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Original Contribution

Nitric oxide synthase inhibitors in experimental ischemic stroke and their effects on infarct size and cerebral blood flow: A systematic review

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Abstract

Nitric oxide produced by the neuronal or inducible isoform of nitric oxide synthase (nNOS, iNOS) is detrimental in acute ischemic stroke (IS), whereas that derived from the endothelial isoform is beneficial. However, experimental studies with nitric oxide synthase inhibitors have given conflicting results. 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 effects of NOS inhibition on lesion volume (mm^3, %) and cerebral blood flow (CBF; %, $ml \cdot min^{-1} \cdot g^{-1}$) were analyzed using the Cochrane Review Manager software. NOS inhibitors reduced total infarct volume in models of permanent (standardized mean difference (SMD) −0.56, 95% confidence interval (95% CI) −0.86, −0.26) and transient (SMD −0.99, 95% CI −1.25, −0.72) ischemia. Cortical CBF was reduced in models of permanent but not transient ischemia. When assessed by type of inhibitor, total lesion volume was reduced in permanent models by nNOS and iNOS inhibitors, but not by nonselective inhibitors. All types of NOS inhibitors reduced infarct volume in transient models. NOS inhibition may have negative effects on CBF but further studies are required. Selective nNOS and iNOS inhibitors are candidate treatments for acute IS.

Keywords: Nitric oxide; Nitric oxide synthase inhibitor; Nitric oxide synthase; Ischemic stroke; Animal; Free radicals

Nitric oxide (NO) is synthesized from its precursor L-arginine by the action of nitric oxide synthase (NOS). NO is produced in the brain after the onset of cerebral ischemia, although its precise role in the pathophysiology of ischemic stroke (IS) is unclear. Gene knockout studies have determined that NO derived from the endothelial isoform of NOS (eNOS) is beneficial in acute IS [1]. This may be due, in part, to anti-platelet effects [2] and preservation of cerebral blood flow (CBF) [3]. In contrast, NO produced by the neuronal and inducible isoforms of NOS (nNOS, iNOS) can be neurotoxic [4,5]. This probably occurs through NO-induced formation of peroxynitrite [6] and toxic free radicals leading to damage by lipid peroxidation [7]. NO further potentiates damage by inhibiting enzymes needed for mitochondrial respiration (cytochrome oxidase), glycolysis (GAPDH), and DNA replication (ribonucleotide reductase) [8–11]. Moreover, NO has been reported to stimulate the release of the neurotransmitter glutamate and could contribute to excitotoxicity [12,13]. Consequently, inhibition of NO production has been considered to be a candidate treatment for acute IS.

The first NOS inhibitors were the guanidino amino acids, many of which act competitively at the NOS

Abbreviations: 7-NI, 7-nitroindinazole; CBF, cerebral blood flow; eNOS, endothelial nitric oxide synthase; FR, Fischer rats; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; L-NIO, N^G-iminoethyl-L-ornithine; L-NMMA, N^G-monomethyl-L-arginine; L-NNA, N^G-nitro-L-arginine; L-NAME, N^G-nitro-L-arginine methyl ester; NOS, nitric oxide synthase; *S*, number of studies; SHR, spontaneously hypertensive rats; SDR, Sprague–Dawley rats; SD, standard deviation; SMD, standardized mean difference; STAIR, Stroke Therapy Academic Industry Roundtable; TRIM, tri(fluoromethylphenyl)imidazole; WR, Wistar rats.

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active site. Examples include N^G-nitro-L-arginine (L-NNA), N^G-nitro-L-arginine methyl ester (L-NAME, a methyl ester prodrug that is activated to become L-NNA), and N^G-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 (Table 1) [14]. However, none of the guanidino amino acids 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 [15]. Agents used to target iNOS include aminoguanidine, N^G-iminoethyl-L-lysine, the bis-isothioureas [16], 1400W (*N*-[3-(aminomethyl)benzyl]acetamidine), GW273629, and GW274150 [17]. Other agents are used to target nNOS and include 7-nitroindinazole (7-NI), tri(fluoromethylphenyl)imidazole (TRIM) [18], ARL 17477, AR-R18512 [19], BN 80933 [20], *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 (about fivefold) and probably affects other molecular targets [15]. Similarly, 7-NI has been found to be an equipotent inhibitor of all three isoforms of NOS at the isolated enzyme level (Table 1) [15,21], although it has more selectivity for nNOS in vivo, possibly a consequence of cell-specific effects (neuronal versus endothelial) [15].

Studies of NOS inhibitors in IS models have given contradictory results for effects on lesion size and CBF, with many demonstrating beneficial effects [20,22–25], whereas others report contradictory findings [26–31]. Hence, the aims of the present investigation were to undertake a systematic review to determine the efficacy of NOS inhibitors to decrease brain injury after cerebral ischemia and to assess whether their effects may be influenced by changes in CBF, timing of administration, type of model, and animal species.

Table 1
In vitro potency (IC_{50}, μM) of commonly recognized inhibitors of NOS isoforms (based on published data [14,15,17–19])

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

IC_{50}, 50% inhibition.

Materials and 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 by M.W. for articles published from 1980 to 2002. For the EMBASE search four primary keywords (nitric oxide, brain, ischemia, nonhuman) 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*, ischemia), which was then limited to animal studies. Other publications were found from reference lists and review articles by C.G., S.M., and P.B. Abstracts were then used to select relevant articles for an examination of the full publication by M.W. Final decisions on inclusion or exclusion were made by M.W. and P.B. Prespecified exclusion criteria were used to minimize 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 ($ml \cdot min^{-1} \cdot g^{-1}$ 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 1 h of occlusion or reperfusion were used in models of permanent and transient ischemia, respectively. Where possible, regional infarct volume and CBF data were obtained separately for total brain, cortex, and subcortex. In cases in which region was not specified 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 in which the number of animals in each experiment was given as a range 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 authors (M.W., C.G.); discrepancies were resolved by P.B. Finally, the methodological quality of the included articles was assessed as an 8-point "STAIR rating" [32] based on published recommendations for