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Development of the Nitrone-Based Spin Trap Agent NXY-059 to Treat Acute Ischemic Stroke

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ABSTRACT

The only current FDA-approved treatment for acute ischemic stroke is thrombolysis with tissue plasminogen activator (tPA). However, there are numerous shortcomings to tPA treatment including an increased incidence of intracerebral hemorrhage (ICH) and a short therapeutic window (3–6 h). In recent years, studies have attempted to identify new therapeutics that might be neuroprotective following ischemic strokes. Free radical scavenging spin trap agents have been proposed as potential candidates for stroke therapy because of the hypothesized role of free radicals in the progression of stroke and ischemia-induced neurodegeneration. Novel spin trap agents like (disodium-[(tert-butylimino) methyl] benzene-1,3-disulfonate N-oxide (NXY-059) are of particular interest, not only because they are broad-spectrum nitrone-based free radical scavengers, but also because of their safety profile in humans. Moreover, the rationale for developing NXY-059 for the treatment of acute ischemic stroke is further supported by the drug's reported neuroprotective effects. In addition, NXY-059 may represent a useful adjunct stroke therapy to tPA, since preclinical studies have demonstrated that NXY-059 increases the therapeutic window for tPA and lowers the occurrence of tPA-induced ICH.

INTRODUCTION

Free radicals, particularly reactive oxygen species (ROS) can accumulate to such a degree as to cause a biochemical chain reaction that leads to cellular injury, death and behavioral deficits (10,23). Accordingly, considerable attention has been given to the possi-

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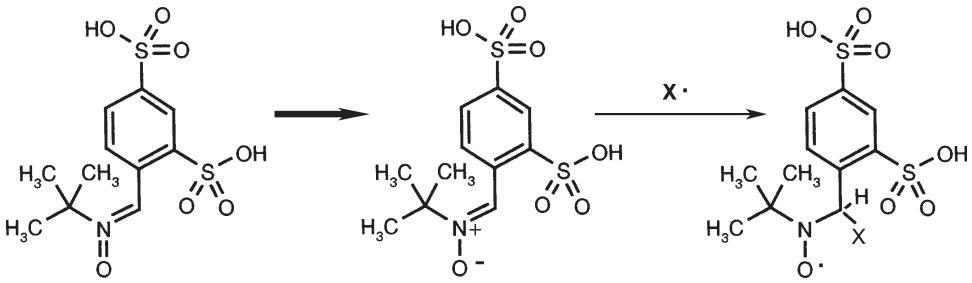

Fig. 1. Chemical structure of NXY-059 and reaction with free radicals. The three panels above show the reaction of NXY-059 with oxygen, sulfur or carbon-based free radicals designated as X•. The panel on the left is the basic structure of NXY-059, which can be represented as a polarized molecule (shown in the middle panel). Because of the charge on the nitrogen and oxygen of the nitrone moiety, free radicals can bind to the N–O to produce a more stable nitroxide (shown on the right). The nitroxide is then readily excreted from the body via the kidneys, as is excess nitrone.

bility of successfully preventing the pathophysiological consequences associated with free radical-induced oxidative stress following ischemic stroke (10,12–14,45,46), with the expectation that this might provide neuroprotection and reduce secondary reperfusion-induced damage after the onset of ischemia. The most widely studied spin trap agents are members of the nitrone class of free radical spin trap agents in which a nitrone moiety traps ROS in addition to other free radical species (3,5,13,20). Nitrone spin traps have become increasingly attractive prospects for the treatment of a variety of pathological conditions in which free radical oxidative stress is suspected to be the major culprit, particularly because of the stable nitroxides that are formed after ROS trapping (5,29).

BIOCHEMICAL PROPERTIES OF SPIN TRAP COMPOUNDS

The parent compound of the nitrone spin trap family is α-phenyl-N-tert-butyl nitrone (PBN), which traps short-lived free radicals such as alkoxyl (54), superoxide (40), and hydroxyl (52) radicals and forms a more stable nitroxide, thereby removing harmful free radicals from circulation. PBN also has the ability to prevent oxidation of lipids like low-density lipoprotein (51). While many PBN-type spin traps have been synthesized, the most extensively studied in vivo have been the analogs of PBN, sodium 2-sulfophenyl-N-tert-butyl nitrone (S-PBN). NXY-059 is also structurally related to the parent compound PBN, but unlike PBN, NXY-059 contains two sulfonyl groups, which reduces the lipid solubility of the compound and appears to alter several aspects of the drug's biochemical properties. A simple and efficient multi-step scheme for the synthesis of NXY-059 from the precursors 2-methyl-2-nitropropane and t-butylhydroxylamine has been published (30). Figure 1 (panel 1) shows the basic chemical structure of NXY-059. NXY-059 in a configuration known as the single occupied molecular orbital (Fig. 1, panel 2) is the reactive nitrone that has a polarized =N(+)–O(–) moiety. The N–O group effectively traps oxygen, carbon, and sulfur-centered radicals to produce a stable nitroxide radical (-N–O.) shown in Fig. 1 (panel 3).

Besides their direct free radical scavenging capabilities, nitrone spin traps are thought to possess a wide range of pharmacological properties primarily related to inhibition of components associated with oxidative stress pathways. For instance, PBN affects enzymes, like inducible cyclooxygenase-2 and nitric oxide synthase (24). The PBN decomposition intermediate, tert-nitrosobutane, can further decompose to nitric oxide, which itself activates many cellular mechanisms in the brain (7). PBN's activities can be as diverse as the regulation of voltage-gated ion channels, the epidermal growth factor receptor pathway, nuclear factor-κB transduction mechanisms, heme oxygenase-1 and mitochondrial complex I, independently of its free radical trapping capability (2,19,21,24,29,39). Although certain properties not associated with ROS trapping that were specific to PBN and not shared by the related S-PBN, such as inhibition of acetylcholinesterase activity, were initially considered to be uncharacteristic, more recent studies have revealed many other such atypical activities (39). Thus, the growing list of pharmacological properties ascribed to PBN includes protection from infection, drug-induced diabetogenesis and certain cancers, all presumed to be attributable to inhibition of various pro-inflammatory genes (24,43,48). Whether NXY-059 has similar pharmacological characteristics as PBN is the subject of ongoing research.

In electron spin resonance (ESR) studies, all nitrone spin trap agents trapped carbon and oxygen free radicals to produce detectable radical adducts, but some were more potent than others. Although NXY-059 binds free radicals with greater affinity than either PBN or S-PBN, resulting in a slower decay of the nitroxide radical adduct thus formed, overall it possesses the least effective free radical scavenging capability (37). Despite the ongoing development of these and other novel spin trap agents, to date there is limited information regarding their pharmacology. In particular, little is known about their potential effects in animal models of human diseases.

PHARMACOLOGY OF SPIN TRAP AGENTS

Pharmacokinetic and Toxicology Profile

Certain aspects of the pharmacokinetic (PK) profile of NXY-059 have been described for the rat, primate and human (38,49,50). In the rat, subcutaneous minipumps were used to deliver NXY-059 at a dose of 10 mg/kg/h, which resulted in a plasma concentration of 28.5 ± 3.0 μmol/L. The authors also showed that the plasma concentration of NXY-059 was linearly related to dose of the drug administered (50). In the primate, Marshall et al. (38) also used minipumps to release NXY-059 at a rate of 16 mg/kg/h. With that administration regimen, plasma unbound concentrations of NXY-059 were 76.3 ± 5.7 μmol/L with a total plasma concentration of 109 ± 8 μmol/L. Based upon animal studies, Strid et al. (49) used a target plasma level of 60 μmol/L to determine the PK properties of NXY-059 in humans. The studies indicated that the clearance rate was 59 mL/min, 53% of NXY-059 was unbound in plasma, the terminal half-life was 4.6 h (range of 2.8–9.5 h) and the steady state distribution was 12.9 L. Additionally, in young and old healthy volunteers, greater than 60% of the administered NXY-059 remained unbound 72 h after i.v. infusion (11). This unbound percentage is consistent with an unpublished observation by Lundstrom (Department of Drug Metabolism and Pharmacokinetics, AstraZeneca,

Sweden). Greater than 90% of the drug is excreted without being metabolized, with elimination occurring almost exclusively via the kidneys.

Since only relatively low doses of NXY-059 have been used in humans for short periods of time, there is little information available regarding acute or chronic toxicities. In rabbits, NXY-059 tended to increase the rate of punctate hemorrhages following an embolic stroke (31) and the same may occur in patients presenting with either acute ischemic stroke or primary ICH. Fortunately, since punctate hemorrhages are not considered to be life threatening (27) an increased incidence of this type of hemorrhage is not expected to have any impact on the well-being of the patient. However, the brief clinical report by Lees et al. (33) suggested that NXY-059 might exacerbate primary ICH. There are no other reported toxicities in humans following acute NXY-059 administration (11,33).

TABLE 1. Pharmacological profile of NXY-059 in stroke models

Animal model	Biomarker	Dosing regimen	Result
Rat transient focal ischemia (26)	Infarct volume	Loading dose of 0.3–30 mg/kg i.v. + infusion for 24–48 h	Reduced infarct size by 50–60%. Window up to 3 h
Rat permanent focal ischemia (59)	Infarct volume	Loading dose of 30–60 mg/kg i.v. + infusion 30–60 mg/kg for 24 h	Slight decrease of infarct size
Rat transient or permanent MCAO (50)	Infarct volume and behavioral test	Loading dose 32.5–75.4 mg/kg + infusion 30–70 mg/kg for 25 h	Dose-dependent decrease of neurological deficit and infarct size
Rabbit small clot embolic stroke (30)	Behavioral test	Dose 100 mg/kg i.v. infused over 30 min	Improved behavior and NXY-059 increased the therapeutic window for tPA
Rat transient focal ischemia (57)	Infarct volume and mitochondrial function	Loading dose 30 mg/kg i.v. + infusion of 30 mg/kg i.v. for 24 h	Reduced infarct volume and improved mitochondrial function
Primate permanent MCAO (38)	Behavioral tests and infarct volume	Loading dose 28 mg/kg i.v. + infusion 16 mg/kg for 24 h	Improved behavioral function, reduced infarct volume
Rat hemorrhage model (collagenase) (42)	Behavioral endpoint and measure of apoptosis	50 mg/kg s.c. + 8.8 mg/kg/h for 3 days	No behavioral improvement. Decreased neutrophil levels and TUNEL positive cells (transient)
Rabbit large clot embolic stroke hemorrhage model (31)	Hemorrhage and infarct volume	Dose 100 mg/kg infused i.v. over 1 h	Slight increase in punctate hemorrhage rate induced by NXY-059. Decreased tPA-induced ICH, no effect on infarct size

Note. s.c., subcutaneous injection; i.v., intravenous injection.

Neuroprotection

Neuroprotective properties of PBN and NXY-059 in animal models of diseases have been reported in the literature. PBN has been shown to reduce infarct size and volume following transient focal cerebral ischemia produced by temporary occlusion and the subsequent reperfusion of the middle cerebral artery occlusion (MCA) in the rat and gerbil (3,4,13,21,25,41,46). Neuroprotection by PBN following more severe insults such as global cerebral ischemia (36,40,53,58) and traumatic brain injury (35) also have been described. In a model of focal embolic cerebral ischemia, in which a thrombus is injected into the rat MCA, PBN decreased infarct volume and consequent tissue damage, which translated into significant improvement in behavioral scores (35,56).

Despite speculation as to the exact mechanism underlying PBN's neuroprotective properties, it has been suggested that it transcends the compound's free radical trapping activity. For instance, since post-ischemic energy state recovers with PBN administration, it is likely that attenuation of mitochondrial dysfunction contributes to neuroprotection (15,25). Moreover, regulation of the mitogen-activated protein kinase-signaling pathway, heat shock proteins (53), caspase-3 (36) and cytokines (14) also has been proposed. Studies of the potential neuroprotective properties of S-PBN are limited, with improved deficits observed following histotoxic hypoxia (44) and focal embolic cerebral ischemia (56).

Even though NXY-059 does not readily penetrate the blood-brain barrier (BBB) early on following an ischemic event (26), it appears that the transport of the drug across the BBB may increase as a function of time (9). Kuroda et al. (26) have postulated that the efficacy of NXY-059 may be related to the ability of the nitrone to react with free radicals (molecules with a single or multiple unpaired electrons such as the superoxide) within the cerebral vasculature. The spin trap may reduce microvascular dysfunction and NXY-059 may prevent oxidation-reduction reactions mediated by free radicals, reactions that may directly or indirectly injure the vascular endothelial cell leading to increased BBB permeability. Additionally, increased BBB permeability may result in the initiation of various types of intracerebral hemorrhages (27,29). This may explain why the anti-ischemic effects of NXY-059 are delayed, but quite prominent, at least in rat and primate MCA occlusion models (26,38,50). In the rabbit embolic stroke model, NXY-059 substantially reduced behavioral deficits, but the therapeutic window was shorter (30) than that observed in the rat. In rats with transient focal ischemia produced by occlusion of the MCA, neuroprotection as judged by improved behavioral scores has been observed when NXY-059 was administered up to 6 hours following occlusion (26,50). In the primate permanent MCA occlusion model, NXY-059 reduced spatial perception neglect and decreased gray and white matter damage (38). NXY-059 was more efficacious in decreasing infarct volume than was PBN despite the latter's more extensive penetration through the BBB, implicating NXY-059 in events occurring at the blood-endothelial interface (26). Similarly, in a rat model of hemorrhagic stroke produced by infusion of the membrane dissolving enzyme collagenase into the caudate-putamen (42), NXY-059 significantly reduced behavioral impairment associated with ICH. However, since stroke models produced by MCA occlusion or collagenase do not mimic the clinical presentations of embolic stroke, a more representative animal model of embolic stroke is necessary to generate a clearer understanding of the potential or spin traps in stroke therapy. Using the rabbit small clot embolic model (RSCEM), in which microclots are delivered into the ce-

rebral circulation through the MCA (30), NXY-059 was shown to ameliorate embolism-induced behavioral dysfunction. The deficits were attenuated when infusion of the spin trap was begun 5 min after embolization, but was not evident when the onset of infusion was delayed by 180 min. Additional studies showed that NXY-059 could significantly increase the therapeutic window for tPA using the RSCEM if NXY-059 was given prior to tPA administration (30). In the RSCEM, tPA has a limited therapeutic effect and is most effective if given within 1 h of embolization (30). If tPA administration is delayed by 3 h post-embolization, then the neuroprotective activity is no longer observed. When experimental rabbits were treated with NXY-059 early on following embolization, which was followed by tPA at 3 h, a significant behavioral improvement was observed. The results suggest that NXY-059 in combination with tPA may provide enhanced neuroprotection and behavioral improvement (30). Accordingly, NXY-059 may be useful not only as a monotherapy, but also as an adjunct to thrombolytic therapy. On the basis of the preclinical evidence, NXY-059 already has been tested for its tolerability in a phase I clinical trial in acute stroke patients (33).

Hemorrhage Studies

Increasingly, clinicians are recommending thrombolytics like tPA for acute stroke management, despite apprehensions regarding exposure of patients to secondary complications of ICH (1,22,27,29,47). Thrombolytics such as tPA present a beneficial therapy for acute stroke patients because they dissolve blood clots and restore perfusion (17). Paradoxically, thrombolytics also can have harmful effects such as causing an increased incidence of secondary ICH (17,22,28,29,55). The incidence of symptomatic ICH (approximately 6.5%) subsequent to tPA therapy for acute stroke reportedly results in a 50% mortality rate, which is substantially higher than in placebo-treated patients (28,47). Because of this and their limited time frame (3–6 h) of therapeutic effectiveness, stroke therapy with thrombolytics alone will have limited success. Clearly, future approaches in stroke research should target therapeutics that minimizes ICH, while affording significant neuroprotection. Several classes of drugs have emerged as potential candidates, with spin trap agents providing some of the most promising preclinical data for neuroprotection (see above). Since preliminary studies in animal models demonstrated promising results for drugs used in combination with tPA (4,29,31), subsequent studies have attempted to identify more drug candidates that may provide neuroprotection and prevent ICH when used in combination with tPA.

In the rabbit large clot embolism (RLCEM), in which parenchymal and punctate (petechial) hemorrhages and hemorrhagic infarctions are observed, NXY-059 was shown to effectively counteract ICH associated with tPA administration (30), suggesting that it may be a useful adjunct therapy to thrombolysis. However, some caution is warranted, since by itself, the spin trap appeared to slightly increase the rate of punctate ICH (30). Furthermore, in other animal models of stroke, NXY-059 produced less striking effects on behavioral improvement. For example, in a rat model of hemorrhagic stroke induced by collagenase infusion into the caudate-putamen (42), NXY-059 only modestly reduced behavioral impairment associated with ICH, but there was no effect on the volume of the hematomas and there was also no significant neuroprotection. The paradoxical results from the rat collagenase are quite difficult to reconcile, since there appears to be no histological basis for the modest behavioral improvement.

CLINICAL TRIALS WITH NXY-059

The pharmaceutical industry giant, Astra-Zeneca (AZ) Inc. has licensed NXY-059 from Centaur Pharmaceuticals (now owned by Renovis Pharmaceuticals) for development for acute ischemic stroke. AZ has approached the development of NXY-059 with fully warranted caution, since all previous clinical trials with "neuroprotective" compounds have failed (16). Over the last year, AZ has sponsored Phase I, II, and IIa trials in order to determine whether NXY-059 will be toxic in humans at steady-state plasma levels that were neuroprotective in animal models. Unfortunately, AZ has released little information concerning the clinical pharmacology and acute or chronic toxicity profiles of NXY-059. The Phase Ia trial, which was a randomized, double-blinded, placebo-controlled, parallel group study, evaluated the safety of two NXY-059 treatment regimens compared with placebo in acute stroke patients (33). NXY-059 given as a 250 mg dose over 1 h followed by 85 mg/h for 71 h or 500 mg over 1 h followed by 170 mg/h for 71 h was well tolerated by young healthy male volunteers and by elderly male and female volunteers for periods of up to 72 h (11). The drug also was well tolerated in acute stroke patients (33), but the study did not have sufficient power to demonstrate beneficial effects of NXY-059. In young and old healthy volunteers, greater than 60% of the administered NXY-059 remained unbound at 72 h after i.v. infusion(11). Greater than 90% of the drug is excreted without being metabolized, with elimination occurring almost exclusively via the kidneys. The pharmacokinetic profile of NXY-059 showed that the clearance rate is 4.6 L/h in acute stroke patients and 4.1 L/h in normal volunteers (11,33).

Understandably, there are concerns about possible toxicities associated with nitrone spin trap agents, particularly when used in high doses. High doses of PBN can cause impaired respiration, abnormal blood chemistry, seizures and tissue damage (18). The broad spectrum of potential side effects may be explained by the drug's pharmacokinetic profile, especially its ability to distribute throughout the body after peripheral administration and its slow clearance rate (8). In contrast, the pharmacokinetic profile of NXY-059 generated from a Phase IIa randomized, double-blinded, placebo-controlled, parallel group, dose-escalation trial, demonstrated that the drug was well tolerated and "safe" in stroke patients (34). In the trial, NXY-059 was administered i.v. either at 915 mg over 1 h followed by 420 mg/h for 71 h or 1820 mg for 1 h followed by 844 mg/h for 71 h. The high dose used exceeded the steady state target of 200 μmol/L that was studied in the Phase I trial (see above), with no serious adverse effects reported. However, caution should be exercised in interpreting the data since the dose of NXY-059 tested still was relatively low (34) and may not have reached the therapeutic level. Of crucial significance will be to determine whether NXY-059 will augment the risk of ICH in patients presenting with primary ICH, as was apparent in rabbits following an embolic stroke (31). In addition, as with the previous trial, this study did not have power to demonstrate beneficial effects of NXY-059 (34).

CONCLUSIONS

Preclinical evidence in standard stroke models including the rabbit embolic stroke model indicate that NXY-059 has a significant neuroprotective profile and may have a

therapeutic window of up to 3–6 h. In the embolic stroke model, NXY-059 has neuroprotective effects with a magnitude comparable to that of tPA. Thus, NXY-059 has been designated as a "good" lead compound for continued development to treat acute ischemic stroke. However, the main problem regarding the potential use of NXY-059 to treat stroke may be the same as that for tPA, namely that the optimal therapeutic window may elapse because of the usually long delay (hours) between stroke onset and presentation in a clinical setting. However, of potential importance, is the preclinical evidence in animal models showing that NXY-059 increases the therapeutic window for tPA treatment and also improves the safety profile of tPA by attenuating tPA-induced ICH. Therefore, while the prospects for NXY-059 monotherapy in acute ischemic stroke may be limited, its adjunct therapy in combination with tPA is promising.

Preliminary results from clinical trials in acute stroke patients suggest that NXY-059 is well tolerated in most patients, but investigators should consider the possibility that NXY-059 will exacerbate the progression of hemorrhagic stroke. Moreover, because certain spin traps appear to have many diverse effects following peripheral administration, prudence is certainly warranted. Finally, the data reported thus far only serve to emphasize the need for further well-designed clinical trials that should include combination studies of NXY-059 used with tPA or other thrombolytics such as Tenecteplase (6,28) or microplasmin (28,32), in order to establish whether this approach to stroke therapy will have any merit.

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