21:1122-1130.

47. Diener H-C. Management of Atherothrombosis with Clopidogrel in High-risk patients with recent TIA or ischaemic stroke (MATCH): rationale and study design. In: *Cerebrovascular Diseases*; 2001:35.

48. Lip GY, Hart RG, Conway DS. Antithrombotic therapy for atrial fibrillation. *Bmj*. 2002;325:1022-5.

49. EAFT (European Atrial Fibrillation Trial) Study Group. Secondary prevention in non-rheumatic atrial fibrillation after TIA or minor stroke. *Lancet*. 1993;342:1255-1262.

50. European Carotid Surgery Trialists' Collaborative Group. Endarterectomy for moderate symptomatic carotid stenosis: interim results from the MRC European Carotid Surgery Trial. *Lancet*. 1996;347:1591-1593.

51. North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *New England Journal of Medicine*. 1991;325:445-453.

52. Rashid P, Leonardi-Bee J, Bath P. Blood pressure reduction and the secondary prevention of stroke and other vascular events: a systematic review. *Stroke*. 2003;34:2741-2749.

53. PROGRESS Collaborative Group. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6105 individuals with previous stroke or transient ischaemic attack. *Lancet*. 2001;358:1033-1041.

54. McInnes GT. Debate: Does it matter how you lower blood pressure? *Curr Control Trials, Cardiovasc Med*. 2001;2:63-66.

55. Barer DH, Cruickshank JM, Ebrahim SB, Mitchell JR. Low dose beta blockade in acute stroke ("BEST" trial): an evaluation. *British Medical Journal*. 1988;296:737-741.

56. Potter J. COSSACS and CHHIPS: Trying to answer the question of how to manage hypertension in acute stroke. *Stroke Matters*. 2004:1.

57. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;360:7-22.

58. Welch KMA, Committee FtSS, 1568. Effect of atorvastatin compared with placebo on cerebrovascular endpoints in patients with a previous stroke or TIA - the SPARCL study. In: *Cerebrovascular Diseases*; 1999:108.

59. Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebrovascular disease in the community: results of a WHO collaborative study. *Bull World Health Organ*. 1980;58:113-30.

60. Stroke Unit Trialists' Collaboration. Organised inpatient (stroke unit) care for stroke. In: *The Cochrane Library*. 3 ed. Oxford: Update Software; 2002.

61. Steultjens EM, Dekker J, Bouter LM, van de Nes JC, Cup EH, van den Ende CH. Occupational therapy for stroke patients: a systematic review. *Stroke*. 2003;34:676-87.

62. Langhorne P, Wagenaar R, Partridge C. Physiotherapy after stroke: more is better? *Physiother Res Int*. 1996;1:75-88.

63. Meek C, Pollock A, Potter J, Langhorne P. A systematic review of exercise trials post stroke. *Clin Rehabil*. 2003;17:6-13.

64. Paci M. Physiotherapy based on the Bobath concept for adults with post-stroke hemiplegia: a review of effectiveness studies. *J Rehabil Med*. 2003;35:2-7.

65. Greener J, Enderby P, Whurr R. Speech and language therapy for aphasia following stroke. *Cochrane Database Syst Rev*. 2000:CD000425.

66. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980;288:373-6.

67. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987;327:524-6.

68. Marin J, Rodriguez-Martinez MA. Role of vascular nitric oxide in physiological and pathological conditions. *Pharmacol Ther*. 1997;75:111-34.

69. Schmidt HH, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. *Biochim Biophys Acta*. 1993;1178:153-75.

70. Denninger JW, Marletta MA. Guanylate cyclase and the .NO/cGMP signaling pathway. *Biochim Biophys Acta*. 1999;1411:334-50.

71. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol*. 1996;271:C1424-37.

72. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet*. 1989;2:997-1000.

73. Fleming I, Busse R. Control and consequences of endothelial nitric oxide formation. *Adv Pharmacol*. 1995;34:187-206.

74. Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci U S A*. 1989;86:3375-8.

75. Welch G, Loscalzo J. Nitric oxide and the cardiovascular system. *J Card Surg*. 1994;9:361-71.

76. McAllister RM, Hirai T, Musch TI. Contribution of endothelium-derived nitric oxide (EDNO) to the skeletal muscle blood flow response to exercise. *Med Sci Sports Exerc*. 1995;27:1145-51.

77. Burnett AL, Tillman SL, Chang TS, Epstein JI, Lowenstein CJ, Bredt DS, Snyder SH, Walsh PC. Immunohistochemical localization of nitric oxide synthase in the autonomic innervation of the human penis. *J Urol*. 1993;150:73-6.

78. Bath PMW, Hassall DG, Gladwin A-M, Palmer RMJ, Martin JF. Nitric oxide and prostacyclin. Divergence of inhibitory effects on monocyte chemotaxis and adhesion to endothelium in vitro. *Arteriosclerosis and Thrombosis*. 1991;11:254-260.

79. Bath PMW. The effect of nitric oxide-donating vasodilators on monocyte chemotaxis and intracellular cGMP concentrations in vitro. *Eur.J.Clin.Pharmacol.* 1993;45:53-58.

80. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest*. 1989;83:1774-7.

81. Cardona-Sanclemente LE, Born GV. Effect of inhibition of nitric oxide synthesis on the uptake of LDL and fibrinogen by arterial walls and other organs of the rat. *Br J Pharmacol*. 1995;114:1490-4.

82. Kim YM, Talanian RV, Billiar TR. Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. *J Biol Chem*. 1997;272:31138-48.

83. Murphy S. Production of nitric oxide by glial cells: regulation and potential roles in the CNS. *Glia*. 2000;29:1-13.

84. Laroux FS, Pavlick KP, Hines IN, Kawachi S, Harada H, Bharwani S, Hoffman JM, Grisham MB. Role of nitric oxide in inflammation. *Acta Physiol Scand*. 2001;173:113-8.

85. Brune B, von Knethen A, Sandau KB. Nitric oxide and its role in apoptosis. *Eur J Pharmacol*. 1998;351:261-72.

86. Sims NR, Anderson MF. Mitochondrial contributions to tissue damage in stroke. *Neurochem Int*. 2002;40:511-26.

87. Manzoni O, Prezeau L, Marin P, Deshager S, Bockaert J, Fagni L. Nitric oxide-induced blockade of NMDA receptors. *Neuron*. 1992;8:653-662.

88. Lipton S. NO - Related species: Neuroprotection verses neurodestruction. In: Welch K, Caplan L, Reis D, Siesjo B, Weir B, eds. *Primer on cerebrovascular diseases*. 1st ed: Academic Press; 1997:210-13.

89. Benz D, Cadet P, Mantione K, Zhu W, Stefano G. Tonal nitric oxide and health: anti-bacterial and -viral actions and implications for HIV. *Med Sci Monit*. 2002;8:RA27-31.

90. Brunet LR. Nitric oxide in parasitic infections. *Int Immunopharmacol*. 2001;1:1457-67.

91. Blottner D, Luck G. Just in time and place: NOS/NO system assembly in neuromuscular junction formation. *Microsc Res Tech*. 2001;55:171-80.

92. Bredt DS. Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Radic Res*. 1999;31:577-96.

93. Symeonides S, Balk RA. Nitric oxide in the pathogenesis of sepsis. *Infect Dis Clin North Am*. 1999;13:449-63, x.

94. Rashid PA, Whitehurst A, Lawson N, Bath PMW. Plasma nitric oxide (nitrate/nitrite) levels in acute stroke and their relationship with severity and outcome. *J.Stroke Cerebrovasc.Dis.* 2003;12:82-87.

95. Panza JA, Garcia CE, Kilcoyne CM, Quyyumi AA, Cannon RO, 3rd. Impaired endothelium-dependent vasodilation in patients with essential hypertension. Evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. *Circulation*. 1995;91:1732-8.

96. Stroes ES, Koomans HA, de Bruin TW, Rabelink TJ. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. *Lancet*. 1995;346:467-71.

97. Castillo J, Rama R, Davalos A. Nitric oxide-related brain damage in acute ischemic stroke. *Stroke*. 2000;31:852-7.

98. Tarkowski E, Ringqvist A, Rosengren L, Jensen C, Ekholm S, Wennmalm A, 1462. Intrathecal release of nitric oxide and its relation to final brain damage in patients with stroke. *Cerebrovascular Diseases*. 2000;10:200-206.

99. Huang Z, Huang PL, Ma J, Meng W, Ayata C, Fishman MC, Moskowitz MA. Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. *Journal of Cerebral Blood Flow and Metabolism*. 1996;16:981-987.

100. Li H, Forstermann U. Nitric oxide in the pathogenesis of vascular disease. *J Pathol*. 2000;190:244-54.

101. Huang Z, Huang PL, Panahian N, Dalkara T, Fishman MC, Moskowitz MA. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. *Science*. 1994;265:1883-1885.

102. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci*. 1999;22:391-7.

103. Iadecola C, Zhang F, Xu S, Casey R, Ross ME. Inducible nitric oxide synthase gene expression in brain following cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism*. 1995;15:378-384.

104. Zhao X, Haensel C, Araki E, Ross ME, Iadecola C. Gene-dosing effect and persistence of reduction in ischemic brain injury in mice lacking inducible nitric oxide synthase. *Brain Res*. 2000;872:215-8.

105. Scott JF, Gray CS. Cerebral and systemic pathophysiological responses to acute stroke. *Age Ageing*. 2000;29:197-202.

106. Wu G, Meininger CJ. Impaired arginine metabolism and NO synthesis in coronary endothelial cells of the spontaneously diabetic BB rat. *Am J Physiol*. 1995;269:H1312-8.

107. Kaposzta Z, Baskerville PA, Madge D, Fraser S, Martin JF, Markus HS. L-arginine and S-nitrosoglutathione reduce embolization in humans. *Circulation*. 2001;103:2371-5.

108. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J*. 2001;357:593-615.

109. Garvey EP, Oplinger JA, Tanoury GJ, Sherman PA, Fowler M, Marshall S, Harmon MF, Paith JE, Furfine ES. Potent and selective inhibition of human nitric oxide synthases. Inhibition by non-amino acid isothioureas. *J Biol Chem*. 1994;269:26669-76.

110. Young RJ, Beams RM, Carter K, Clark HA, Coe DM, Chambers CL, Davies PI, Dawson J, Drysdale MJ, Franzman KW, French C, Hodgson ST, Hodson HF, Kleanthous S, Rider P, Sanders D, Sawyer DA, Scott KJ, Shearer BG, Stocker R, Smith S, Tackley MC, Knowles RG. Inhibition of inducible nitric oxide synthase by acetamidine derivatives of hetero-substituted lysine and homolysine. *Bioorg Med Chem Lett*. 2000;10:597-600.

111. Handy RL, Wallace P, Gaffen ZA, Whitehead KJ, Moore PK. The antinociceptive effect of 1-(2-trifluoromethylphenyl) imidazole (TRIM), a potent inhibitor of neuronal nitric oxide synthase in vitro, in the mouse. *Br J Pharmacol*. 1995;116:2349-50.

112. Reif DW, McCarthy DJ, Cregan E, Macdonald JE. Discovery and development of neuronal nitric oxide synthase inhibitors. *Free Radic Biol Med*. 2000;28:1470-7.

113. Chabrier PE, Auguet M, Spinnewyn B, Auvin S, Cornet S, Demerle-Pallardy C, Guilmard-Favre C, Marin JG, Pignol B, Gillard-Roubert V, Roussillot-Charnet C, Schulz J, Viossat I, Bigg D, Moncada S. BN 80933, a dual inhibitor of neuronal nitric oxide synthase and lipid peroxidation: a promising neuroprotective strategy. *Proc Natl Acad Sci U S A*. 1999;96:10824-9.

114. Escott KJ, Beech JS, Haga KK, Williams SCR, Meldrum BS, Bath PMW. Cerebroprotective effect of the nitric oxide synthase inhibitors, 1-(2-trifluoromethylphenyl) imidazole (TRIM) and 7-nitro indazole, after transient focal cerebral ischemia in the rat. *J Cereb Blood Flow Metab*. 1998;18:281-287.

115. CAST (Chinese Acute Stroke Trial) Collaborative Group. CAST: randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke. *Lancet*. 1997;349:1641-1649.

116. Wallace JD, Levy LL. Blood pressure after stroke. *JAMA*. 1981;246:2177-2180.

117. Britton M, Carlsson A, de Faire U. Blood pressure course in patients with acute stroke and matched controls. *Stroke*. 1986;17:861-864.

118. Carlberg B, Asplund K, Hagg E. Factors influencing admission blood pressure levels in patients with acute stroke. *Stroke*. 1991;4:527-530.

119. Harper G, Castleden CM, Potter JF. Factors affecting changes in blood pressure after acute stroke. *Stroke*. 1994;25:1726-1729.

120. Murros K, Fogelholm R, Kettunen S, Vuorela A-L. Serum cortisol and outcome of ischemic brain infarction. *Journal Neurological Sciences*. 1993;116:12-17.

121. Fassbender K, Schmidt R, Mobner R, Daffertshofer M, Hennerici M. Pattern of activation of the hypothalamic-pituitary-adrenal axis in acute stroke. *Stroke*. 1994;6:1105-1108.

122. Treib J, Hass A, Krammer L, Stoll M, Grauer MT, Schimrigk K. Cardiac output in patients with acute stroke. *J.Neurol.* 1996;243:575-578.

123. Ahmed N, Wahlgren NG. High intial blood pressure after acute stroke is associated with poor functional outcome. *Journal of Internal Medicine*. 2001;249:467-473.

124. Britton M, Carlsson A. Very high blood pressure in acute stroke. *J Intern Med*. 1990;228:611-5.

125. Bourestom NC. Predictors of long term recovery in cerebrovascular disease. *Arch.Phys.Med.Rehabil.* 1967;48:415-459.

126. Panayiotou BN, Taub NA, Fotherby MD. Twenty-four-hour blood pressure profiles following stroke. *Blood Press Monit*. 1996;1:409-414.

127. Dunne JW, Chakera T, Kermode S. Cerebellar haemorrhage--diagnosis and treatment: a study of 75 consecutive cases. *Q J Med*. 1987;64:739-54.

128. Harmsen P, Tibblin G. A stroke register in Gotteborg, Sweden. *Acta Med Scand*. 1972;191:463-70.

129. Qureshi AI, Safdar K, Weil EJ, Barch C, Bliwise DL, Colohan AR, McKay B, Frankel MR. Predictors of early deterioration and mortality in black americans with spontaneous intracerebral hemorrhage. *Stroke*. 1995;1995:1764-1767.

130. Robinson T, Waddington A, Ward-Close S, Taub N, Potter J. The predictive role of 24-hour compared to casual blood pressure levels on outcome following acute stroke. *Cerebrovascular Diseases*. 1997;7:264-272.

131. Tuhrim S, Dambrosia J, Rice T, Mohr J, Wolf P, Heyman A, Kase C, 1894. Prediction of intracerebral hemorrhage survival. *Ann.Neurol.* 1988;24 (2):258-263.

132. Dandapani BK, Suzuki S, Kelley RE, Reyes-Iglesias Y, Duncan R. Relation between blood pressure and outcome in intracerebral haemorrhage. *Stroke*. 1995;26:21-24.

133. Rankin J. Cerebral vascular accidents in patients over the age of 60. 2. Prognosis. *Scottish Medical Journal*. 1957;2:200-215.

134. Acheson J. Factors affecting the natural history of "focal cerebral vascular disease". *Q J Med*. 1971;40:25-46.

135. Longo-Mbenza B, Tonduangu K, Muyeno K, Phanzu M, Kebolo Baku A, Muvova D, Lelo T, Odio W, Lukoki L, Bikangi Nkiabungu F, Kilembe M, Tshiamala P, Katalay L, Mwema M, Muyembe T. Predictors of stroke - associated mortality in Africans. *Rev Epidemiol Sante Publique*. 2000;48:31-9.

136. M'Buyamba-Kabangu JR, Longo-Mbenza B, Tambwe MJ, Dikassa LN, Mbala-Mukendi M. J-shaped relationship between mortality and admission blood pressure in black patients with acute stroke. *J Hypertens*. 1995;13:1863-8.

137. Boreas AMHP, Lodder J, Kessels F, de Leeuw PW, Troost J. Predictors of poststroke blood pressure level and course. *Journal Stroke Cerebrovascular Diseases*. 2001;10:85-91.

138. Leonardi-Bee J, Bath PMW, Phillips SJ, Sandercock PAG, for the IST Collaborative Group. Blood pressure and clinical outcomes in the International Stroke Trial. *Stroke*. 2002;33:1315-1320.

139. Bath FJ, Bath PMW. What is the correct management of blood pressure in acute stroke? The Blood pressure in Acute Stroke Collaboration. *Cerebrovascular Diseases*. 1997;7:205-213.

140. Sacco RL, Foulkes MA, Mohr JP, Wolf PA, Hier DB, Price TR. Determinants of early recurrence of cerebral infarction. The Stroke Data Bank. *Stroke*. 1989;20:983-9.

141. Krieger DW, Demchuk AM, Kasner SE, Jauss M, Hantson L. Early clinical and radiological predictors of fatal brain swelling in ischemic stroke. *Stroke*. 1999;30:287-92.

142. Kazui S, Minematsu K, Yamamoto H, Sawada T, Yamaguchi T. Predisposing factors to enlargement of spontaneous intracerebral hematoma. *Stroke*. 1997;28:2370-2375.

143. Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS. Stroke in patients with diabetes. *Stroke*. 1994;25:1977-1984.

144. Allen CMC. Predicting the outcome of acute stroke: a prognostic score. *Journal of Neurology, Neurosurgery and Psychiatry*. 1984;47:475-480.

145. Osaki Y, Matsubayashi K, Yamasaki M, Okumiya K, Yoshimura K, Hamashige N, Doi Y. Post-stroke hypertension correlates with neurologic recovery in patients with acute ischemic stroke. *Hypertens Res*. 1998;21:169-73.

146. Merrett JD, Adams GF. Comparison of mortality rates in elderly hypertensive and normotensive hemiplegic patients. *Br Med J*. 1966;2:802-5.

147. Meyer JS, Shimazu K, Fukuuchi Y, Ohuchi T, Okamoto S, Koto A, Ericsson AD. Impaired neurogenic cerebrovascular control and dysautoregulation after stroke. *Stroke*. 1973;4:169-186.

148. Dawson SL, Manktelow BN, Robinson TG, Panerai RB, Potter JF. Which parameters of beat-to-beat blood pressure and variability best predict early outcome after acute ischemic stroke? *Stroke*. 2000;31:463-468.

149. Fieschi C, Agnoli A, Battistini N, Bozzao L, Prencipe M. Derangement of regional cerebral blood flow and its regulatory mechanisms in acute cerebrovascular lesions. *Neurology*. 1968;18:1166-1179.

150. Robinson TG, James M, Youde J, Panerai R, Potter J. Cardiac baroreceptor sensitivity is impaired after acute stroke. *Stroke*. 1997;28:1671-1676.

151. Wahlgren NG, MacMahon DG, de Keyser J, Indredavik B, Ryman T, INWEST Study Group. Intravenous Nimodipine West European Stroke

Trial (INWEST) of nimodipine in the treatment of acute ischaemic stroke. *Cerebrovascular Diseases*. 1994;4:204-210.

152. The Intercollegiate Working Party for Stroke. *National Clinical Guidelines for Stroke*. London: Royal College of Physicians London. March; 2000.

153. O'Connell JE, Gray GS. Treating hypertension after stroke. *BMJ*. 1994;308:1523-1524.

154. Brott T, Lu M, Kothari R, Fagan SC, Frankel M, Grotta JC, Broderick J, Kwiatkowski T, Lewandowski C, Haley E, Marler J, Tilley B, The NINDS rt-PA Stroke Study Group. Hypertension and its treatment in the NINDS rt-PA stroke trial. *Stroke*. 1998;29:1504-1509.

155. Blood pressure in Acute Stroke Collaboration (BASC). Vasoactive drugs for acute stroke (Cochrane Review). In: *The Cochrane Library*. 4 ed. Oxford: Update Software; 2001.

156. Ahmed N, Nasman P, Wahlgren NG. Effect of intravenous nimodipine on blood pressure and outcome after acute stroke. *Stroke*. 2000;31:1250-1255.

157. Lisk DR, Grotta JC, Lamki LM, Tran HD, Taylor JW, Molony DA, Barron BJ. Should hypertension be treated after acute stroke? A randomised controlled trial using single photon emission computed tomography. *Archives Neurology*. 1993;50:855-862.

158. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJA, van Gijn J, 1519. Interobserver agrteement for the assessment of handicap in stroke patients. *Stroke*. 1988;19:604-607.

159. Patel RV, Kertland HR, Jahns BE, 1770. Labetalol: Response and safety in criticaly ill hemorrhagic stroke patients. *The Annals of Pharmacotherapy*. 1993;27:180-181.

160. Evans P, et al. Bendrofluazide does not reduce arterial blood pressure in the acute stroke period (abstract). In: *6th Scientific Meeting of The Stroke Association*. Oxford; 2001.

161. Dahl A, Russell D, Nyberg-Hansen R, Rootwelt K. Effect of nitroglycerin on cerebral circulation measured by transcranial doppler and SPECT. *Stroke*. 1989;20:1733-1736.

162. Butterworth RJ, Cluckie A, Jackson SHD, Buxton-Thomas M, Bath PMW. Pathophysiological assessment of nitric oxide (given as sodium nitroprusside) in acute ischaemic stroke. *Cerebrovascular Diseases*. 1998;8:158-165.

163. Salom JB, Orti M, Centeno JM, Torregrosa G, Alborch E. Reduction of infarct size by the NO donors sodium nitroprusside and spermine/NO after transient focal cerebral ischemia in rats. *Brain Res*. 2000;865:149-56.

164. Gelb AW, Boisvert DP, Tang C, Lam AM, Marchak BE, Dowman R, Mielke BW. Primate brain tolerance to temporary focal cerebral ischemia during isoflurane- or sodium nitroprusside-induced hypotension. *Anesthesiology*. 1989;70:678-83.

165. Smith RP, Kruszyna H. Nitroprusside produces cyanide poisoning via reaction with hemoglobin. *J Pharmacol Exp Ther*. 1974;191:557-63.

166. Bath PMW, Pathansali R, Iddenden R, Bath FJ. The effect of transdermal glyceryl trinitrate, a nitric oxide donor, on blood pressure

and platelet function in acute stroke. *Cerebrovascular Diseases*. 2001;11:265-272.

167. Rashid P, Leonardi-Bee J, Weaver C, Bath F, Bath P. The effect of transdermal glyceryl trinitrate on blood pressure and middle cerebral artery volocity in acute stroke. *Cerebrovasc.Dis.* 2002;13 (suppl 3):53 (abstract).

168. Bath PMW, Willmot M, Bath FJ. Nitric oxide donors (nitrates), L-arginine, or nitric oxide synthase inhibitors in acute ischaemic stroke (Cochrane Review). In: *The Cochrane Library*. 4 ed. Oxford: Update Software; 2002.

169. Bath PMW, Bath FJ, Investigators TE. Efficacy of Nitric Oxide in Stroke (ENOS) trial - a prospective large randomised controlled trial in acute stroke. *Cerebrovascular Diseases*. 2000;10 (suppl 2):81 (abstract).

170. Gomma AH, Purcell HJ, Fox KM. Potassium channel openers in myocardial ischaemia: therapeutic potential of nicorandil. *Drugs*. 2001;61:1705-10.

171. Kobayashi S, Yamaguchi S, Okada K, Suyama N, Bokura K, Murao M. Effects of nicorandil on regional cerebral blood flow in patients with chronic cerebral infarction. Preliminary communication. *Arzneimittelforschung*. 1992;42:1086-9.

172. Chalmers J, MacMahon S, Committee obotPM. PROGRESS - perindopril protection against recurrent stroke study: main results. *Journal of Hypertension*. 2001;19 (suppl 2):S260.

173. Bosch J, Yusuf S, Pogue J, Probstfield J, Diaz R, Hoeschen R, Ostergren J. Impact of ramipril and vitamin E on cerebrovascular

events in the HOPE (Heart Outcomes Prevention Evaluation) Trial. *J Am Coll Cardiol*. 2000;35 (suppl A):324.

174. Waldemar G, Vorstrup S, Anderson AR, Pedersen H, Paulson OB. Angiotensin-converting enzyme inhibition and regional cerebral blood flow in acute stroke. *Journal Cardiovascular Pharmacology*. 1989;14:722-729.

175. Dyker AG, Grosset DG, Lees K. Perindopril reduces blood pressure but not cerebral blood flow in patients with recent cerebral ischemic stroke. *Stroke*. 1997;28:580-583.

176. Schrader J, Luders S, Kulchewski A, Berger J, Zidek W, Treib J, Einhaupl K, Diener H-C, Dominiak P. The ACCESS Study. Evaluation of acute candesartan therapy in stroke survivors. *Stroke*. 2003;34:1699-1703.

177. Nazir FS, Overell JR, Bolster A, Hilditch TE, Reid JL, Lees KR. The effect of losartan on global and focal cerebral perfusion and on renal function in hypertensives in mild early ischaemic stroke. *J Hypertens*. 2004;22:989-95.

178. Blood pressure in Acute Stroke Collaboration (BASC). Interventions for deliberately altering blood pressure in acute stroke (Cochrane Review). In: *The Cochrane Library*. 4 ed. Oxford: Update Software; 2001.

179. Goldstein LB. Effects of amphtamines and small related molecules on recovery after stroke in animals and man. *Neuro Pharmacology*. 2000;39:852-859.

180. Rordorf G, Cramer SC, Efird JT, Schwamm LH, Buonanno F, Koroshetz WJ. Pharmacological elevation of blood pressure in acute stroke. Clinical effects and safety. *Stroke*. 1997;28:2133-2138.

181. Duke BJ, Breeze RE, Rubenstein D, Tranmer BI, Kindt GW. Induced hypervolemia and inotropic support for acute cerebral arterial insufficiency: an underused therapy. *Surg Neurol*. 1998;49:51-4; discussion 54-7.

182. Schwarz S, Georgiadis D, Aschoff A, Schwab S. Effects of induced hypertension on intracranial pressure and flow velocities of the middle cerebral arteries in patients with large hemispheric stroke. *Stroke*. 2002;33:998-1004.

183. Wise G, Sutter R, Burkholder J. The treatment of brain ischaemia with vasopressor drug. *Stroke*. 1972;3:135-140.

184. Asplund K, Israelsson K, Schampi I. Haemodilution for acute ischaemic stroke (Cochrane Review). In: *The Cochrane Library*. 2 ed. Oxford: Update Software; 1999.

185. Muir KW, Grosset DG, Lees KR. Effects of prolonged infusions of the NMDA antagonist aptiganel hydrochloride (CNS 1102) in normal volunteers. *Clinical Neuropharmacology*. 1997;20, No.4:311-321.

186. Saxena R, Wijnhoud AD, Carton H, Hacke W, Kaste M, Przybelski RJ, Stern KN, Koudstaal PJ. Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb, in acute ischemic stroke. *Stroke*. 1999;30:993-996.

187. Ramos-Zuniga R, Velazquez-Santana H, Mercado-Pimentel R, Cerda-Camacho F. Neuroprotection in selective focal ischemia in rats by

nitrates, an alternative redox manipulation on nitric oxide: experimental model. *Minim Invasive Neurosurg*. 1998;41:152-60.

188. Zhang F, Iadecola C. Nitroprusside improves blood flow and reduces brain damage after focal ischemia. *Neuroreport*. 1993;4:559-62.

189. Zhang F, White JG, Iadecola C. Nitric oxide donors increase blood flow and reduce brain damage in focal ischemia: evidence that nitric oxide is beneficial in the early stages of cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism*. 1994;14:217-226.

190. Multicenter Acute Stroke Trial - Europe (MAST-E). *Cerebrovasc.Dis.* 1994;4:241 (Abstract).

191. Coert BA, Anderson RE, Meyer FB. A comparative study of the effects of two nitric oxide synthase inhibitors and two nitric oxide donors on temporary focal cerebral ischemia in the Wistar rat. *J Neurosurg*. 1999;90:332-8.

192. Chi OZ, Wei HM, Weiss HR. Effects of CAS 754, a new nitric oxide donor, on regional cerebral blood flow in focal cerebral ischemia. *Anesthesia Analgesia*. 1995;80:703-708.

193. Molloy J, Martin JF, Baskerville PA, Fraser SC, Markus HS. S-nitrosoglutathione reduces the rate of embolization in humans. *Circulation*. 1998;98:1372-5.

194. Kiris T, Karasu A, Yavuz C, Erdem T, Unal F, Hepgul K, Baloglu H. Reversal of cerebral vasospasm by the nitric oxide donor SNAP in an experimental model of subarachnoid haemorrhage. *Acta Neurochir (Wien)*. 1999;141:1323-8; discussion 1328-9.

195. Zhang R, Zhang L, Zhang Z, Wang Y, Lu M, Lapointe M, Chopp M. A nitric oxide donor induces neurogenesis and reduces functional deficits after stroke in rats. *Ann Neurol*. 2001;50:602-11.

196. Tashima K, Fujita A, Umeda M, Takeuchi K. Lack of gastric toxicity of nitric oxide-releasing aspirin, NCX-4016, in the stomach of diabetic rats. *Life Sci*. 2000;67:1639-52.

197. Lechi C, Andrioli G, Gaino S, Tommasoli R, Zuliani V, Ortolani R, Degan M, Benoni G, Bellavite P, Lechi A, Minuz P. The antiplatelet effects of a new nitroderivative of acetylsalicylic acid--an in vitro study of inhibition on the early phase of platelet activation and on TXA2 production. *Thromb Haemost*. 1996;76:791-8.

198. Lechi C, Gaino S, Tommasoli R, Zuliani V, Bonapace S, Fontana L, Degan M, Lechi A, Minuz P. In vitro study of the anti-aggregating activity of two nitroderivatives of acetylsalicylic acid. *Blood Coagul Fibrinolysis*. 1996;7:206-9.

199. Fredduzzi S, Mariucci G, Tantucci M, Del Soldato P, Ambrosini MV. Nitro-aspirin (NCX4016) reduces brain damage induced by focal cerebral ischemia in the rat. *Neurosci Lett*. 2001;302:121-4.

200. Haynes WG, Noon JP, Walker BR, Webb DJ. Inhibition of nitric oxide synthesis increases blood pressure in healthy humans. *J Hypertens*. 1993;11:1375-80.

201. Albert J, Schedin U, Lindqvist M, Melcher A, Hjemdahl P, Frostell C. Blockade of endogenous nitric oxide production results in moderate hypertension, reducing sympathetic activity and shortening bleeding time in healthy volunteers. *Acta Anaesthesiol Scand*. 1997;41:1104-13.

202. White RP, Deane C, Vallance P, Markus HS. Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia. *Stroke*. 1998;29:467-72.

203. Margaill I, Allix M, Boulu RG, Plotkine M. Dose- and time-dependence of L-NAME neuroprotection in transient focal cerebral ischaemia in rats. *Br J Pharmacol*. 1997;120:160-3.

204. Ashwal S, Cole DJ, Osborne TN, Pearce WJ. Dual effects of L-NAME during transient focal cerebral ischemia in spontaneously hypertensive rats. *Am J Physiol*. 1994;267:H276-84.

205. Ashwal S, Cole DJ, Osborne S, Osborne TN, Pearce WJ. L-NAME reduces infarct volume in a filament model of transient middle cerebral artery occlusion in the rat pup. *Pediatr Res*. 1995;38:652-6.

206. Ashwal S, Cole DJ, Osborne TN, Pearce WJ. Low dose L-NAME reduces infarct volume in the rat MCAO/reperfusion model. *J Neurosurg Anesthesiol*. 1993;5:241-9.

207. Iuliano BA, Anderson RE, Meyer FB. Effect of intermittent reperfusion and nitric oxide synthase inhibition on infarct volume during reversible focal cerebral ischemia. *J Neurosurg*. 1995;83:491-5.

208. Kuluz JW, Prado RJ, Dietrich WD, Schleien CL, Watson BD. The effect of nitric oxide synthase inhibition on infarct volume after reversible focal cerebral ischemia in conscious rats. *Stroke*. 1993;24:2023-9.

209. Stagliano NE, Zhao W, Prado R, Dewanjee MK, Ginsberg MD, Dietrich WD. The effect of nitric oxide synthase inhibition on acute platelet accumulation and hemodynamic depression in a rat model of thromboembolic stroke. *J Cereb Blood Flow Metab*. 1997;17:1182-90.

210. Kamii H, Mikawa S, Murakami K, Kinouchi H, Yoshimoto T, Reola L, Carlson E, Epstein CJ, Chan PH. Effects of nitric oxide synthase inhibition on brain infarction in SOD-1-transgenic mice following transient focal cerebral ischemia. *J Cereb Blood Flow Metab*. 1996;16:1153-7.

211. Sadoshima S, Nagao T, Okada Y, Fujii K, Ibayashi S, Fujishima M. L-arginine ameliorates recirculation and metabolic derangement in brain ischemia in hypertensive rats. *Brain Res*. 1997;744:246-52.

212. Carreau A, Duval D, Poignet H, Scatton B, Vige X, Nowicki JP. Neuroprotective efficacy of N omega-nitro-L-arginine after focal cerebral ischemia in the mouse and inhibition of cortical nitric oxide synthase. *Eur J Pharmacol*. 1994;256:241-9.

213. Nagafuji T, Matsui T, Koide T, Asano T. Blockade of nitric oxide formation by N omega-nitro-L-arginine mitigates ischemic brain edema and subsequent cerebral infarction in rats. *Neurosci Lett*. 1992;147:159-62.

214. Gursoy-Ozdemir Y, Bolay H, Saribas O, Dalkara T. Role of endothelial nitric oxide generation and peroxynitrite formation in reperfusion injury after focal cerebral ischemia. *Stroke*. 2000;31:1974-80; discussion 1981.

215. Spinnewyn B, Cornet S, Auguet M, Chabrier PE. Synergistic protective effects of antioxidant and nitric oxide synthase inhibitor in transient focal ischemia. *J Cereb Blood Flow Metab*. 1999;19:139-43.

216. Trifiletti RR. Neuroprotective effects of NG-nitro-L-arginine in focal stroke in the 7-day old rat. *Eur J Pharmacol*. 1992;218:197-8.

217. Skaper SD, Ancona B, Facci L, Franceschini D, Giusti P. Melatonin prevents the delayed death of hippocampal neurons induced by enhanced excitatory neurotransmission and the nitridergic pathway. *Faseb J*. 1998;12:725-31.

218. Zhang ZG, Reif D, Macdonald J, Tang WX, Kamp DK, Gentile RJ, Shakespeare WC, Murray RJ, Chopp M. ARL 17477, a potent and selective neuronal NOS inhibitor decreases infarct volume after transient middle cerebral artery occlusion in rats. *J Cereb Blood Flow Metab*. 1996;16:599-604.

219. Kidd GA, Dobrucki LW, Brovkovych V, Bohr DF, Malinski T. Nitric oxide deficiency contributes to large cerebral infarct size. *Hypertension*. 2000;35:1111-8.

220. Buchan AM, Gertler SZ, Huang ZG, Li H, Chaundy KE, Xue D. Failure to prevent selective CA1 neuronal death and reduce cortical infarction following cerebral ischemia with inhibition of nitric oxide synthase. *Neuroscience*. 1994;61:1-11.

221. Izuta M, Clavier N, Kirsch JR, Traystman RJ. Cerebral blood flow during inhibition of brain nitric oxide synthase activity in normal, hypertensive, and stroke-prone rats. *Stroke*. 1995;26:1079-85.

222. Iadecola C, Zhang F, Xu X. Inhibition of inducible nitric oxide synthase ameliorates cerebral ischemic damage. *American Journal of Physiology*. 1995;268:R286-R292.

223. Cockroft KM, Meistrell M, 3rd, Zimmerman GA, Risucci D, Bloom O, Cerami A, Tracey KJ. Cerebroprotective effects of aminoguanidine in a rodent model of stroke. *Stroke*. 1996;27:1393-8.

224. Nagayama M, Zhang F, Iadecola C. Delayed treatment with aminoguanidine decreases focal cerebral ischemic damage and enhances neurologic recovery in rats. *J Cereb Blood Flow Metab*. 1998;18:1107-13.

225. Parmentier S, Bohme GA, Lerouet D, Damour D, Stutzmann JM, Margaill I, Plotkine M. Selective inhibition of inducible nitric oxide synthase prevents ischaemic brain injury. *Br J Pharmacol*. 1999;127:546-52.

226. Goyagi T, Goto S, Bhardwaj A, Dawson VL, Hurn PD, Kirsch JR. Neuroprotective effect of sigma(1)-receptor ligand 4-phenyl-1-(4-phenylbutyl) piperidine (PPBP) is linked to reduced neuronal nitric oxide production. *Stroke*. 2001;32:1613-20.

227. Yoshida T, Limmroth V, Irikura K, Moskowitz MA. The NOS inhibitor, 7-nitroindazole, decreases focal infarct volume but not the response to topical acetylcholine in pial vessels. *J Cereb Blood Flow Metab*. 1994;14:924-9.

228. Kelly PAT, Ritchie IM, Arbuthnott GW. Inhibition of neuronal nitric oxide synthase by 7-nitroindazole: effects upon local cerebral blood flow and glucose use in the rat. *Journal Cerebral Blood Flow Metabolism*. 1995;15:766-773.

229. van Egmond J, Lenders JW, Weernink E, Thien T. Accuracy and reproducibility of 30 devices for self-measurement of arterial blood pressure. *Am J Hypertens*. 1993;6:873-9.

230. Borow KM, Newburger JW. Noninvasive estimation of central aortic pressure using the oscillometric method for analyzing systemic artery

pulsatile blood flow: comparative study of indirect systolic, diastolic, and mean brachial artery pressure with simultaneous direct ascending aortic pressure measurements. *Am Heart J*. 1982;103:879-86.

231. Foster C, McKinlay S, Cruickshank JM, Coats AJ. Accuracy of the Omron HEM 706 portable monitor for home measurement of blood pressure. *J Hum Hypertens*. 1994;8:661-4.

232. O'Brien E, Mee F, Atkins N, O'Malley K. Inaccuracy of seven popular sphygmomanometers for home measurement of blood pressure. *J Hypertens*. 1990;8:621-34.

233. *Association for the advancement of medical instrumentation, American national standard; Electronic or automated sphgmomanometers*. Arlington VA: Association for the advancement of medical instrumentation; 1993.

234. Cooper R, Puras A, Tracy J, Kaufman J, Asuzu M, Ordunez P, Mufunda J, Sparks H. Evaluation of an electronic blood pressure device for epidemiological studies. *Blood Press Monit*. 1997;2:35-40.

235. O'Brien E, Mee F, Atkins N, Thomas M. Evaluation of three devices for self-measurement of blood pressure according to the revised British Hypertension Society Protocol: the Omron HEM-705CP, Philips HP5332, and Nissei DS-175. *Blood Press Monit*. 1996;1:55-61.

236. *TNO finger pressure reference guide; A guide for the non-invasive measurement and recording systems Portapress and Finometer*. Amsterdam, the Netherlands: TNO Biomedial instrumentation; 2001.

237. Imholz BP, van Montfrans GA, Settels JJ, van der Hoeven GM, Karemaker JM, Wieling W. Continuous non-invasive blood pressure

monitoring: reliability of Finapres device during the Valsalva manoeuvre. *Cardiovasc Res*. 1988;22:390-7.

238. Parati G, Casadei R, Groppelli A, Di Rienzo M, Mancia G. Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. *Hypertension*. 1989;13:647-55.

239. Schmidt TF, Wittenhaus J, Steinmetz TF, Piccolo P, Lupsen H. Twenty-four-hour ambulatory noninvasive continuous finger blood pressure measurement with Portapres: a new tool in cardiovascular research. *Journal Cardiovascular Pharmacology*. 1992;19:S117-S145.

240. Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol*. 1993;74:2566-73.

241. Jellema WT, Wesseling KH, Groeneveld AB, Stoutenbeek CP, Thijs LG, van Lieshout JJ. Continuous cardiac output in septic shock by simulating a model of the aortic input impedance: a comparison with bolus injection thermodilution. *Anesthesiology*. 1999;90:1317-28.

242. Schreuder JJ, van der Veen FH, van der Velde ET, Delahaye F, Alfieri O, Jegaden O, Lorusso R, Jansen JR, van Ommen V, Finet G, et al. Beat-to-beat analysis of left ventricular pressure-volume relation and stroke volume by conductance catheter and aortic Modelflow in cardiomyoplasty patients. *Circulation*. 1995;91:2010-7.

243. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J*. 1993;14:160-7.

244. Chen CH, Nevo E, Fetics B, Pak PH, Yin FC, Maughan WL, Kass DA. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation*. 1997;95:1827-36.

245. Fetics B, Nevo E, Chen CH, Kass DA. Parametric model derivation of transfer function for noninvasive estimation of aortic pressure by radial tonometry. *IEEE Trans Biomed Eng*. 1999;46:698-706.

246. Davies JI, Struthers AD. Pulse wave analysis and pulse wave velocity: a critical review of their strengths and weaknesses. *J Hypertens*. 2003;21:463-72.

247. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol*. 2000;525 Pt 1:263-70.

248. Gatzka CD, Cameron JD, Dart AM, Berry KL, Kingwell BA, Dewar EM, Reid CM, Jennings GL. Correction of carotid augmentation index for heart rate in elderly essential hypertensives. ANBP2 Investigators. Australian Comparative Outcome Trial of Angiotensin-Converting Enzyme Inhibitor- and Diuretic-Based Treatment of Hypertension in the Elderly. *Am J Hypertens*. 2001;14:573-7.

249. Smulyan H, Marchais SJ, Pannier B, Guerin AP, Safar ME, London GM. Influence of body height on pulsatile arterial hemodynamic data. *J Am Coll Cardiol*. 1998;31:1103-9.

250. Wilkinson IB, MacCallum H, Rooijmans DF, Murray GD, Cockcroft JR, McKnight JA, Webb DJ. Increased augmentation index and systolic stress in type 1 diabetes mellitus. *Qjm*. 2000;93:441-8.

251. Wilkinson IB, Prasad K, Hall IR, Thomas A, MacCallum H, Webb DJ, Frenneaux MP, Cockcroft JR. Increased central pulse pressure and augmentation index in subjects with hypercholesterolemia. *J Am Coll Cardiol*. 2002;39:1005-11.

252. Cameron JD, McGrath BP, Dart AM. Use of radial artery applanation tonometry and a generalized transfer function to determine aortic pressure augmentation in subjects with treated hypertension. *J Am Coll Cardiol*. 1998;32:1214-20.

253. London GM, Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME. Arterial wave reflections and survival in end-stage renal failure. *Hypertension*. 2001;38:434-8.

254. Buckberg G, Eber L, Herman M, Gorlin R. Ischemia in aortic stenosis: hemodynamic prediction. *Am J Cardiol*. 1975;35:778-84.

255. Saito M, Kasuya A. [Relationship between the subendocardial viability ratio and risk factors for ischemic heart disease]. *Sangyo Eiseigaku Zasshi*. 2003;45:114-9.

256. Gunstensen J, Scully HE, Kelly T, Adelman AG, Williams WG, Wigle ED, Goldman BS. Prognostic significance of endocardial viability ratio in aortocoronary bypass surgery. *Can J Surg*. 1976;19:93-6.

257. Martin PJ, Evans DH, Naylor AR. Transcranial color-coded sonography of the basal cerebral circulation. Reference data from 115 volunteers. *Stroke*. 1994;25:390-6.

258. Demchuk AM, Christou I, Wein TH, Felberg RA, Malkoff M, Grotta JC, Alexandrov AV. Specific transcranial Doppler flow findings related to the presence and site of arterial occlusion. *Stroke*. 2000;31:140-6.

259. Rorick MB, Nichols FT, Adams RJ. Transcranial Doppler correlation with angiography in detection of intracranial stenosis. *Stroke*. 1994;25:1931-4.

260. Sorteberg W, Lindegaard KF, Rootwelt K, Dahl A, Russell D, Nyberg-Hansen R, Nornes H. Blood velocity and regional blood flow in defined cerebral artery systems. *Acta Neurochir (Wien)*. 1989;97:47-52.

261. Hartmann A, Ries F, Tsuda Y, Lagreze H, Seiler R, Grolimund P. Correlation of regional cerebral blood flow and blood flow velocity in normal volunteers and patients with cerebro-vascular disease. *Neurochirurgia (Stuttg)*. 1991;34:6-13.

262. Bishop CC, Powell S, Rutt D, Browse NL. Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. *Stroke*. 1986;17:913-5.

263. Dahl A, Lindegaard KF, Russell D, Nyberg-Hansen R, Rootwelt K, Sorteberg W, Nornes H. A comparison of transcranial Doppler and cerebral blood flow studies to assess cerebral vasoreactivity. *Stroke*. 1992;23:15-9.

264. Larsen FS, Olsen KS, Hansen BA, Paulson OB, Knudsen GM. Transcranial Doppler is valid for determination of the lower limit of cerebral blood flow autoregulation. *Stroke*. 1994;25:1985-8.

265. Jorgensen LG, Perko M, Hanel B, Schroeder TV, Secher NH. Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. *J Appl Physiol*. 1992;72:1123-32.

266. Brooks DJ, Redmond S, Mathias CJ, Bannister R, Symon L. The effect of orthostatic hypotension on cerebral blood flow and middle cerebral

artery velocity in autonomic failure, with observations on the action of ephedrine. *J Neurol Neurosurg Psychiatry*. 1989;52:962-6.

267. Winkler SS, Spira J. Radiopacity of xenon under hyperbaric conditions. *Am J Roentgenol Radium Ther Nucl Med*. 1966;96:1035-40.

268. Haughton V, Harrington G, Schmidt J. Xenon contrast enhacement for CT scanning in multiple sclerosis (abstract). In: *Proceedings of international symposium on computer assisted tomongraphy in non tumoural diseases of the brain, spinal cord and eye*. Bethseda; 1976:127.

269. Kety SS. The theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacol Rev*. 1951;3:1-41.

270. *XeCT System 2 User Manual; Revision C*. Houston, Texas: Diversified Diagnostic Products; 2001.

271. Yonas H, Pindzola RP, Johnson DW. Xenon/computed tomography cerebral blood flow and its use in clinical management. *Neurosurg Clin N Am*. 1996;7:605-16.

272. DeWitt DS, Fatouros PP, Wist AO, Stewart LM, Kontos HA, Hall JA, Kishore PR, Keenan RL, Marmarou A. Stable xenon versus radiolabeled microsphere cerebral blood flow measurements in baboons. *Stroke*. 1989;20:1716-23.

273. Fatouros PP, Wist AO, Kishore PR, DeWitt DS, Hall JA, Keenan RL, Stewart LM, Marmarou A, Choi SC, Kontos HA. Xenon/computed tomography cerebral blood flow measurements. Methods and accuracy. *Invest Radiol*. 1987;22:705-12.

274. Gur D, Yonas H, Jackson DL, Wolfson SK, Jr., Rockette H, Good WF, Cook EE, Arena VC, Willy JA, Maitz GS. Simultaneous measurements of cerebral blood flow by the xenon/CT method and the microsphere method. A comparison. *Invest Radiol*. 1985;20:672-7.

275. Wolfson SK, Jr., Clark J, Greenberg JH, Gur D, Yonas H, Brenner RP, Cook EE, Lordeon PA. Xenon-enhanced computed tomography compared with [14C]iodoantipyrine for normal and low cerebral blood flow states in baboons. *Stroke*. 1990;21:751-7.

276. Yonas H, Gur D, Claassen D, Wolfson SK, Jr., Moossy J. Stable xenon enhanced computed tomography in the study of clinical and pathologic correlates of focal ischemia in baboons. *Stroke*. 1988;19:228-38.

277. Yonas H, Gur D, Claassen D, Wolfson SK, Jr., Moossy J. Stable xenon-enhanced CT measurement of cerebral blood flow in reversible focal ischemia in baboons. *J Neurosurg*. 1990;73:266-73.

278. Haku T, Hosoya T, Komatani A, Watanabe N, Yamaguchi K. [Correlation of regional cerebral blood flow between Xe-CT and 133Xe-SPECT: validity of Xe-CT in evaluating rCBF]. *Nippon Igaku Hoshasen Gakkai Zasshi*. 1996;56:828-33.

279. Hellman RS, Collier BD, Tikofsky RS, Kilgore DP, Daniels DL, Haughton VM, Walsh PR, Cusick JF, Saxena VK, Palmer DW, et al. Comparison of single-photon emission computed tomography with [123I]iodoamphetamine and xenon-enhanced computed tomography for assessing regional cerebral blood flow. *J Cereb Blood Flow Metab*. 1986;6:747-55.

280. Machida K, Nakano H. [Comparison of cerebral blood flow with Xe-inhalation CT and perfusion CT]. *Nippon Hoshasen Gijutsu Gakkai Zasshi*. 2003;59:302-8.

281. Wintermark M, Thiran JP, Maeder P, Schnyder P, Meuli R. Simultaneous measurement of regional cerebral blood flow by perfusion CT and stable xenon CT: a validation study. *AJNR Am J Neuroradiol*. 2001;22:905-14.

282. Hagen T, Bartylla K, Piepgras U. Correlation of regional cerebral blood flow measured by stable xenon CT and perfusion MRI. *J Comput Assist Tomogr*. 1999;23:257-64.

283. Cullen SC, Gross EG. The anesthetic properties of xenon in animals and human beings, with additional observations on krypton. *Science*. 1951;113:580-2.

284. Lynch C, 3rd, Baum J, Tenbrinck R. Xenon anesthesia. *Anesthesiology*. 2000;92:865-8.

285. Latchaw RE, Yonas H, Pentheny SL, Gur D. Adverse reactions to xenon-enhanced CT cerebral blood flow determination. *Radiology*. 1987;163:251-4.

286. Plougmann J, Astrup J, Pedersen J, Gyldensted C. Effect of stable xenon inhalation on intracranial pressure during measurement of cerebral blood flow in head injury. *J Neurosurg*. 1994;81:822-8.

287. Siesjo BK, 1664. Pathophysiology and treatment of focal cerebral ischaemia Part 1: pathophysiology. *J Neurosurg*. 1992;77:169-184.

288. Baron J. Perfusion thresholds in human cerebral ischemia: historical perspective and therapeutic implications. *Cerebrovasc Dis*. 2004;11:2-8.

289. Hossmann K. Viability thresholds and the penumbra of focal ischemia. *Ann Neurol*. 1994;36:557-565.

290. Touho H, Karasawa J. Evaluation of time-dependent thresholds of cerebral blood flow and transit time during the acute stage of cerebral embolism: a restrospective study. *Surg Neurol*. 1996;46.

291. Archer S. Measurement of nitric oxide in biological models. *FASEB J.* 1993;7:349-360.

292. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A*. 1987;84:9265-9.

293. Tracey WR, Linden J, Peach MJ, Johns RA. Comparison of spectrophotometric and biological assays for nitric oxide (NO) and endothelium-derived relaxing factor (EDRF): nonspecificity of the diazotization reaction for NO and failure to detect EDRF. *J Pharmacol Exp Ther*. 1990;252:922-8.

294. Arroyo CM, Kohno M. Difficulties encountered in the detection of nitric oxide (NO) by spin trapping techniques. A cautionary note. *Free Radic Res Commun*. 1991;14:145-55.

295. Greenberg SS, Wilcox DE, Rubanyi GM. Endothelium-derived relaxing factor released from canine femoral artery by acetylcholine cannot be identified as free nitric oxide by electron paramagnetic resonance spectroscopy. *Circ Res*. 1990;67:1446-52.

296. Kelm M, Feelisch M, Spahr R, Piper HM, Noack E, Schrader J. Quantitative and kinetic characterization of nitric oxide and EDRF released from cultured endothelial cells. *Biochem Biophys Res Commun*. 1988;154:236-44.

297. Shibuki K. An electrochemical microprobe for detecting nitric oxide release in brain tissue. *Neurosci Res*. 1990;9:69-76.

298. Myers PR, Minor RL, Jr., Guerra R, Jr., Bates JN, Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature*. 1990;345:161-3.

299. *Nitric oxide analyser NOA 280 operation and maintenance manual*. Revision DLM 01401-02A ed. Colorado: Sievers Instruments, Inc; 1999.

300. Aoki T. Continuous flow determination of nitrite with membrane separation/chemiluminescence detection. *Biomed Chromatogr*. 1990;4:128-30.

301. Brien JF, McLaughlin BE, Nakatsu K, Marks GS. Quantitation of nitric oxide formation from nitrovasodilator drugs by chemiluminescence analysis of headspace gas. *J Pharmacol Methods*. 1991;25:19-27.

302. Archer SL, Cowan NJ. Measurement of endothelial cytosolic calcium concentration and nitric oxide production reveals discrete mechanisms of endothelium-dependent pulmonary vasodilatation. *Circ Res*. 1991;68:1569-81.

303. Dickersin K, Scherer R, Lefebvre C. Identifying relevant studies for systematic reviews. *Bmj*. 1994;309:1286-91.

304. Edwards P, Clarke M, DiGuiseppi C, Pratap S, Roberts I, Wentz R. Identification of randomized controlled trials in systematic reviews: accuracy and reliability of screening records. *Stat Med*. 2002;21:1635-40.

305. Alderson P, Green S, Higgins J. *Cochrane Reviewers' Handbook 4.2.1 [updated December 2003]*. Chichester, UK: John Wiley & Sons, Ltd.; 2004.

306. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *Journal of National Cancer Institiute*. 1959;22:719-748.

307. Yusuf S, Peto R, Lewis J, Collins R, Sleight P. Beta blockade during and after myocardial infarction: an overview of the randomized trials. *Progress in Cardiovascular Diseases*. 1985;27:335-371.

308. Kirkwood B, Sterne J. *Essential Medical Statistics*. 2nd ed. Massachusetts, USA: Blackwell Science Ltd; 2003.

309. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Controlled Clinical Trials*. 1986;7:177-188.

310. Horn J, deHaan R, Vermeulen M, Luiten PGM, Limburg M, 2045. Nimodipine in animal model experiments of focal cerebral ischemia. *Stroke*. 2001;32:2433-2438.

311. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315:629-634.

312. Chamorro A, Vila N, Ascaso C, Elices E, Schonewille W, Blanc R. Blood pressure and functional recovery in acute ischemic stroke. *Stroke*. 1998;29:1850-1853.

313. Yatsu FM, Zivin J. Hypertension in acute ischaemic strokes - not to treat. *Arch.Neurol.* 1985;42:999-1000.

314. Spence JD, Del Maestro RF. Hypertension in acute ischaemic strokes - Treat. *Arch.Neurol.* 1985;42:1000-1002.

315. Carlberg B, Asplund K, Hagg E. The prognostic value of admission blood-pressure in patients with acute stroke. *Stroke*. 1993;24:1372-1375.

316. Marquarsden J. The natural history of acute cerebrovascular disease: A retrospective study of 769 patients. *Acta.Neurol.Scand.* 1969;45:118-124.

317. Marshall J, Shaw DA. The natural history of cerebrovascular disease. *British Medical Journal*. 1959;i:1614-1617.

318. Portenoy RK, Lipton RB, Berger AR, Lesser ML, Lantos G. Intracerebral haemorrhage: a model for the prediction of outcome. *J Neurol Neurosurg Psychiatry*. 1987;50:976-9.

319. Armario P, Ceresuela LM, Bello J, Martin-Baranera M, Hernandez del Rey R, Avila A. The role of blood pressure in the prognosis of acute ischaemic stroke (abstract),. *Journal of Hypertension*. 2001;19 (suppl 2):S13.

320. Bhalla A, Wolfe CD, Rudd AG. The effect of 24 h blood pressure levels on early neurological recovery after stroke. *J Intern Med*. 2001;250:121-30.

321. Lopez-Gonzalez FJ, Aldrey JM, Pardellas H, Castillo J. [Morbidity due to intracerebral hemorrhage]. *Rev Neurol*. 1998;27:755-8.

322. Jorgensen H, Nakayama H, Raaschou H, Olsen T. Effect of blood pressure and diabetes on stroke in progression. *Lancet*. 1994;344:156-159.

323. Toni D, Fiorelli M, Gentile M, Bastianello S, Sacchetti ML, Argentino C, Pozzilli C, Fieschi C. Progressing neurological deficit secondary to acute ischemic stroke. A study on predictability, pathogenesis, and prognosis. *Arch Neurol*. 1995;52:670-5.

324. Bowes MP, Zivin JA, Thomas GR, Thibodeaux H, Fagan SC. Acute hypertension, but not thrombolysis, increases the incidence and severity of hemorrhagic transformation following experimental stroke in rabbits. *Exp Neurol*. 1996;141:40-6.

325. Saku Y, Choki J, Waki R, Masuda J, Tamaki K, Fujishima M, Ogata J. Hemorrhagic infarct induced by arterial hypertension in cat brain following middle cerebral artery occlusion. *Stroke*. 1990;21:589-95.

326. Fagan SC, Bowes MP, Lyden PD, Zivin JA. Acute hypertension promotes hemorrhagic transformation in a rabbit embolic stroke model: effect of labetalol. *Exp Neurol*. 1998;150:153-8.

327. Fujii Y, Tanaka R, Takeuchi S, Koike T, Minakawa T, Sasaki O. Hematoma enlargement in spontaneous intracerebral hemorrhage. *J Neurosurg*. 1994;80:51-57.

328. Brott T, Broderick J, Kothari R, Barsan W, Tomsick T, Sauerbeck L, Spilker J, Duldner J, Khoury J. Early hemorrhage growth in patients with intracerebral hemorrhage. *Stroke*. 1997;28:1-5.

329. Fogelhol R, Aho K. Characteristics and survival of patients with brain stem infarction. *Stroke*. 1975;6:328-33.

330. Fogelholm R, Avikainen S, Murros K. Prognostic value and determinants of first-day mean arterial pressure in spontaneous supratentorial intracerebral hemorrhage. *Stroke*. 1997;28:1396-1400.

331. Fullerton KJ, Mackenzie G, Stout RW. Prognostic indices in stroke. *Quarterly Journal of Medicine*. 1988;New series 66:147-162.

332. Latorre PP, Jr., Boyle RW. Prognosis for survival after a stroke. *Geriatrics*. 1968;23:106-11.

333. Mbala-Mukendi M, Tambwe MJ, Dikassa LN, M'Buyamba-Kabangu JR. [Initial arterial pressure and prognosis of cerebrovascular accidents]. *Arch Mal Coeur Vaiss*. 1995;88:21-5.

334. Tennent EC, Harman JW. A study of factors affecting the prognosis of cerebrovascular accident. *The American Journal of the Medical Sciences*. 1949;October:361-68.

335. Terayama Y, Tanahashi N, Fukuuchi Y, Gotoh F. Prognostic value of admission blood pressure in patients with intracerebral hemorrhage. Keio Cooperative Stroke Study. *Stroke*. 1997;28:1185-1188.

336. Iadecola C, Pelligrino DA, Moskowitz MA, Lassen NA. Nitric oxide synthase inhibition and cerebrovascular regulation. *J Cereb Blood Flow Metab*. 1994;14:175-92.

337. Zhang F, Iadecola C. Reduction of focal cerebral ischemic damage by delayed treatment with nitric oxide donors. *Journal of Cerebral Blood Flow and Metabolism*. 1994;14:574-580.

338. Mason RB, Pluta RM, Walbridge S, Wink DA, Oldfield EH, Boock RJ. Production of reactive oxygen species after reperfusion in vitro and in vivo: protective effect of nitric oxide. *J Neurosurg*. 2000;93:99-107.

339. Pluta RM, Rak R, Wink DA, Woodward JJ, Khaldi A, Oldfield EH, Watson JC. Effects of nitric oxide on reactive oxygen species production and infarction size after brain reperfusion injury. *Neurosurgery*. 2001;48:884-92; discussion 892-3.

340. Wink DA, Cook JA, Pacelli R, Liebmann J, Krishna MC, Mitchell JB. Nitric oxide (NO) protects against cellular damage by reactive oxygen species. *Toxicol Lett*. 1995;82-83:221-6.

341. Lipton SA, Rayudu PV, Choi YB, Sucher NJ, Chen HS. Redox modulation of the NMDA receptor by NO-related species. *Prog Brain Res*. 1998;118:73-82.

342. He Z, Ibayashi S, Nagao T, Fujii K, Sadoshima S, Fujishima M. L-arginine ameliorates cerebral blood flow and metabolism and decreases infarct volume in rats with cerebral ischemia. *Brain Res*. 1995;699:208-13.

343. Willmot MR, Bath PMW. The potential of nitric oxide therapeutics in stroke. *Expert Opinion Investigational Drugs*. 2003;12:455-470.

344. Stroke Therapy Academic Industry Roundtable (STAIR). Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke*. 1999;30:2752-2758.

345. Richard Green A, Odergren T, Ashwood T. Animal models of stroke: do they have value for discovering neuroprotective agents? *Trends Pharmacol Sci*. 2003;24:402-8.

346. Bednar MM, Gross CE, Howard DB, Russell SR, Thomas GR. Nitric oxide reverses aspirin antagonism of t-PA thrombolysis in a rabbit model of thromboembolic stroke. *Exp Neurol*. 1997;146:513-7.

347. Morikawa E, Moskowitz MA, Huang Z, Yoshida T, Irikura K, Dalkara T. L-arginine infusion promotes nitric oxide-dependent vasodilation, increases regional cerebral blood flow, and reduces infarction volume in the rat. *Stroke*. 1994;25:429-35.

348. Dalkara T, Morikawa E, Panahian N, Moskowitz MA. Blood flow-dependent functional recovery in a rat model of focal cerebral ischemia. *American Journal of Physiology*. 1994;267:H678-H683.

349. Humphreys SA, Koss MC. Role of nitric oxide in post-ischemic cerebral hyperemia in anesthetized rats. *Eur J Pharmacol*. 1998;347:223-9.

350. Morikawa E, Rosenblatt S, Moskowitz MA. L-arginine dilates rat pial arterioles by nitric oxide-dependent mechanisms and increases blood flow during focal cerebral ischaemia. *Br J Pharmacol*. 1992;107:905-7.

351. Zhao H, Asai S, Ishikawa K. Neither L-NAME nor L-arginine changes extracellular glutamate elevation and anoxic depolarization during global ischemia and reperfusion in rat. *Neuroreport*. 1999;10:313-8.

352. Prado R, Watson BD, Zhao W, Yao H, Busto R, Dietrich WD, Ginsberg MD. L-arginine does not improve cortical perfusion or histopathological outcome in spontaneously hypertensive rats subjected to distal middle cerebral artery photothrombotic occlusion. *J Cereb Blood Flow Metab*. 1996;16:612-22.

353. Coert BA, Anderson RE, Meyer FB. Effects of the nitric oxide donor 3-morpholinosydnonimine (SIN-1) in focal cerebral ischemia dependent on intracellular brain pH. *J Neurosurg*. 2002;97:914-21.

354. Zhang F, Casey RM, Ross ME, Iadecola C. Aminoguanidine ameliorates and L-arginine worsens brain damage from intraluminal middle cerebral artery occlusion. *Stroke*. 1996;27:317-23.

355. Buisson A, Margaill I, Callebert J, Plotkine M, Boulu RG. Mechanisms involved in the neuroprotective activity of a nitric oxide synthase inhibitor during focal cerebral ischemia. *J Neurochem*. 1993;61:690-6.

356. Barbul A. *Physiology and pharmacology of arginine*. New York: Elsevier Science Publishing Co; 1990.

357. Morgan DM. Polyamines, arginine and nitric oxide. *Biochem Soc Trans*. 1994;22:879-83.

358. Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ. Agmatine: an endogenous clonidine-displacing substance in the brain. *Science*. 1994;263:966-9.

359. Ginsberg MD, Busto R. Rodent models of cerebral ischemia. *Stroke*. 1989;20:1627-42.

360. Zhang F, Iadecola C. Nitroprusside improves blood flow and reduces brain damage after focal ischaemia. *Clinical Neuroscience and Neuropathology*. 1993;4:559-562.

361. Hillis AE, Ulatowski JA, Barker PB, Torbey M, Ziai W, Beauchamp NJ, Oh S, Wityk RJ. A pilot randomised trial of induced blood pressure elevation: Effects on function and focal perfusion in acute and subacute stroke. *Cerebrovasc Dis*. 2003;16:236-246.

362. Rashid P, Weaver C, Leonardi-Bee JA, Fletcher S, Bath FJ, Bath PMW. The effects of transdermal glyceryl trinitrate, a nitric oxide donor on blood pressure, cerebral and cardiac haemodynamics and plasma

nitric oxide levels in acute stroke. *J.Stroke Cerebrovasc.Dis.* 2003;13:143-151.

363. Bath PM. Major ongoing stroke trials. Efficacy of Nitric Oxide in Stroke (ENOS) trial. *Stroke*. 2001;32:2450-2451 (abstract).

364. Morikawa E, Huang Z, Moskowitz MA. L-arginine decreases infarct size caused by middle cerebral arterial occlusion in SHR. *Am J Physiol*. 1992;263:H1632-5.

365. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A*. 1990;87:1620-4.

366. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys*. 1991;288:481-7.

367. Garthwaite J. Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci*. 1991;14:60-7.

368. Dawson TM, Dawson VL, Snyder SH. A novel neuronal messenger molecule in brain: the free radical, nitric oxide. *Ann.Neurol.* 1992;32:297-311.

369. Nathan C. Nitric oxide as a secretory product of mammalian cells. *Faseb J*. 1992;6:3051-64.

370. Dimmeler S, Brune B. Nitric oxide preferentially stimulates auto-ADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase compared to alcohol or lactate dehydrogenase. *FEBS Lett*. 1993;315:21-4.

371. Sorkin LS. NMDA evokes an L-NAME sensitive spinal release of glutamate and citrulline. *Neuroreport*. 1993;4:479-82.

372. Montague PR, Gancayco CD, Winn MJ, Marchase RB, Friedlander MJ. Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. *Science*. 1994;263:973-7.

373. Nagafuji T, Sugiyama M, Matsui T, Muto A, Naito S. Nitric oxide synthase in cerebral ischemia. Possible contribution of nitric oxide synthase activation in brain microvessels to cerebral ischemic injury. *Mol Chem Neuropathol*. 1995;26:107-57.

374. Nakashima M, Niwa M, Iwai T, Uematsu T. Involvement of free radicals in cerebral vascular reperfusion injury evaluated in a transient focal cerebral ischemia model of rat. *Free Radic Biol Med*. 1999;26:722-9.

375. Yamamoto S, Golanov EV, Berger SB, Reis DJ. Inhibition of nitric oxide synthesis increases focal ischemic infarction in rat. *J Cereb Blood Flow Metab*. 1992;12:717-26.

376. Batteur-Parmentier S, Margaill I, Plotkine M. Modulation by nitric oxide of cerebral neutrophil accumulation after transient focal ischemia in rats. *J Cereb Blood Flow Metab*. 2000;20:812-9.

377. Xu SJ, Zhang JX, Li Lf, Du XL. The effects of nitric oxide synthase inhibitors on the early stage of focal cerebral ischaemic injury in rats. *Chinese Pharm Bull*. 2000;16:645-7.

378. Dawson DA, Graham DI, McCulloch J, Macrae IM. Anti-ischaemic efficacy of a nitric oxide synthase inhibitor and a N-methyl-D-aspartate receptor antagonist in models of transient and permanent focal cerebral ischaemia. *Br J Pharmacol*. 1994;113:247-53.

379. Anderson RE, Meyer FB. Is intracellular brain pH a dependent factor in NOS inhibition during focal cerebral ischemia? *Brain Res*. 2000;856:220-6.

380. Anderson RE, Meyer FB. Nitric oxide synthase inhibition by L-NAME during repetitive focal cerebral ischemia in rabbits. *Am J Physiol*. 1996;271:H588-94.

381. Clavier N, Kirsch JR, Hurn PD, Traystman RJ. Cerebral blood flow is reduced by N omega-nitro-L-arginine methyl ester during delayed hypoperfusion in cats. *Am J Physiol*. 1994;267:H174-81.

382. Nishikawa T, Kirsch JR, Koehler RC, Bredt DS, Snyder SH, Traystman RJ. Effect of nitric oxide synthase inhibition on cerebral blood flow and injury volume during focal ischemia in cats. *Stroke*. 1993;24:1717-24.

383. Nishikawa T, Kirsch JR, Koehler RC, Miyabe M, Traystman RJ. Nitric oxide synthase inhibition reduces caudate injury following transient focal ischemia in cats. *Stroke*. 1994;25:877-85.

384. Nowicki JP, Duval D, Poignet H, Scatton B. Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. *Eur J Pharmacol*. 1991;204:339-40.

385. Sugimoto K, Iadecola C. Effects of aminoguanidine on cerebral ischemia in mice: comparison between mice with and without inducible nitric oxide synthase gene. *Neurosci Lett*. 2002;331:25-8.

386. Ding-Zhou L, Marchand-Verrecchia C, Croci N, Plotkine M, Margaill I. L-NAME reduces infarction, neurological deficit and blood-brain barrier disruption following cerebral ischemia in mice. *Eur J Pharmacol*. 2002;457:137-46.

387. Zhu DY, Deng Q, Yao HH, Wang DC, Deng Y, Liu GQ. Inducible nitric oxide synthase expression in the ischemic core and penumbra after transient focal cerebral ischemia in mice. *Life Sci*. 2002;71:1985-96.

388. O'Neill MJ, Murray TK, McCarty DR, Hicks CA, Dell CP, Patrick KE, Ward MA, Osborne DJ, Wiernicki TR, Roman CR, Lodge D, Fleisch JH, Singh J. ARL 17477, a selective nitric oxide synthase inhibitor, with neuroprotective effects in animal models of global and focal cerebral ischaemia. *Brain Res*. 2000;871:234-44.

389. Spatz M, Stanimirovic D, Strasser A, McCarron RM. Nitro-L-arginine augments the endothelin-1 content of cerebrospinal fluid induced by cerebral ischemia. *Brain Res*. 1995;684:99-102.

390. Hiramatsu T, Jonas RA, Miura T, duPlessis A, Tanji M, Forbess JM, Holtzman D. Cerebral metabolic recovery from deep hypothermic circulatory arrest after treatment with arginine and nitro-arginine methyl ester. *J Thorac Cardiovasc Surg*. 1996;112:698-707.

391. Schleien CL, Kuluz JW, Gelman B. Hemodynamic effects of nitric oxide synthase inhibition before and after cardiac arrest in infant piglets. *Am J Physiol*. 1998;274:H1378-85.

392. Greenberg RS, Helfaer MA, Kirsch JR, Traystman RJ. Effect of nitric oxide synthase inhibition on postischemic cerebral hyperemia. *Am J Physiol*. 1995;269:H341-7.

393. Segawa D, Hatori N, Yoshizu H, Uriuda Y, Shimizu M, Tanaka S. The effect of nitric oxide synthase inhibitor on reperfusion injury of the brain under hypothermic circulatory arrest. *J Thorac Cardiovasc Surg*. 1998;115:925-30.

394. Dorrepaal CA, Shadid M, Steendijk P, Van der Velde ET, Van de Bor M, Baan J, Van Bel F. Effect of post-hypoxic-ischemic inhibition of nitric oxide synthesis on cerebral blood flow, metabolism and electrocortical brain activity in newborn lambs. *Biol Neonate*. 1997;72:216-26.

395. Quast MJ, Wei J, Huang NC. Nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester decreases ischemic damage in reversible focal cerebral ischemia in hyperglycemic rats. *Brain Res*. 1995;677:204-12.

396. Wei J, Quast MJ. Effect of nitric oxide synthase inhibitor on a hyperglycemic rat model of reversible focal ischemia: detection of excitatory amino acids release and hydroxyl radical formation. *Brain Res*. 1998;791:146-56.

397. Cash D, Beech JS, Rayne RC, Bath PMW, Meldrum BS, Williams SCR. Neuroprotective effect of aminoguanidine on transient focal ischaemia in the rat brain. *Brain Research*. 2001;905:91-103.

398. Santizo R, Baughman VL, Pelligrino DA. Relative contributions from neuronal and endothelial nitric oxide synthases to regional cerebral blood flow changes during forebrain ischemia in rats. *Neuroreport*. 2000;11:1549-53.

399. Jiang MH, Kaku T, Hada J, Hayashi Y. 7-Nitroindazole reduces nitric oxide concentration in rat hippocampus after transient forebrain ischemia. *Eur J Pharmacol*. 1999;380:117-21.

400. Sugimura T, Sako K, Tohyama Y, Yonemasu Y. Consecutive in vivo measurement of nitric oxide in transient forebrain ischemic rat under normothermia and hypothermia. *Brain Res*. 1998;808:313-6.

401. Sakashita N, Ando Y, Yonehara T, Tanaka Y, Sato K, Uchino M, Ando M. Role of superoxide dismutase and nitric oxide on the interaction between brain and systemic circulation during brain ischemia. *Biochim Biophys Acta*. 1994;1227:67-73.

402. Hashimoto H, Ohta S, Utsunomiya H, Kumon Y, Sakaki S, Matsuda S, Sakanaka M. Changes in guanylate cyclase activity in arteriolar smooth muscle cells and hemodynamics after ischemia-reperfusion in rats. *Acta Neuropathol (Berl)*. 1999;98:603-13.

403. Iadecola C, Zhang F, Casey R, Clark HB, Ross ME. Inducible nitric oxide synthase gene expression in vascular cells after transient focal cerebral ischemia. *Stroke*. 1996;27:1373-80.

404. Prado R, Watson BD, Wester P. Effects of nitric oxide synthase inhibition on cerebral blood flow following bilateral carotid artery occlusion and recirculation in the rat. *J Cereb Blood Flow Metab*. 1993;13:720-3.

405. Wei HM, Chi OZ, Liu X, Sinha AK, Weiss HR. Nitric oxide synthase inhibition alters cerebral blood flow and oxygen balance in focal cerebral ischemia in rats. *Stroke*. 1994;25:445-9; discussion 449-50.

406. Uetsuka S, Fujisawa H, Yasuda H, Shima H, Suzuki M. Severe cerebral blood flow reduction inhibits nitric oxide synthesis. *J Neurotrauma*. 2002;19:1105-16.

407. Matsui T, Nagafuji T, Mori T, Asano T. N omega-nitro-L-arginine attenuates early ischemic neuronal damage of prolonged focal cerebral ischemia and recirculation in rats. *Neurol Res*. 1997;19:192-203.

408. Hamada J, Greenberg JH, Croul S, Dawson TM, Reivich M. Effects of central inhibition of nitric oxide synthase on focal cerebral ischemia in rats. *J Cereb Blood Flow Metab*. 1995;15:779-86.

409. Caldwell M, O'Neill M, Earley B, Kelly JP, Leonard BE. NG-nitro-L-arginine methyl ester protects against lipid peroxidation in the gerbil following cerebral ischaemia. *Eur J Pharmacol*. 1995;285:203-6.

410. Gumuslu S, Serteser M, Ozben T, Balkan S, Balkan E. Inhibitory role of N omega-nitro-L-arginine methyl ester (L-NAME), a potent nitric oxide synthase inhibitor, on brain malondialdehyde and conjugated diene levels during focal cerebral ischemia in rats. *Clin Chim Acta*. 1997;267:213-23.

411. Seif-el-Nasr M, Fahim AT. Antioxidant effect of N omega-nitro-L-arginine methyl ester (L-NAME) on global cerebral ischemia in a rat model. *Arzneimittelforschung*. 2001;51:628-32.

412. Solenski NJ, Kwan A. Attenuation of free radical generation during reversible focal cerebral ischemia with the nitric oxide inhibitor, L-NAME (L-N(G)-nitro-L-arginine methyl ester). *Brain Res*. 2000;862:262-5.

413. Peeters-Scholte C, Koster J, Veldhuis W, van den Tweel E, Zhu C, Kops N, Blomgren K, Bar D, van Buul-Offers S, Hagberg H, Nicolay K, van Bel F, Groenendaal F. Neuroprotection by selective nitric oxide synthase inhibition at 24 hours after perinatal hypoxia-ischemia. *Stroke*. 2002;33:2304-10.

414. Charriaut-Marlangue C, Margaill I, Borrega F, Plotkine M, Ben-Ari Y. NG-nitro-L-arginine methyl ester reduces necrotic but not apoptotic cell

death induced by reversible focal ischemia in rat. *Eur J Pharmacol*. 1996;310:137-40.

415. O'Neill MJ, Hicks C, Ward M. Neuroprotective effects of 7-nitroindazole in the gerbil model of global cerebral ischaemia. *Eur J Pharmacol*. 1996;310:115-22.

416. O'Neill MJ, Hicks C, Ward M, Panetta JA. Neuroprotective effects of the antioxidant LY231617 and NO synthase inhibitors in global cerebral ischaemia. *Brain Res*. 1997;760:170-8.

417. Caldwell M, O'Neill M, Earley B, Leonard B. NG-Nitro-L-arginine protects against ischaemia-induced increases in nitric oxide and hippocampal neuro-degeneration in the gerbil. *Eur J Pharmacol*. 1994;260:191-200.

418. Kohno K, Ohta S, Furuta S, Kumon Y, Sakaki S. Intraventricular administration of nitric oxide synthase inhibitors prevents delayed neuronal death in gerbil hippocampal CA1 neurons. *Neurosci Lett*. 1995;199:65-8.

419. Jones PA, Smith RA, Stone TW. Nitric oxide synthase inhibitors L-NAME and 7-nitroindazole protect rat hippocampus against kainate-induced excitotoxicity. *Neurosci Lett*. 1998;249:75-8.

420. Nanri K, Montecot C, Springhetti V, Seylaz J, Pinard E. The selective inhibitor of neuronal nitric oxide synthase, 7-nitroindazole, reduces the delayed neuronal damage due to forebrain ischemia in rats. *Stroke*. 1998;29:1248-53; discussion 1253-4.

421. Loihl AK, Asensio V, Campbell IL, Murphy S. Expression of nitric oxide synthase (NOS)-2 following permanent focal ischemia and the role of

nitric oxide in infarct generation in male, female and NOS-2 gene-deficient mice. *Brain Res*. 1999;830:155-64.

422. Horn J, de Haan RJ, Vermeulen M, Luiten PGM, Limburg M. Systematic review of nimodipine in animal experiments. *Cerebrovascular Diseases*. 2001;11 (suppl 4):110.

423. Pound P, Ebrahim S, Sandercock P, Bracken MB, Roberts I. Where is the evidence that animal research benefits humans? *Bmj*. 2004;328:514-7.

424. Roberts I. Animal research. Three Rs should be registration randomisation, and reviews (systematic). *Bmj*. 2001;322:1604.

425. Roberts I, Kwan I, Evans P, Haig S. Does animal experimentation inform human healthcare? Observations from a systematic review of international animal experiments on fluid resuscitation. *Bmj*. 2002;324:474-6.

426. Buisson A, Plotkine M, Boulu RG. The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischaemia. *Br J Pharmacol*. 1992;106:766-7.

427. Dawson DA, Kusumoto K, Graham DI, McCulloch J, Macrae IM. Inhibition of nitric oxide synthesis does not reduce infarct volume in a rat model of focal cerebral ischaemia. *Neurosci Lett*. 1992;142:151-4.

428. Puisieux F, Deplanque D, Pu Q, Souil E, Bastide M, Bordet R. Differential role of nitric oxide pathway and heat shock protein in preconditioning and lipopolysaccharide-induced brain ischemic tolerance. *Eur J Pharmacol*. 2000;389:71-8.

429. Sercombe R, Issertial O, Seylaz J, Pinard E. Effects of chronic L-NAME treatment on rat focal cerebral ischemia and cerebral vasoreactivity. *Life Sci*. 2001;69:2203-16.

430. Zhang F, Xu S, Iadecola C. Time dependence of effect of nitric oxide synthase inhibition on cerebral ischaemic damage. *J Cereb Blood Flow Metab*. 1995;15:595-01.

431. Harukuni I, Traystman RJ, Kirsch JR. Effect of AR-R 17477, a potent neuronal nitric oxide synthase inhibitor, on infarction volume resulting from permanent focal ischemia in rats. *Crit Care Med*. 1999;27:2508-11.

432. Han HS, Qiao Y, Karabiyikoglu M, Giffard RG, Yenari MA. Influence of mild hypothermia on inducible nitric oxide synthase expression and reactive nitrogen production in experimental stroke and inflammation. *J Neurosci*. 2002;22:3921-8.

433. Tsuji M, Higuchi Y, Shiraishi K, Kume T, Akaike A, Hattori H. Protective effect of aminoguanidine on hypoxic-ischemic brain damage and temporal profile of brain nitric oxide in neonatal rat. *Pediatr Res*. 2000;47:79-83.

434. Zhang F, Iadecola C. Temporal characteristics of the protective effect of aminoguanidine on cerebral ischemic damage. *Brain Res*. 1998;802:104-10.

435. Weyland A, Grune F, Buhre W, Kazmaier S, Stephan H, Sonntag H. [The effect of nitroglycerin on cerebrovascular circulation, cerebrovascular CO2-reactivity and blood flow rate in basal cerebral arteries]. *Anaesthesist*. 1996;45:1037-44.

436. Menon N, Wolf A, Zehetgruber M, Bing R. An improved chemiluminescence assay suggests non nitric oxide mediated action of lysophoshatidylcholine and acetylcholine. *Proceedings Society Experimental Medicine Biology*. 1989;191:316-319.

437. Lavin P. Management of hypertension in patients with acute stroke. *Archives of Internal Medicine*. 1986;146:66-68.

438. Bashir A, Lewis MJ, Henderson AH. Pharmacokinetic studies of various preparations of glyceryl trinitrate. *Br J Clin Pharmacol*. 1982;14:779-84.

439. Green LC, Deluzuriaga KR, Wagner DA, Rand W, Istfan N, Young VR, Tannenbaum SR. Nitrate Biosynthesis in Man. *Proceedings National Academy Sciences USA - Biological Sciences*. 1981;78:7764-7768.

440. Greig LD, Leslie SJ, Gibb FW, Tan S, Newby DE, Webb DJ. Comparative effects of glyceryl trinitrate and amyl nitrite on pulse wave reflection and augmentation index. *Br J Clin Pharmacol*. 2005;59:265-70.

441. Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. *Hypertension*. 2001;37:1429-1433.

442. Mason DT. Afterload reduction and cardiac performance. Physiologic basis of systemic vasodilators as a new approach in treatment of congestive heart failure. *Am J Med*. 1978;65:106-25.

443. Miller RR, Vismara LA, Williams DO, Amsterdam EA, Mason DT. Pharmacological mechanisms for left ventricular unloading in clinical congestive heart failure. Differential effects of nitroprusside,

phentolamine, and nitroglycerin on cardiac function and peripheral circulation. *Circ Res*. 1976;39:127-33.

444. Christensson B, Nordenfelt I, Westling H, White T. Intravenous infusion of nitroglycerin in normal subjects. *Scand J Clin Lab Invest*. 1969;23:49-53.

445. Kawakami H, Sumimoto T, Hamada M, Mukai M, Shigematsu Y, Matsuoka H, Abe M, Hiwada K. Acute effect of glyceryl trinitrate on systolic blood pressure and other hemodynamic variables. *Angiology*. 1995;46:151-6.

446. Williams JF, Jr., Glick G, Braunwald E. Studies on cardiac dimensions in intact unanesthetized man. V. Effects of nitroglycerin. *Circulation*. 1965;32:767-71.

447. Dirnberger E, Lucan H, Eichler HG, Kastner J, Pernerstorfer T, Jilma B. Effects of nitroglycerin and sodium nitroprusside on endexpiratory concentrations of nitric oxide in healthy humans. *Life Sci*. 1998;62:PL103-8.

448. Robin E, Cowan C, Puri P, Ganguly S, DeBoyrie E, Martinez M, Stock T, Bing RJ. A comparative study of nitroglycerin and propranolol. *Circulation*. 1967;36:175-86.

449. Loscalzo J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. *Circ Res*. 2001;88:756-62.

450. Hogan JC, Lewis MJ, Henderson AH. Glyceryl trinitrate and platelet aggregation: effects of N-acetyl-cysteine. *British Journal of Clinical Pharmacology*. 1989;27:617-619.

451. Booth BP, Jacob S, Bauer JA, Fung H-L. Sustained antiplatelet properties of nitroglycerin during hemodynamic tolerance in rats. *Journal of cardiovascular pharmacology*. 1996;28:432-438.

452. Langford EJ, Wainwright RJ, Martin JF. Platelet activation in acute myocardial infarction and unstable angina is inhibited by nitric oxide donors. *Arterioscler Thromb Vasc Biol*. 1996;16:51-5.

453. Bishop C, Powell S, Rutt D, Browse N. Transcranial doppler measurement of middle cerebral artery blood flow velocity. *Stroke*. 1986;17, No 5:913-915.

454. White RP, Deane C, Hindley C, Bloomfield PM, Cunningham VJ, Vallance P, Brooks DJ, Markus HS. The effect of the nitric oxide donor glyceryl trinitrate on global and regional cerebral blood flow in man. *J Neurol Sci*. 2000;178:23-8.

455. Micieli G, Bosone D, Costa A, Cavallini A, Marcheselli S, Pompeo F, Nappi G. Opposite effects of L-arginine and nitroglycerin on cerebral blood velocity: nitric oxide precursors and cerebral blood velocity. *Journal Neurological Sciences*. 1997;150:71-75.

456. Chien YW. Pharmaceutical considerations of transdermal nitroglycerin delivery: the various approaches. *Am Heart J*. 1984;108:207-16.

457. Willmot M, Leonardi-Bee J, Bath PM. High blood pressure in acute stroke and subsequent outcome: a systematic review. *Hypertension*. 2004;43:18-24.

458. Lisk DR, Grotta JC, Lamki LM, Tran HD, Taylor JW, Molony DA, Barron BJ. Should hypertension be treated after acute stroke? A

randomized controlled trial using single photon emission computed tomography. *Arch Neurol*. 1993;50:855-62.

459. Vorstrup S, Andersen G, S., Blegvad N, Paulson OO. Calcium antagonist (PY 108-068) treatment may further decrease flow in ischaemic ares in acute stroke. *J.Cereb.Blood Flow Metab.* 1986;6:222-229.

460. Waldemar G, Vorstrup S, Andersen AR, Pedersen H, Paulson OB. Angiotensin-converting enzyme inhibition and regional cerebral blood flow in acute stroke. *J Cardiovasc Pharmacol*. 1989;14:722-9.

461. Hancock SM, Eastwood JR, Mahajan RP. Effects of inhaled nitrous oxide 50% on estimated cerebral perfusion pressure and zero flow pressure in healthy volunteers. *Anaesthesia*. 2005;60:129-32.

462. Hennerici M, Rautenberg W, Sitzer G, Schwartz A. Transcranial doppler for the assessment of intracranial arterial flow velocity - Part 1. *Surgical Neurology*. 1987;27:439-448.

463. Athanassiou L, Hancock SM, Mahajan RP. Doppler estimation of zero flow pressure during changes in downstream pressure in a bench model of a circulation using pulsatile flow. *Anaesthesia*. 2005;60:133-8.

464. Optimising Analysis of Stroke Trials (OAST) Collaborators, Bath PMW. Optimising the statistical analysis of functional outcome in stroke trials. *Stroke*. 2003;34:316 (abstract).

465. Ohwaki K, Yano E, Nagashima H, Hirata M, Nakagomi T, Tamura A. Blood pressure management in acute intracerebral hemorrhage: relationship between elevated blood pressure and hematoma enlargement. *Stroke*. 2004;35:1364-7.

466. Ding-Zhou L, Marchand-Verrecchia C, Palmier B, Croci N, Chabrier PE, Plotkine M, Margaill I. Neuroprotective effects of (S)-N-[4-[4-[(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)carbonyl]-1-piperazinyl]phenyl]-2-thiophenecarboximid-amide (BN 80933), an inhibitor of neuronal nitric-oxide synthase and an antioxidant, in model of transient focal cerebral ischemia in mice. *J Pharmacol Exp Ther*. 2003;306:588-94.