THE ROLE OF NITRIC OXIDE IN STROKE PATHOPHYSIOLOGY

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LIST OF ABBREVIATIONS

Bilateral common carotid artery occlusion (BCCAO)
Calcium/calmodulin-dependent protein kinase type II subunit alpha (CaMKIIα)
Cerebral blood flow (CBF)
Endothelial nitric oxide synthase (eNOS)
Glucagon-like peptide-1 receptor (GLP-1R)
Haemorrhagic strokes (HS)
Hypoxia-inducible factor 1-alpha (HIF-α)
Inducible nitric oxide synthase (iNOS)
Inhaled nitric oxide (iNO)
Intracerebral haemorrhage (ICH)
Ischaemic stroke (IS)
L-arginine (L-Arg)
Matrix metalloproteinase (MMP-9)
Mixed-lineage kinase 3 (MLK3)
Neuronal nitric oxide synthase (nNOS)
Nitric Oxide (NO)
Nitric Oxide Synthase (NOS)
N-methyl-D-aspartate (NMDA)
Peroxynitrite (ONOO-)
Postsynaptic density protein 95 (PSD-95)
Subarachnoid haemorrhage (SAH)
Transient ischaemic attacks (TIA)
Zona occludens (ZO-1)

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Stroke is one of the leading causes of death and disability worldwide. Strokes can be classified as being either ischaemic strokes (IS) or haemorrhagic stokes (HS), mini strokes or transient ischaemic attacks (TIA). Ischaemic strokes tend to be the most frequent, with an incidence of about 60-70% (1). Nitric oxide (NO) has been indicated to contribute to the pathogenesis of both ischaemic and haemorrhagic forms of stroke.

THE ROLE OF NO IN ISCHAEMIC STROKE AND TRANSIENT ISCHAEMIC ATTACKS

Brain damage in the case of IS and TIA occurs in the form of ischaemic/reperfusion which has a multitude of possible causes such as excitotoxicity, calcium dysregulation, or due to either nitrosative or oxidative stress (2). Moreover, the aetiology is somewhat different in the sense that ISs are thrombotic in nature whereas TIAs are embolic and therefore of short duration or transient. Serum NO levels have been indicated to rise exponentially during the onset of ischaemia in Northern Indian patients (3). NO may either be neuroprotective or neurotoxic in the ischaemic environment, depending on the isoform through which it is produced. There are three different forms of nitric oxide synthase (NOS) the enzyme responsible for the synthesis of NO. These are: endothelial nitric oxide synthase (eNOS); neuronal nitric oxide synthase (nNOS); and inducible nitric oxide synthase (iNOS). Whilst eNOS has been indicated to reduce ischaemic injury, on the other hand, NO produced by either nNOS or iNOS tend to aggravate neuronal damage.

nNOS has been shown to increase during the onset of ischaemia in bilateral common carotid artery occlusion (BCCAO) stroke models in mice. However, research still has to be carried out to determine how nNOS may worsen outcome as the administration of L-citrulline was shown to be overall beneficial to improve outcome, despite the concurrent elevation in the levels of nNOS (4). Therefore, the exact mechanism through which nNOS is harmful still requires further research. A death-promoting pathway through which nNOS could potentially lead to neuronal death is via the interaction of NMDA receptors with the postsynaptic density protein 95 (PSD-95), and that is tethered to nNOS. In a series of experiments involving the use of stroke rodent models, administration of nNOS-N1-133 together with the drug ZL006 prevents the dimerization of the nNOS-PDZ-95 complex, were found to both reduce the size of the infarct and improve neurological function (5). A further mechanism that contributes to neuronal death during ischaemia is thought to be through the nitrosylation of the GluR6 subunit of the NMDA receptor as a result of the increased upregulation of nNOS. This interaction is thought to promote the assembly of the complex between the PDZ-95 domain and, GluR6 with mixed-lineage kinase 3 (MLK3), which increases the activation of the downstream signalling molecule c-Jun N-terminal kinase (JNK) and consequently the phosphorylation of the protein c-Jun (6). The therapeutic importance of this mechanism is likely conferred through the suppression of the activity of the downstream pathways potentiated by nitrosylation of GluR6 (7–10). The inhibition of the PDZ-95 domain also has been indicated to have the potential to block this pathway (6).

iNOS upregulation has also been indicated to indicate bad outcome following ischaemia. Administration on rats of the iNOS inhibitor s-methylisothiourea on rats has been previously shown to cause a reduction in apoptosis following ischaemia, as well as a combined decrease in the levels of NO and peroxynitrite (ONOO-) (11). This is supported by another study in rats which showed that the administration of the angiotensin 2 type 1 receptor blocker, Losartan, caused a reduction is ischaemic damage in part through attenuation of iNOS (12)

Despite research convincingly implicates nNOS and iNOS activation with neuronal damage following IS, numerous studies provide contrasting results. A study conducted on rats showed that transient ischaemia exhibited improved outcome when the effected brain area contained a greater number of nNOS-producing neurons that were colocalized with iNOS. The improved outcome might, in this case, be related to the potential in promoting neurogenesis (13). Another study showed that post-conditioning rats with the general anaesthetic isoflurane exerted a protective effect through the increase of hypoxia-inducible factor 1-alpha (HIF-α) and the consequent elevation in iNOS. Inhibition of iNOS prevented this protective effect, indicating that higher levels of iNOS may be neuroprotective. However, there is need for further research (14).

On the other hand, upregulation of eNOS has been associated with decreased ischaemic damage. A study conducted on diabetic rats showed that the glucagon-like peptide-1 receptor (GLP-1R) proteins was associated with beneficial outcome as a result of a combined reduction of iNOS and an increase in eNOS (15). L-Citrulline, which may serve as a precursor for NO though promoting generation of L-Arginine, has also been associated with increased beneficial outcome in BCCAO rats. It prevents reduced production of eNOS during ischaemia either in low-dose (40mg/kg) co-administration with glutathione (16), or when administered at a higher dose (100mg/kg) (4). This occurrence may be explained through the mechanism where the combined administration of both L-Citrulline and Glutathione prevent the S-glutathionylation of eNOS (16). S-glutathionylation may cause the uncoupling of eNOS dimers by inhibiting eNOS dimerization (17–19). In contrast, however, a study showed that, initially, ischaemia causes an upregulation of eNOS. However, eNOS levels return to the normal level within a day. The administration of losartan was shown to maintain this elevated level of eNOS, reducing ischaemia induced damage. The suggested mechanism of function of losartan is that it increased the phosphorylation of both eNOS, as well as its downstream products causing the activation of the PI3K/Akt pathway. This may lead to neuroprotective effects with respect to ischaemic injury (12). These studies indicate the need for further research on the association between eNOS mechanisms and beneficial ischaemic damage outcome, and which can lead to the development of a treatment to improve outcomes in patients.

THE ROLE OF NITRIC OXIDE IN HAEMORRHAGIC STROKE PATHOPHYSIOLOGY

Haemorrhagic stroke is less common than ischaemic stroke. Haemorrhagic strokes are divided into two subgroups: those which are the more common intracerebral haemorrhage (ICH) which makes up around 10-15% of global strokes (20); and the less common subarachnoid haemorrhage (SAH) which accounts for approximately 7% of global strokes. SAH is associated with a relatively high of negative outcome even in among the younger population. (21). SAH has been associated with a number of secondary problems such as vasospasms. These were thought to be a delayed consequence of SAH, and were found to occur approximately 5 days after injury (22). However, more recent research has found that these vasoconstrictions may even begin to occur right after SAH (23). This finding is of great significance due to the high mortality rate of SAH within the first two days following the insult (24). In addition, SAH may cause other secondary pathological effects such as causing thrombus formation and dysfunction in the microcirculation, causing a delayed ischaemia (25–27).

NO has been indicated to affect both forms of haemorrhage. However, more research has been conducted with respect to SAH. In SAH, NO levels have been shown to decrease between the onset of SAH and lasting several hours (28). However, other research has indicated that there may be a surplus of nitric oxide following this period of NO deficiency (29). This NO deficiency has been linked to reduced blood supply, and consequently leading to vasospasms (30). Therefore, treatment for these vasospasm in animals models has been attempted via NO administration of NO donors (23) as well as NO inhalation (31). Inhaled nitric oxide (iNO) was able to reverse the majority of micro-vasospasms. iNO exerted a dilatory effect in the remainder of microvessels, and was also able to reduce vasospasm in larger vessels, with this effect typically wearing out after one day (31). Both studies indicated an elevated cerebral blood flow (CBF), which is important to restore blood supply (23,31). However, one benefit of using iNO as a treatment is that it has no effect on the systemic blood pressure, making it a safer option (31).

The different isoforms of NOS, like in the case of brain ischaemia, have been indicated to be either beneficial or pathological. eNOS deficiency has been indicated in SAH to cause vasospasms (32). This is supported by research indicating that the upregulation of eNOS is beneficial (33). Vasospasm should induce the production of eNOS due to the stress experienced by the endothelium. Research conducted on mice indicated that eNOS was upregulated following the onset of SAH due to an increased phosphorylation. However, eNOS dimers were uncoupled, and this resulted in the production of ONOO- instead of NO, resulting in the decrease in eNOS physiological function (34). Given the beneficial role of eNOS in haemorrhagic pathology, further research may help develop a new form of treatment for the augmentation of eNOS levels.

nNOS in both SAH and ICH appears to be pathological. In rat models of SAH, nNOS phosphorylation appeared to be increased at the Ser847 site. This is associated with a decrease in nNOS activity. The mechanism through which this may occur is via the SAH-induced increased intra-cerebral pressure which may stimulate Calcium/calmodulin-dependent protein kinase type II subunit alpha (CaMKIIα activation), resulting in nNOS phosphorylation (35). The importance of this occurrence is that a decreased activity may be an adaptive mechanism to prevent neurotoxicity, possibly by stimulating the expression of heme oxygenase-1 (36). In rat ICH models, there appeared to be upregulation of nNOS activity. The application of the nNOS inhibitor S-methyl-L-thiocutrulline gave positive results due to the reduction of both neuronal death and behavioural deficits (37).

iNOS is apparently upregulated in both SAH (34,38) and ICH (39) mice models. The application of Baicilin, which is a traditional ingredient in Chinese medicine, was shown to reduce iNOS and NOX-2. iNOS and NOX-2 are associated with oxidative damage under the pathological state, aiding in reducing the proinflammatory state, and also contributing to restore the blood-brain barrier (38). Furthermore, Baicilin has also been indicated to reduce infarct volume in SAH (40). This is, in part, due to the inhibition of iNOS, indicating that it typically has a pathological role in SAH. In ICH, iNOS has been shown to increase, and the inhibition of iNOS has been shown to be beneficial to ICH outcome (35,41–43). The presence

of recombinant osteopontin, which is an inhibitor of iNOS, had a beneficial effect on the outcome of ICH in mice as it reduced brain oedema, as well as increased the number of surviving neurons. The mechanism through which recombinant osteopontin appears to carry out its effect involves reducing phosphorylated Stat-1 levels, possibly by increasing its rate of ubiquitination (44). In addition, recombinant osteopontin reduced matrix metalloproteinase (MMP)-9 levels), which is responsible for causing the disruption of tight junctions by reducing the levels of zona occludens (ZO)-1 (39). This research indicates that iNOS and nNOS inhibitors functioning are promising fields for further research for developing new treatments to improve outcome of HS patients.

CONCLUSION

Both nNOS and iNOS have also been associated with worsened outcomes in cases of ischaemic and haemorrhagic stroke. In contrast, eNOS has been implicated to contribute to smaller infarct sizes and better long-term outcomes. Therefore, NOS-targeted treatment which increases levels of eNOS and/or decreasing levels of both nNOS and iNOS could be a possible therapeutic target for treatment.

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