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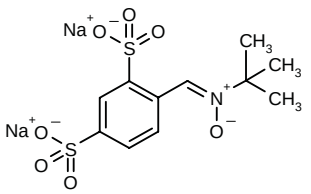
CCC: 0377-8282/2002

NXY-059

Treatment of Ischemic Stroke
Free Radical Scavenger

ARL-16556
CPI-22
CXY-059

4-(*tert*-Butyliminiomethyl)benzene-1,3-disulfonic acid *N*-oxide disodium salt
N-(*tert*-Butyl)-α-(2,4-disulfophenyl)nitrone disodium salt



$C_{11}H_{13}NNa_2O_7S_2$
Mol wt: 381.3357
CAS: 168021-79-2
CAS: 168021-77-0 (as free acid)
CAS: 168021-80-5 (as dipotassium salt)
CAS: 168021-81-6 (as diammonium salt)
CAS: 168021-82-7 (as calcium salt)
CAS: 168021-83-8 (as magnesium salt)
EN: 259634

Abstract

Stroke is considered the third leading cause of death and the major cause of disability in the U.S. There are 2 major therapeutic options available for the treatment of stroke and they are targeting of the insufficient arterial oxygen and glucose resulting from stroke by enhancing blood flow and neuroprotection. One group of neuroprotective agents are the free radical scavengers and free radical production inhibitors; within this group the novel spin trap class of agents have emerged. NXY-059 is a spin trap agent that was designed to treat ischemic stroke but is not contraindicated in hemorrhagic stroke. NXY-059 has demonstrated considerable neuroprotective effects in preclinical studies and has been shown to be safe and effective in clinical trials involving patients with acute stroke.

Synthesis

NXY-059 is synthesized by condensation of *N*-*tert*-butylhydroxylamine (I) (1, 2) or its acetate (3) or hydrochloride (4) salts and disodium benzaldehyde-2,4-disulfonate (II) directly in refluxing MeOH or mixtures of MeOH/water or *i*-PrOH/MeOH/water (1-3) or by means of MeONa in refluxing MeOH/water or *i*-PrOH/MeOH/water (4). Scheme 1.

N-*tert*-butylhydroxylamine (I) can be prepared as follows:

a) Reduction of 2-methyl-2-nitropropane (III) with either Zn in AcOH/EtOH or aluminum foil and $HgCl_2$ in EtOH/ether/H_2O (1, 2).

b) Condensation of benzaldehyde (IV) with *tert*-butylamine (V) in refluxing toluene provides *N*-benzylidene-*N*-*tert*-butylamine (VI), which is oxidized with *meta*-chloroperbenzoic acid and Na_2CO_3 in water/toluene/EtOH to furnish the phenyloxaziridine derivative (VII). Finally, the oxaziridine ring of (VII) is opened by treatment with H_2SO_4/AcOH in EtOH/H_2O (5).

c) Heating of the phenyloxaziridine derivative (VII) at 130 °C gives *N*-*tert*-butylphenylnitrone (VIII), which is finally treated with H_2SO_4/AcOH in toluene (5).

Disodium benzaldehyde-2,4-disulfonate (II) can be obtained as follows:

a) Heating of 2,4-dichlorobenzaldehyde (IX) with sodium sulfite at 170 °C in water, followed by oxidation with sodium hypochlorite (6).

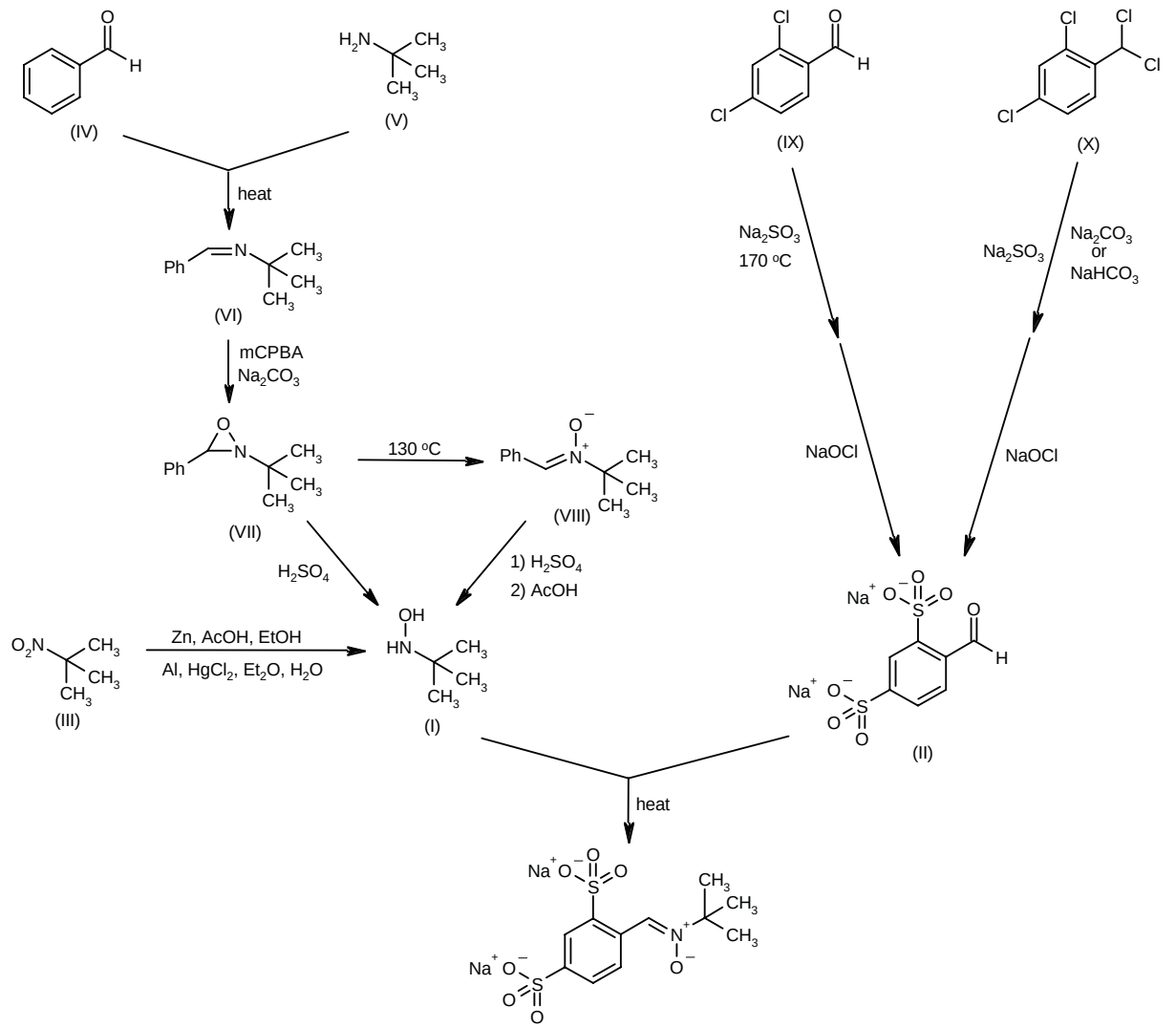
b) Reaction of 2,4-dichlorobenzal chloride (X) with sodium sulfite and Na_2CO_3 or $NaHCO_3$ at 170 °C in water, followed by oxidation with sodium hypochlorite (7).

Introduction

Stroke is defined as an abrupt impairment of brain function resulting from occlusion (*i.e.*, thrombus) or

L.A. Sorbera, P.A. Leeson, J. Castañer, M. del Fresno. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of NXY-059



rupture of intra- or extracranial blood vessels and is considered the third leading cause of death and the major cause of disability in the U.S. According to the American Heart Association, approximately 600,000 Americans suffer a stroke each year with about 500,000 of these first attacks and 100,000 recurrent strokes. In the general population, the incidence of stroke is 1/1000 individuals. However, incidence doubles in individuals who are 80 years of age or older (8, 9).

There are 4 types of stroke: cerebral thrombosis and cerebral embolism are classified as ischemic stroke due to blockage of blood flow and they account for almost 80% of all cases, and subarachnoid hemorrhage and intracerebral hemorrhage are classified as hemorrhagic strokes and are due to an aneurysm in the brain or head injury. Cerebral thrombosis is the most common type of stroke and occurs when a thrombus develops on the wall of a cerebral artery (usually damaged by atherosclerosis) and grows to eventually block the flow of blood. Cerebral embolism results when a migratory thrombus or particle forms in a blood vessel located away from the brain (usually in the heart) and is transported toward the brain until it becomes lodged within an artery leading to or in the brain. Subarachnoid hemorrhage occurs when a blood vessel on the surface of the brain ruptures, resulting in bleeding into the subarachnoid space and contamination of the cerebrospinal fluid (CSF) within. The contaminated CSF causes extensive subsequent damage as it flows through the cranium. Intracerebral hemorrhage occurs due to the rupturing of a damaged artery within the brain,

causing the flooding of blood into the surrounding tissue which becomes compressed from the resulting pressure. Other less common types of stroke include transient ischemic attacks also known an mini-strokes, lacunar infarcts and recurrent stroke (8).

Due to the extensive research generated in recent years, stroke has become treatable with many new potential therapies continuing to be developed. In general, 2 major therapeutic options are currently available for the treatment of stroke. One method involves targeting the insufficient arterial oxygen and glucose resulting from stroke by enhancing blood flow. This can be achieved by lysing the arterial thrombus within hours of symptom onset or by reducing tissue back-pressure hours to days later. The other method is based on neuroprotection and attempts to decrease the intrinsic vulnerability of brain tissue to ischemia. This latter method is a relatively newer therapeutic approach and it concentrates on reducing neuronal death due to excitotoxicity by suppressing those cerebral mechanisms that are responsible for the heightening vulnerability of the CNS to ischemia. Agents for the treatment of stroke that affect excitotoxicity include glutamate receptor antagonists, NMDA antagonists, AMPA antagonists, glutamate release inhibitors, NAALADase (*N*-acetylated α-linked acidic dipeptidase) inhibitors, GABA agonists and ion channel modulators (*i.e.*, Ca^{2+} channel antagonists, Na^{+} channel antagonists and K^{+} channel agonists). Another group of neuroprotective agents are the free radical scavengers and free radical production inhibitors. Free radicals have been implicated in more than 50 diseases including stroke. They play a major role in the damage caused by hypoxia and reperfusion during cerebral ischemia, affecting a late stage of the ischemic process. Thus, agents which scavenge free radicals or prevent their production may be able to prolong the therapeutic time window. Table I shows several antioxidants and free radical scavengers launched and under development for the treatment of stroke.

One novel class of free radical scavengers are the spin trap agents also known as free radical spin traps. These agents possess multiple pharmacological activities including the ability to trap alkoxyl, superoxide and hydroxyl radicals. Preclinical studies have shown that these agents in combination with tissue plasminogen activator (t-PA) may be effective for the prevention of secondary intracerebral hemorrhage. The nitrone, NXY-059 (CPI-22, CXY-059), is a spin trap agent that was designed to treat ischemic stroke. However, it is not contraindicated in hemorrhagic stroke. This is extremely beneficial because often, when a stroke first occurs, it is not evident whether it is ischemic or hemorrhagic. As a consequence, prior to administration of t-PA which is contraindicated in hemorrhagic stroke, time-consuming tests must be conducted to determine the type of stroke. This would not be the case with NXY-059. Moreover, NXY-059 has shown considerable neuroprotective effects and has been chosen for further development.

Pharmacological Actions

An *in vitro* study has compared the radical trapping abilities of NXY-059 with 2 other nitrones, α-phenyl-*N*-*tert*-butyl nitrone (PBN) and sodium 2-sulfophenyl-*N*-*tert*-butyl nitrone (S-PBN). Electron spin resonance analysis was performed after generation of hydroxyl radicals using ultraviolet light and hydrogen peroxide and after generation of secondary radicals via addition of methanol, ethanol, isopropanol, dimethylsulfoxide, tetrahydrofuran or 1,4-dioxane. Competition spin trapping studies were also executed using PBN-α-(13) C and either S-PBN or NXY-059 as well as salicylate studies in which the ability of the 3 agents to prevent the formation of 2,3- and 2,5-dihydroxybenzoic acid in hydroxyl radical generating systems was compared to other antioxidants and reference compounds (cysteine, glutathione, ascorbate, uric acid, Tempo, Trolox and Tirilizad). Results showed that all 3 agents trapped carbon and oxygen-centered radicals producing radical adducts and each prevented salicylate oxidation in a manner similar to the reference antioxidants (10).

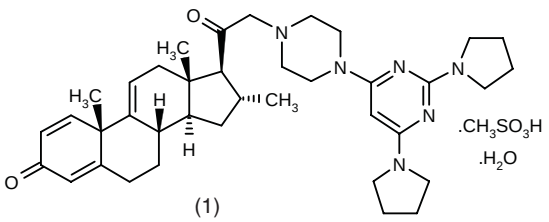
The neuroprotectant effects of NXY-059 (0.30, 3 or 30 mg/kg i.v. bolus followed by 0.30, 3 or 30 mg/kg/h continuous infusion over 24 or 48 h) were compared to PBN (1.4 mg/kg i.v. bolus followed by 1.4 g/kg/h continuous infusion over 24 or 48 h) in a study using a rat model of transient focal cerebral ischemia (*i.e.*, transient middle cerebral artery occlusion [MCAO] for 2 h followed by 2 h of reperfusion). NXY-059 given after 1 h of recirculation and continued for 24 h significantly reduced infarct volume from 35.7 ± 9% of the contralateral hemisphere to 17.3 ± 16.1, 15.3 ± 12.8 and 8.3 ± 3.7% for doses of 0.3, 3 and 30 mg/kg, respectively. In contrast, PBN at a dose equimolar to 3 mg/kg NXY-059 had no effect on infarct volume (33.7 ± 4.9%). NXY-059 continued to significantly reduce infarct size even when administered at 3 h of recirculation (13.8 ± 15.1%); no significant effect was observed when the agent was given at 6 h of recirculation (31.3 ± 16.4%). Similar results were obtained when animals were allowed to recover for 7 days after 2 h of MCAO and treated with the agents starting at 1 h of recirculation. NXY-059 when given at 6 h of reperfusion was also shown to significantly improve neurological deficits (*i.e.*, forelimb flexion); no significant improvement was observed at 48 h when treatment was delayed 3 or 6 h. From these results, the therapeutic window of opportunity for NXY-059 was concluded to be 3-6 h after the start of recirculation (11).

In this same study, the ability of NXY-059 to cross the blood-brain barrier was examined by deriving the transfer coefficient (K_{in}) from the rate of influx of [^{14}C]-labeled NXY-059. Unlike PBN, the more water-soluble NXY-059 did not easily penetrate the blood-brain barrier. These results suggest that the antiischemic effects of NXY-059 may occur at the blood-endothelial interface (11).

The neuroprotective efficacy of NXY-059 in a rat model of transient (2 h) MCAO was further demonstrated in a study in which the agent (1, 10 and 30 mg/kg/h

Table I: Antioxidants and free radical scavengers under development for stroke (Prous Science Integrity®).

Drug	Source	Mechanism of Action	Phase
1. Tirilazad Mesylate	Pharmacia	Lipid peroxidation inhibitor/NOS inhibitor	L-1995
2. Edaravone	Mitsubishi Pharma	Antioxidant/Free radical scavenger	L-2001
3. Ebselen	Daiichi Pharmaceutical	Antioxidant	Preregistered
4. Nicaraven	Chugai/Novartis	Lipid peroxidation inhibitor/Free radical scavenger	Preregistered
5. NS-7	Nippon Shinyaku/Schering AG	NOS inhibitor/Na^+/Ca^{2+} channel blocker	II
6. NXY-059	Centaur/AstraZeneca	Free radical scavenger	II
7. AEOL-10113	Incara	Antioxidant	Preclinical
8. AEOL-10150	Incara	Antioxidant	IND
9. BN-80933	Beaufour-Ipsen	Free radical scavenger/nNOS inhibitor	Preclinical
10. L-Dehydroascorbic Acid	Progenics	Free radical scavenger	Preclinical



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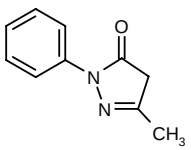

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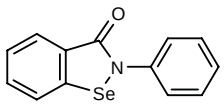

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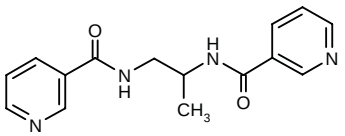

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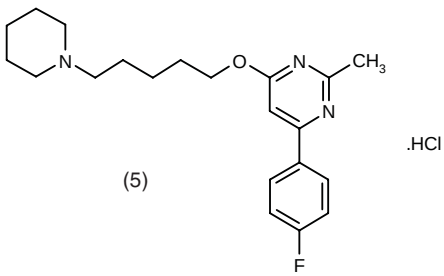

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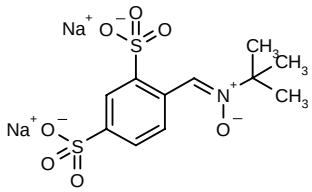

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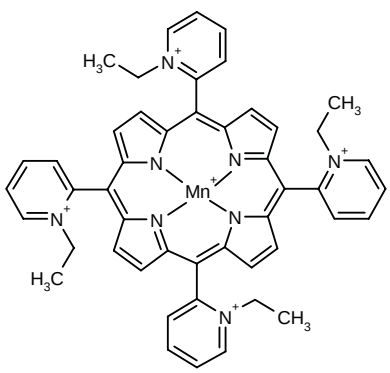

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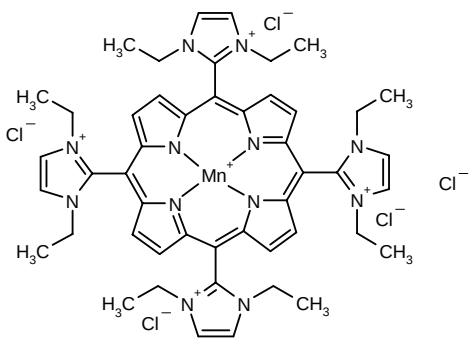

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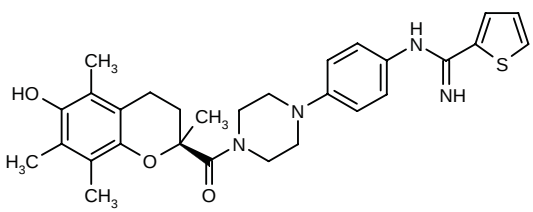

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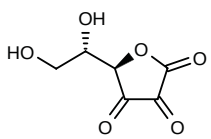

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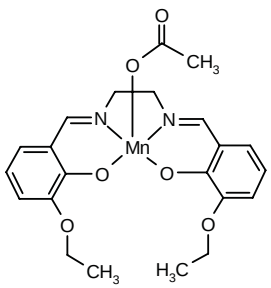
Table I (Cont.): Antioxidants and free radical scavengers under development for stroke (Prous Science Integrity®).

Drug	Source	Mechanism of Action	Phase
11. EUK-189	Eukarion	Antioxidant	Preclinical
12. GT-015[1]	GoBang	Nitrate	Preclinical
13. GT-715	GoBang	Nitrate	Preclinical
14. SPBN	Centaur/Eisai	Free radical scavenger	Preclinical
15. SUN-N8075	Suntory/Taisho	Antioxidant/Na^+/Ca^{2+} channel blocker	Preclinical
16. KR-31378	Korea Res. Inst. Chem. Technol.	Antioxidant/K^+_{ATP} channel activator	Preclinical



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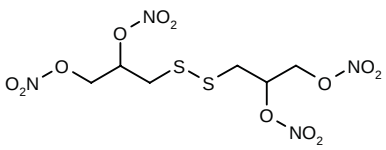

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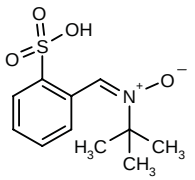

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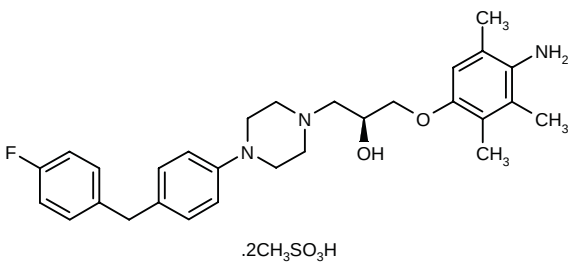

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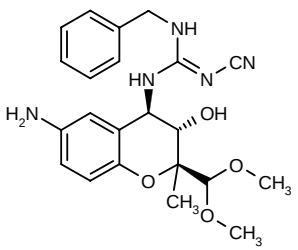

(16)

[1]Structure not yet detected.

infusion) was administered for 21.75 h starting 2.25 h postocclusion. Dose-dependent decreases in neurological impairment (*i.e.*, forelimb flexion, spontaneous rotation and absence of response to contralateral whisker stimulation) and infarct volume were reported, with significant effects observed at doses of 10 (59% decrease) and 30 mg/kg (12).

Another study also using the rat model of transient (2 h) MCAO examined the mechanism of action of NXY-059 (30 mg/kg bolus followed by 30 mg/kg/h infusion for 24 h starting 1 h after reperfusion). Neuronal damage was found to increase progressively after transient MCAO which was accompanied by significant increases in Erk activation in microglial cells from 4-48 h postreperfusion and in endothelial p65 at 48 h postreperfusion. In addition, Akt phosphorylation was significantly decreased 24 h postreperfusion in the core and penumbra of the infarct region and cortical neuron cytosolic cytochrome c levels in the infarct area were significantly increased at 48 h postocclusion. Treatment with the agent not only prevented an increase in infarct volume but also prevented the downregulation of neuronal Akt activation at 24 h postreperfusion and the increases in neuronal cytosolic cytochrome c at both 24 and 48 h postreperfusion (13).

NXY-059 was also effective in the rat model of permanent focal ischemia. In one study, NXY-059 (32.5, 53.8 or 75.4 mg/kg s.c. bolus starting 5 min after MCAO followed by 30, 50 or 70 mg/kg/h via implanted osmotic minipumps) dose-dependently protected both cortical and subcortical tissue (*e.g.*, 63% with 53.8/50 mg/kg). Neuroprotection afforded by the agent was linearly related to NXY-059 plasma concentrations (196 ± 35, 387 ± 52 and 450 ± 687 μmol/l at 1 h for doses of 32.5/30, 53.8/50 and 75.4/70 mg/kg, respectively). Significant and marked protection was also observed when 53.8/50 mg/kg was administered between 5 min and 4 h postocclusion (*e.g.*, 44% protection in the cortex at 4 h) but not after 6 h (12).

A study using male spontaneously hypertensive rats (SHR) subjected to permanent MCAO also demonstrated the neuroprotective efficacy of NXY-059 (30 or 60 mg/kg i.v. bolus at 5 min postocclusion followed by 30 or 60 mg/kg/h 24-h continuous infusion). Treatment with 60 mg/kg NXY-059 significantly ameliorated cortical infarction from 22.6 ± 6.8% of contralateral hemisphere to

14.5 ± 5%; the 30 mg/kg dose also decreased infarct volume (17.4 ± 6.8%) although results were not statistically significant. Plasma concentrations of the agent at the 30 and 60 mg/kg doses were 80.2 ± 52.2 and 391 ± 207 μM/l, respectively (14).

NXY-059 (50 mg/kg s.c. followed by 8.8 mg/kg/h for 3 days s.c. via implanted osmotic pump) showed efficacy in rat models of intracerebral hemorrhagic stroke (*i.e.*, infusion of collagenase into the caudate putamen). Treatment with the agent significantly decreased neurological impairment (*i.e.*, beam walking, circling and posture reflex) on days 1, 3, 7, 14 and 21 after intracerebral hemorrhage (ICH). However, treatment did not improve deficits in skilled forelimb use (*i.e.*, a staircase apparatus) 4-5 weeks post-ICH or striatal function (*i.e.*, rotorod test) 6 weeks postI-CH. Additional effects of the agent included significantly decreased neutrophil infiltrate in the area of the hematoma and the number of TUNEL-positive cells (*i.e.*, cells with DNA damage) at the hematoma margin at 48 h posthemorrhage. No differences in neuronal densities were noted at 6 weeks between NXY-059-treated animals and controls (15).

NXY-059 was shown to have neuroprotective effects in other animal models of ischemic stroke. A study using a rabbit small clot thromboembolic stroke model (*i.e.*, injection of a blood clot suspension into the internal carotid artery) examined the efficacy of NXY-059 (100 mg/kg i.v. starting 5 min after embolization) and t-PA alone (starting 60 min postembolization) or in combination. Treatment with NXY-059 or t-PA significantly increased the effective stroke dose value (ES50; after behavioral assessment) from 1.10 ± 0.41 mg in controls to 2.54 ± 0.72 and 2.19 ± 0.31 mg, respectively; combination treatment did not result in any significant additional improvement in ES50 values (3.15 ± 0.59 mg). However, both NXY-059 and t-PA alone increased the hemorrhage rate after embolic stroke to 80 and 87%, respectively, as compared to 55% in controls; combination treatment resulted in a 46% hemorrhage rate, suggesting that NXY-059 may reduce t-PA-induced hemorrhage. The mechanism of the deleterious effect of NXY-059 on increasing the hemorrhage rate is not known but is similar to effects seen with PBN (16, 17).

NXY-059 was shown to decrease long-term functional disability in a primate model of stroke. Marmosets were treated with the agent (58 mg/kg i.v. followed by 16 mg/kg/h i.v. for 48 h) starting 5 min following permanent MCAO. Unbound plasma and total plasma NXY-059 concentrations were 76.3 ± 5.7 and 109 ± 8 μmol/l, respectively, 24 h following pump implantation. Treatment with the agent resulted in an overall reduction in brain damage of more than 50% with comparable protection seen in white and gray matter. Behavioral testing at 3 and 10 weeks postsurgery showed that NXY-059 treatment significantly improved reaching with the hemiparetic arm and significantly reduced the degree of spatial perceptual neglect as compared to untreated controls (18).

Clinical Studies

The pharmacokinetics, safety and tolerability of NXY-059 (250 mg i.v. loading dose over 1 h followed by 85 mg/h as 71-h continuous infusion or 500 mg i.v. over 1 h followed by 170 mg/h as 71-h continuous infusion) were examined in a multicenter, randomized, double-blind, placebo-controlled, parallel-group trial involving 147 patients with a clinical diagnosis of acute stroke (NIH stroke scale score = 7.9 ± 6.2). Mean unbound plasma NXY-059 concentrations were 25 and 45 μmol/l for the respective dosing regimens and a clearance of 4.6 l/h and volume of the central compartment of 7.2 l were estimated using population pharmacokinetic analysis. Pharmacokinetics were not affected by gender, age or body mass index. NXY-059 was well tolerated. Serious adverse events were observed in 23 and 16% of the low- and high-dose NXY-059-treated patients, respectively, as compared to 16% on placebo. Deaths occurred in 10 and 4% of patients treated with low- and high-dose NXY-059 as compared to 0% in the placebo group. All deaths except for 2 occurred in patients with extensive MCA infarctions or hemorrhagic stroke. Death rates followed the proportions observed for incidence of primary intracerebral hemorrhage in each group (16 and 8% *vs.* 6% in placebo). Other adverse events included hyperglycemia, headache and fever which appeared not to be related to treatment. Outcomes according to Barthel Index Scores were not statistically different between groups (19).

The safety and tolerability of higher target concentrations of NXY-059 (unbound plasma concentration of the agent at steady state [pCu_{ss}] = 100 and 200 μM) were examined in a placebo-controlled, dose-escalation study conducted in 134 patients with acute stroke. Patients were administered either 420 or 844 mg/h NXY-059 for 71 h after a 1-h loading dose at triple rate within 24 h of stroke onset. The pCu_{ss} value obtained for patients receiving the higher dose exceeded the target (260 ± 79 μM). Outcome scores of mortality (4 and 3 *vs.* 0), number of patients with serious adverse events (15 and 13 *vs.* 5), adverse events (42 and 39 *vs.* 29), treatment discontinuations due to adverse events (1 and 3 *vs.* 0) were similar for the low- and high-dose NXY-059 and placebo groups, respectively. Good outcomes were 53 and 29% in the low- and high-dose groups as compared to 40% in placebo. However, further analysis revealed that the differences in outcome between groups were attributed mainly to baseline differences in age and severity of stroke for patients. One hemorrhagic transformation occurred in a patient receiving low-dose NXY-059. No clinically significant changes in body temperature, blood pressure or other laboratory parameters were noted. It was concluded that NXY-059 was well tolerated at these doses which are adequate for subsequent efficacy trials (20).

NXY-059 is completing phase IIa trials for ischemic stroke (21, 22).

Sources

Discovered by the Oklahoma Medical Research Foundation, Oklahoma City, OK (US) and the University of Kentucky, Lexington, KY (US); licensed to Centaur Pharmaceuticals, Inc. (US) in codevelopment with AstraZeneca plc (GB).

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