Tolerability of NXY-059 at Higher Target Concentrations in Patients With Acute Stroke

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Background and Purpose—NXY-059 is a nitrone-based free radical–trapping agent in development for acute stroke. In patients with acute stroke, NXY-059 is well tolerated at concentrations known to be associated with neuroprotection in animal models of transient cerebral ischemia; however, higher target concentrations appear necessary on the basis of animal models of permanent ischemia.

Methods—This was a randomized, double-blind, placebo-controlled, parallel-group, dose-escalation, multicenter study that evaluated safety, tolerability, and plasma concentrations of 2 NXY-059 dosing regimens within 24 hours of acute stroke. NXY-059 was administered as either 915 mg over 1 hour followed by 420 mg/h for 71 hours or 1820 mg for 1 hour followed by 844 mg/h for 71 hours; plasma concentrations were monitored. Neurological and functional outcomes were recorded for up to 30 days.

Results—one hundred thirty-five patients were recruited, of whom 134 received study treatment and completed assessments (844 mg/h, n = 39; 420 mg/h, n = 48; placebo, n = 47). Mean age was 69 years (range, 34 to 92 years), and baseline National Institutes of Health Stroke Scale score was 8.5 (SD, 6.6). Serious adverse events occurred in 3, 17, and 13 patients, respectively, with deaths in 0, 4, and 3 patients and treatment discontinuations because of adverse events in 0, 1, and 3 patients. Good outcome, defined by modified Rankin Scale score of 0 or 1, was seen in 53%, 29%, and 40%, respectively. No safety concern was identified in analysis of body temperature, blood pressure, or other laboratory parameters. The unbound plasma concentration at steady state was 260 ± 79 µmol/L, exceeding the target of 200 µmol/L in the high-dose group.

Conclusions—NXY-059 was well tolerated in patients with an acute stroke at and above concentrations shown to be neuroprotective in an animal model when initiated 4 hours after onset of permanent focal ischemia. (Stroke. 2003;34: 482-487.)

Key Words: free radicals ■ neuroprotection ■ NXY-059 ■ safety

There has been recent criticism of the strategy generally used to develop neuroprotective drugs for acute stroke. In particular, pharmaceutical companies have been criticized for abandoning laboratory data on pharmacological issues such as dose when proceeding to clinical trials.1 It takes time for lessons to be learned from clinical trials, however, and recent recommendations from academic and industry experts such as those of the Stroke Therapy Academic Industry Roundtable (STAIR) group2 have not yet been evaluated in the field. NXY-059 is a novel nitrone-based free radical–trapping agent that is under development for use in acute stroke.3–7 This is 1 of the first compounds to be evaluated against the STAIR recommendations and to incorporate suggestions based on recent trial experience.1,8,9 As part of that package, although NXY-059 was already known to be tolerated by stroke patients10 at concentrations of 8 to 40 µmol/L, which are associated with neuroprotection in a rat model of transient focal cerebral ischemia,3 there was a need to evaluate tolerability at and, if appropriate, above the higher concentrations of 50 to 150 µmol/L needed to achieve a dose-dependent increased degree of neuroprotection in the more rigorous permanent focal ischemia model.4 This phase Ila trial was designed to establish tolerability and plasma concentration data at that higher target concentration.

Subjects and Methods

Study Design

This was a multicenter, double-blind, placebo-controlled, dose-escalation trial with central randomization in which an interactive voice response system (Clinphone) was used. The trial was con-
ducted in 2 parts. In the first, patients were randomized between placebo and an intermediate dose of NXY-059, ie, a dose aimed at producing concentrations approximately twice those that had previously been studied but still lower than believed necessary for maximal neuroprotection. In the second part, which took place only after favorable safety review by an independent data monitoring committee, the dose was selected to achieve and maintain unbound plasma concentrations >150 μmol/L in most patients, ie, a mean target concentration of 200 μmol/L. For both stages of this dose-escalation study, unequal randomization was used, favoring active treatment over placebo in a 2:1 ratio. Patients were stratified according to a composite score that was based on age and stroke severity to maintain balance on prognostic variables across the treatment groups. Because a dose-escalation design was used with recruitment continuing during safety analysis of the first dose group, the number of patients at this dose could exceed the number recruited to the higher dose cohort.

Serious adverse events (SAEs) were reported by investigators according to the standard objective criteria; on-site monitoring of source documents was used to ensure complete reporting. Adjudication of SAEs was completed before study unblinding took place. A data safety monitoring board (DSMB) comprising 2 independent clinicians and 1 independent statistician (see the Appendix) had access to unblinded information and undertook formal safety reviews of the data throughout the trial. Individual data from previous clinical trials with NXY-059 were also provided to the DSMB. Using the reviews, the DSMB was responsible for deciding whether and at what stage dose escalation could take place. The DSMB members had no other role in the design, conduct, or analysis of the trial or in the development of NXY-059.

Patients
Patients with a clinical diagnosis of acute ischemic stroke confirmed by CT scan within the last 24 hours were considered eligible. Stroke symptoms had to have been present for at least 1 hour and still be present at the start of treatment with a measurable deficit on the National Institutes of Health Stroke Scale (NIHSS).11,12 Exclusion criteria were reduced level of consciousness or evidence of cerebral herniation, known severe hepatic disorder, known or estimated creatinine clearance of <30 mL/min, alcohol or substance abuse, women in whom recent or current pregnancy could not be excluded, and other presence of significant life-threatening conditions. Patients were also excluded if they had pathology other than cerebral infarction on any admission imaging tests, if they had participated in a trial of an investigational drug or thrombolytic agent since admission to the hospital, or if they had been started on antihypertensive therapy after stroke onset. In addition, patients were withdrawn from study treatment if they had been incorrectly included, if the patient experienced an intolerable adverse drug reaction, if creatinine clearance fell to <30 mL/min, if a concomitant illness developed or required treatment that would interfere with the trial, or if the patient asked to be withdrawn. Patients who withdrew from continued treatment were encouraged to complete follow-up assessments. Stratification used at randomization divided patients into 2 strata reflecting the mortality risk at baseline according to age and the NIHSS motor scores.

Study Treatment
Before randomization, the creatinine clearance was calculated according to the formula of Cockcroft and Gault.13 Patients with an estimated creatinine clearance of <30 mL/min were excluded because no data were available on safety, tolerability, or pharmacokinetics at this level of renal function and because NXY-059 is predominantly cleared by urinary excretion.6,7

Study treatment was given as a continuous intravenous infusion for 72 hours, including a 1-hour loading dose. A clear, phosphate-buffered, nonviscous solution was used as placebo. Study treatments

| TABLE 1. Incidence of Adverse Events, Serious Adverse Events (SAE), and Deaths; n (%) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | NXY-059 844 mg/h | NXY-059 420 mg/h | Placebo Total   | Placebo Part 1  | Placebo Part 2  |
|                                | (n=39)          | (n=48)          | (n=47)          | (n=25)          | (n=22)          |
| No. of adverse event reports   | 71 (1.8)        | 144 (3.0)       | 130 (2.8)       | 83 (3.3)        | 47 (2.2)        |
| (events per patient)           |                 |                 |                 |                 |                 |
| No. of patients (%) with adverse event(s) | 29 (74%) | 42 (88%) | 39 (83%) | 22 (88%) | 17 (77%) |
| No. of SAE reports*            | 3               | 25              | 15              | 9               | 6               |
| No. of SAE reports assessed as related to study treatment | 0 | 1 | 3 | 2 | 1 |
| Deaths (% of patients)†        | 0 (0%)          | 4 (8%)          | 3 (6%)          | 3               | 0               |

*Each report could include more than one SAE and each patient could have more than 1 SAE report.
†All deaths occurred after end of the study treatment.

| TABLE 2. Serious Adverse Event (SAE) Reports,* Grouped by Main Body System and Time of Occurrence |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | NXY-059 844 mg/h | NXY-059 170 mg/h | Placebo         |
|                                | (n=39)          | (n=48)          | (n=47)          |
| SAE Reports†                   | Treatment Day 4–30 | Treatment Day 4–30 | Treatment Day 4–30 |
| Cerebral conditions            | 0 1             | 4 1             | 2 1             |
| Cardiovascular conditions      | 0 2             | 4 3             | 2 4             |
| Respiratory conditions         | 1 0             | 5 2             | 2 3             |
| Other conditions               | 0 0             | 3 3             | 1 0             |
| Total                          | 0 3             | 16 9            | 7 8             |

*Each report could include more than one SAE and each patient could have more than 1 SAE report.
†Main diagnosis grouped by clinical conditions.
Of the 43 SAE reports, 4 were recorded by investigators as causally related to study treatment: 3 after placebo, and 1 after NXY-059 420 mg/h. Of the 15 SAE reports in the placebo group, 9 occurred in part 1 (1 cerebral, 4 cardiovascular, 3 respiratory, and 1 other conditions) and 6 occurred in part 2 (2 cerebral, 2 cardiovascular, 2 respiratory).
were prepared for infusion by dilution in a 500-mL bag of 0.9% sodium chloride solution. The infusion rates for active treatment were 420 and 844 mg/h, with 1-hour loading infusions of 915 and 1820 mg/h, respectively. The patients with creatinine clearance values of 51 to 80 mL/min had a reduction in the maintenance infusion dose of 38%; patients with creatinine clearance of 30 to 50 mL/min had the maintenance dose reduced by 61%.

The infusion rates were chosen on the basis of the results of volunteer studies and a prior study in stroke patients. The projected mean target unbound plasma concentrations were 100 and 200 μmol/L in the 2 dose groups of NXY-059. Hence, the dose chosen for the higher-dose group aimed to attain NXY-059 unbound plasma concentrations >150 μmol/L in most patients. Based on the results in a rat model of permanent focal ischemia, exposures ≥150 μmol/L appear to have the potential to provide the most prominent neuroprotective effects within 4 hours of ischemia onset.

Outcome Measures
A CT or MRI brain scan was performed before randomization to confirm the diagnosis. Vital signs were recorded on admission and at intervals throughout the dosing period. A 12-lead ECG was obtained at admission, 24 to 30 hours after the start of treatment, and 4 to 7 days after the end of infusion (7 to 10 days after stroke). Blood samples for clinical chemistry and hematology were collected on admission and after 1, 3, and 7 to 10 days. All adverse events were recorded within the first 7 to 10 days and were followed up until day 30. NIHSS score was recorded on admission and after 1, 3, and 7 to 10 days.11,12 Barthel Index and modified Rankin Scale scores were recorded after 7 to 10 and 30 days14; these assessments were collected for descriptive purposes only.

Blood samples were collected at baseline and at 0.5, 1, 24, and 72 hours after the start of treatment for analysis of NXY-059 concentration; up to 4 additional samples after the end of infusion were collected in selected centers. Plasma concentrations of NXY-059 were determined by high-performance liquid chromatography at a central laboratory. The limit of quantification was between 0.08 and 0.2 μmol/L, with a coefficient of variation of <5% in the concentration range of 76.1 to 501 μmol/L for plasma samples. Free concentrations of NXY-059 were determined by use of ultrafiltration; the limit of quantification was 0.08 μmol/L with a coefficient of variation of <2.44% at a total plasma concentration of 400 μmol/L.

Study sites were monitored regularly for data verification and compliance with the protocol. Study blinding was maintained until the database was locked. Until that time, only the independent DMSB had access to unblinded data.

Statistical Analysis
The analysis of safety was based on all patients who received study drug and had any postrandomization safety data available. All adverse events were analyzed, whether or not they were considered typical complications of stroke. The evaluation of clinical adverse events, including deaths, and laboratory adverse events used statistical tests and estimation procedures as descriptive indicators of treatment group differences.

One patient in the 844-mg/h NXY-059 group was diagnosed with encephalitis 1 day after randomization and discontinued study drug at that time. The analysis of efficacy was based on all other patients who received study drug and who had any postrandomization outcome data available. The efficacy of the 2 doses of NXY-059 and placebo was compared by calculating odds ratios and associated 95% confidence intervals for different definitions of a favorable outcome on the NIHSS, Barthel Index, and modified Rankin Scale, but the study was neither designed nor powered to determine efficacy. Because of imbalances in stroke severity within and among groups, stratified analyses were used for mortality, adverse event incidence, and categorized results for favorable outcome at last rating.

Results
This study began in August 2000 and was completed 9 months later, with 11 active centers (4 in the United Kingdom, 4 in Sweden, 3 in Germany) recruiting between 2 and 27 patients each (UK, 84 patients; Germany, 25; Sweden, 26). One hundred thirty-five patients were randomized, but 1 patient allocated placebo was discontinued before treatment began. A further 11 patients were enrolled but not randomized: 9 because of ineligibility and 2 who withdrew consent. The first part of the study (NXY-059 420 mg/h versus placebo) was intended to include only ≈30 patients but, on the advice of the DSMB, was expanded. In total, 74 patients

### Table 3. Distribution of Outcome According to Barthel Index Scores at Day 30/Last Observation Carried Forward

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Good 95–100</th>
<th>Moderate 60–90</th>
<th>Poor 0–55</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>NXY-059 444 mg/h (n = 38)</td>
<td>22 (58%)</td>
<td>8 (21%)</td>
<td>8 (21%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>NXY-059 442 mg/h (n = 48)</td>
<td>22 (46%)</td>
<td>7 (15%)</td>
<td>15 (31%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Placebo part 1 (n = 25)</td>
<td>11 (44%)</td>
<td>5 (20%)</td>
<td>6 (24%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Placebo part 2 (n = 22)</td>
<td>11 (50%)</td>
<td>6 (27%)</td>
<td>5 (23%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Placebo total (n = 47)</td>
<td>22 (47%)</td>
<td>11 (23%)</td>
<td>11 (23%)</td>
<td>3 (6%)</td>
</tr>
</tbody>
</table>

All patients eligible for the efficacy analyses. There was no statistically significant difference between placebo and active groups.
(48 active, 26 placebo) were included before progression to part II (844 mg/h versus placebo; 39 active, 22 placebo).

The mean ± SD age of the patients was 69 ± 11 years (range, 34 to 92 years); 58% were male. Patients had a mean weight of 76 ± 15 kg (range, 42 to 110 kg) and a mean baseline NIHSS score of 8.5 ± 6.6 to 4.6 ± 2.9 in stratum 1 and 13.4 ± 6.8 in stratum 2. Of the patients, 11% had current atrial fibrillation, and 13% had current diabetes mellitus; 50% were hypertensive. Treatment began at a mean of 15 hours after symptom onset, with no patient being treated <4 hours from stroke onset. Within the low-dose group, 10 received the full maintenance dose, 20 had maintenance dose reduction by 38%, and 18 had maintenance dose reduction by 61% as a result of their creatinine clearance; in the higher-dose group, the numbers were 17, 14, and 8, respectively.

The patients were well balanced between treatment groups with respect to large middle cerebral artery stroke and urinary continence, but the mean NIHSS score was slightly lower in the high-dose group (6.9 ± 5.7) compared with the lower-dose and placebo groups (9.9 ± 7.0 and 8.4 ± 6.8, respectively). German sites joined the study after the first part was concluded and recruited milder stroke patients; thus, the stratified analysis was justified. The proportions with acute infarction visible on the admission CT scan were 44%, 67%, and 54% respectively; 24% of patients across groups also had evidence of old infarction.

Safety and Tolerability
Treatment was stopped early because of adverse events in 3 patients (6%) in the placebo group and 1 patient (2%) in the low-dose NXY-059 group. There was no increase in the overall incidence of adverse events during treatment with NXY-059 compared with placebo (Table 1). There were fewer SAEs in the high-dose group than in the other 2 groups.

Fever, headache, and hypertension were common, but the incidences were similar among all groups. None of these events was clearly related to treatment. Signs of vessel or tissue irritation were evenly distributed among the 3 treatment groups and across strata. Laboratory tests of hematology or clinical chemistry showed no consistent clinically relevant trend related to treatment group allocation within the study. SAEs are summarized in Tables 1 and 2. Only 4 of the 43 SAEs were assessed by the investigators as causally related to the treatment with the investigational product. Of these patients, 3 received placebo and 1 received the low dose of NXY-059.

In total, there were 7 deaths among the 134 patients during the study, giving a total mortality rate of 5.2%. No patient died in the NXY-059 high-dose group, 4 deaths occurred in the low-dose group, and 3 patients died in the placebo group during the 30-day study period. There were no significant differences among the groups. The deaths were due to respiratory infection, sepsis, pulmonary edema, or direct results of stroke.

Vital Signs
There was no evidence for an effect of NXY-059 on blood pressure, heart rate, temperature, or ECG parameters.

Stroke Outcome
The median improvement on the NIHSS was 3 points in all treatment groups. There was no statistically significant difference between placebo and active groups with regard to outcome on NIHSS score, Barthel score ≥60 at last rating, or modified Rankin score ≤1 at last rating. The distribution of Barthel scores at final rating is shown in Table 3.

Exposure
The mean unbound steady-state plasma concentration of NXY-059 in the higher-dose group was ≈260 µmol/L (30% above the target unbound concentration of 200 µmol/L); in the lower-dose group, it was 109 µmol/L (9% above the target concentration of 100 µmol/L). The unbound fraction was 0.70 ± 0.06 and 0.67 ± 0.08 in the high- and low-dose groups, respectively. More than 90% of patients in the high-dose group had unbound concentrations of >150 µmol/L, and >25% had concentrations of >300 µmol/L.
Discussion

The primary aim of the present study was to determine whether NXY-059 would be tolerated by acute stroke patients at the higher target plasma concentration that was associated with neuroprotection in a rat model of permanent focal ischemia. The unbound plasma concentrations achieved in this study exceeded the target by 30% without any evidence of intolerance related to drug treatment. The adverse events observed were unremarkable for an acute stroke population and appeared evenly distributed across groups, including placebo. SAEs were more common in the placebo and low-dose groups, consistent with the slightly greater stroke severity in these groups. Mortality was also restricted to these 2 groups. The absence of effect on vital signs or laboratory tests was reassuring; in particular, there was no evidence of an effect on temperature, confirming that minor temperature elevations during the previous study with NXY-059 were likely to be a chance finding related to hemorrhagic stroke in that study. Thus, the strategy of undertaking cerebral imaging before randomization in this study reduced the potential confounding effect of stroke type on outcome. Follow-up CT scans were rarely undertaken, but the lack of SAEs during treatment in the high-dose group, particularly those resulting from cerebral complications, provides reassurance that hemorrhagic transformation is not a significant clinical problem.

The plasma concentrations achieved in this study compared favorably with those required for neuroprotection in both reperfusion animal models of ischemic stroke (8 to 40 mg/h).
μmol/L)\(^3\)\(^{16}\) and permanent models of ischemic stroke (50 to 150 μmol/L)\(^7\) (Figure 1). The mean unbound plasma concentrations of NXY-059 were higher than targeted, particularly in the high-dose group. This is partly a consequence of a high fraction of unbound NXY-059 in the high-dose group compared with that observed in an earlier study in stroke patients (0.61 ± 0.04)\(^10\). The clearance of NXY-059 correlated closely with creatinine clearance, thus supporting the decision to adjust the dose according to renal function. Further population kinetic analyses are planned of the pooled data from the 2 studies in stroke patients to define the appropriate dosing regimen for further studies.

This study was not designed to examine the effects of NXY-059 on stroke outcome and would have had insufficient power to do so even if the treatment window had been shorter and the follow-up duration longer. The encouraging trend at the higher-dose group also observed in the stratified analyses may still be confounded by the milder stroke severity in that group, and overall, there was no significant difference in outcome among the 3 groups (Figure 2).

A phase IIa study such as this should not be interpreted on its own. All of the data for the drug should be analyzed in a similar way and considered together. Table 4 displays an overview of selected outcomes with each of the 4 doses of NXY-059 that have been studied in acute stroke to date. It reveals that studies of this size, even with n=200 patients receiving active treatment, are much too small to draw reliable conclusions about either efficacy or safety. The most that can be concluded is that there is not significant evidence of either harm or benefit and that neither the major outcomes nor the serious adverse event data provide any suggestion of a dose-related effect, particularly on safety outcomes, for which power is higher. The dimensioning of the present study was guided by ensuring the appropriate power to detect significant differences in laboratory tests that could indicate a tolerability issue.

In conclusion, NXY-059 was well tolerated in patients with an acute stroke, and no concern was raised by the safety evaluation. NXY-059 appears to satisfy all of the criteria that have recently been suggested before formal efficacy studies in patients with acute stroke are undertaken. Whether efficacy will eventually be determined in human stroke remains to be seen. Certainly, a rigorous approach to further clinical testing is still required that uses doses that have now been shown to be tolerated in suitably selected patients initiating treatment within the same timeframe as shown to be efficacious in the animal models.

Appendix

SA-NXY-0004 Investigators

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