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A Phase I Clinical Trial of Tiopronin, a Putative Neuroprotective Agent, in Aneurysmal Subarachnoid Hemorrhage

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Abstract

BACKGROUND—The neurotoxic aldehyde 3-aminopropanal (3-AP) contributes to brain injury following cerebral ischemia. Tiopronin (N-2-mercaptopropionyl-glycine[N-2-MPG]) is a US Food and Drug Administration (FDA)-approved drug for the treatment of cystinuria and a putative

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neuroprotective agent that has been shown to bind and neutralize 3-AP and reduce infarct volumes.

OBJECTIVE—The objective of this trial was to establish the safety of tiopronin administration in patients with aneurysmal subarachnoid hemorrhage (aSAH) in preparation for further trials of its efficacy as a neuroprotective agent in this disease process.

METHODS—This Phase I dose-escalation trial enrolled three-patient cohorts using a conventional “3 + 3” study design. Tiopronin dose began at 1 g/d until aSAH Day 14. Each subsequent cohort received a dose of tiopronin based on predetermined guidelines. A maximum dose of 3 g/d was selected, because this is the maximum FDA-approved dose for long-term cystinuria treatment. Subjects were monitored for known side effects of tiopronin.

RESULTS—Nine patients were enrolled, the minimum number required based on the study design. None of these patients experienced serious side effects attributable to tiopronin, and no adverse events were noted that could not be attributed to the pathophysiology of aSAH.

CONCLUSION—The administration of 3 g/d of tiopronin following aSAH for up to 14 days appears to be safe and without the side effects associated with long-term use. Plans for a randomized, placebo-controlled Phase II trial of tiopronin for neuroprotection following aSAH are underway.

Keywords

Aneurysm; Neuroprotection; Subarachnoid hemorrhage; Tiopronin

Following aneurysmal subarachnoid hemorrhage (aSAH), cerebral vasospasm and ischemia are major causes of morbidity and mortality.^{1,2} During cerebral ischemia, polyamine oxidase activity in the brain increases significantly, leading to the production of 3-aminopropanal (3-AP), a potent neurotoxin, and the death of potentially viable brain cells in oxygen-deprived tissue.^{3,4} In rodent models of cerebral ischemia, 3-AP levels increased in a time-dependant manner concomitant with spreading neuronal and glial death.⁴ Clinically, elevated 3-AP levels have been detected in the cerebrospinal fluid (CSF) of patients with aSAH.⁵

Tiopronin (N-2-mercaptopropionyl glycine [N-2-MPG], marketed as Thiola in the United States, is a U.S. Food and Drug Administration (FDA)–approved drug for the treatment of cystinuria that has been shown to bind and neutralize 3-AP in vitro.⁵ Intraperitoneal treatment with tiopronin in a rodent model of cerebral ischemia resulted in reduced infarct volumes compared to vehicle.⁵ The role of 3-AP in neurologic injury and the potential therapeutic implications of inhibiting its cytotoxic effects remain to be studied in humans. In preparation for further trials of its efficacy as a neuroprotective agent in a critically ill population, we conducted a prospective, nonrandomized, open-label, dose-escalation study to determine the safety of tiopronin administration in patients with aSAH.

PATIENTS AND METHODS

FDA and institutional review board approval was obtained for this Phase I study. Forty-six patients admitted with aSAH to the neurologic ICU at Columbia University Medical Center between July 2006 and August 2007 were screened for enrollment. Of these, 9 subjects

ultimately were enrolled and received the study drug. Inclusion and exclusion criteria are shown in Table 1. The most common reason for not enrolling a screened patient was inability of the patient to provide informed consent. Only patients with an aneurysmal etiology of SAH, confirmed via CT or conventional angiography, were enrolled in this study. The study design was based on a conventional “3 + 3” dose-escalation model, with the initial 3 patients receiving 1 g/d of oral tiopronin divided three times daily beginning on the day of enrollment until Day 14 after the aSAH ictus. Each subsequent cohort received a dose of tiopronin determined by the guidelines in Table 2. A maximum dose of 3 g/d was pre-selected during the design of the study, because this is the highest FDA-approved dose of tiopronin. All enrolled subjects were administered the study drug within 96 hours of aSAH onset.

All patients received standard-of-care treatment for aSAH, with definitive surgical or endovascular repair of their aneurysm, and hypertensive-hypervolemic therapy or endovascular therapy for cerebral vasospasm. Patients were evaluated on a daily basis and assessed for potential adverse effects of tiopronin, which were used as drop-out criteria (Table 3). The primary endpoint of this safety study was the occurrence of adverse events attributable to tiopronin administration. Secondary endpoints of interest were functional outcome at discharge or aSAH day 14, whichever occurred first, and infarct from cerebral vasospasm. Adverse events were recorded by the study coordinator. A drug safety monitoring committee (DSMC) consisting of a non-study neurosurgeon, a non-study neurologist, and a biostatistician reviewed all clinical research forms at the end of each drug tier. Functional outcome was determined by a study coordinator based on the modified Rankin Scale (mRS). Good outcome was defined as mRS 1 to 3 and poor outcome as mRS 4 to 6.

RESULTS

Demographics and clinical course for the 9 enrolled subjects are shown in Table 4. All of the patients were Hunt-Hess grades 1 to 3 on admission, with most having diffuse thin or thick sub-arachnoid blood (Fisher grade 2 or 3) on admission CT imaging. All patients underwent definitive treatment of their ruptured aneurysm.

No patients experienced significant side effects of tiopronin administration that warranted drop-out from the study. All patients completed a full course of the study drug, except Patient 2, who experienced fever for 6 days without an obvious cause. Tiopronin was withheld from Patient 2 on aSAH day 12; however, the fever continued and ultimately was attributed by the clinical team to presumed ventriculitis and therefore was not considered a side effect of tiopronin by the DSMC.

Five of the 9 (55.6%) patients had evidence of cerebral vasospasm during their neurologic ICU course (Table 4). Of these, only 3 patients had documented angiographic vasospasm. A total of 3 patients exhibited clinical signs of cerebral ischemia (2 of them with angiographic vasospasm). No patients in the study developed infarct from cerebral vasospasm. At discharge, all patients had a favorable outcome of mRS 1 to 3, except for Patient 2, who had developed ventriculitis during the course of her hospital admission and had a discharge mRS

of 5, and Patient 9, who experienced a rebleed before her ruptured aneurysm was secured and had a discharge mRS of 4. Although outside the scope of the original design of this Phase I trial, long-term functional outcomes were obtained for most of the patients enrolled in this study (Table 4). Of the 8 patients with 3-month data and 6 patients with 12-month data available, only 1 patient (Patient 2) had a poor outcome at these time points. Five of 6 (83.3%) patients with available data had an mRS of 0 or 1 at 12 months post-aSAH.

DISCUSSION

During cerebral ischemia, the induction of polyamine oxidase and subsequent development of neurotoxic aldehydes, such as 3-AP, contributes to neuronal death. Elevated CSF levels of 3-AP have been correlated with the severity of cerebral injury in aSAH.⁵ Tiopronin, an FDA-approved drug for the treatment of cystinuria, has been shown in preclinical studies to bind and inactivate 3-AP in vitro and reduce infarct volumes in vivo.⁵ Although these studies fulfilled only 3 of the 6 Stroke Therapy Academic Industry Roundtable (STAIR) criteria for neuroprotective drugs, clinical data published strongly correlate the levels of the toxin 3-AP with clinical grade of subarachnoid hemorrhage in humans.⁶ Due to a high incidence of delayed cerebral ischemia, aSAH provides an ideal model to test potential neuroprotective agents, especially since agents can be administered before the onset of ischemia.

The aim of this Phase I study was to provide sufficient evidence that short-term (14 d) tiopronin administration is safe in this population. The study was based on a conventional “3 + 3” dose-escalation design, and the initial dose of 1 g/d for the first patient cohort was titrated for each subsequent cohort based on pre-designated criteria (Table 2). In total, 9 patients with aSAH were enrolled in this study. In general, the demographics of the cohort were consistent with the demographics of aSAH overall. All enrolled patients were of good or intermediate Hunt-Hess grade on admission due to the requirement that patients had to be able to consent for themselves in order to participate. Patients were monitored daily for the development of known significant side effects of tiopronin, all of which are sequelae of long-term (>3 mo) treatment.^{7,8} Development of any of these side effects mandated the prompt cessation of the study drug and reporting to the DSMC. No patients in this study, however, experienced these adverse effects. One patient, receiving 1 g/d of tiopronin, had the study drug discontinued on SAH Day 12 due to a fever of unknown origin, however, the fever persisted and was attributed to presumed ventriculitis by the clinical team and DSMC. No other subjects had the study drug discontinued, and the maximal FDA-approved dose of 3 g/d was attained with the minimum number of patients.

While no patient experienced side effects attributable to tiopronin, several patients experienced known neurologic or medical complications of aSAH. Five patients demonstrated transcranial Doppler or angiographic evidence of cerebral vasospasm during their time in the neurologic ICU; however, only 3 patients exhibited clinical symptoms of cerebral ischemia. No patients developed infarction from cerebral vasospasm. Infections were the most prevalent medical complications in our cohort, including 7 urinary tract infections, 1 case of pneumonia, and 1 instance of sepsis. Most of the patients had good discharge outcomes (mRS 1–3), with two notable exceptions: Patient 2 developed

ventriculitis and was discharged with an mRS of 5, and Patient 4 suffered an aneurysmal rebleed and was discharged with an mRS of 4. Of patients with available long-term outcome data, only Patient 2 continued to have a poor outcome at 3 and 12 months post-aSAH.

Given the noncontrolled, nonrandomized nature of this study, it is impossible to ascertain whether the administration of tiopronin improved outcome following aSAH. Our results, however, indicate that short-term (14 d) administration of tiopronin to patients with aSAH up to the maximum FDA-approved dose of 3 g/d appears to be safe, with no adverse effect on outcome. Plans for a randomized, placebo-controlled, Phase II trial to establish that tiopronin crosses the blood-brain barrier in humans and reduces CSF concentration of the neurotoxin 3-AP are underway. This trial will also continue to monitor the safety of the drug in the aSAH population.

ABBREVIATIONS

3-AP	3-aminopropanal
aSAH	aneurysmal subarachnoid hemorrhage
DSMC	drug safety monitoring committee
mRS	modified Rankin Scale

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TABLE 1**Phase I Inclusion and Exclusion Criteria^a**

Inclusion Criteria
<i>Patient 18 years or older</i>
<i>Admitted to the hospital with acute (within 96 hours) SAH diagnosed by head CT or lumbar puncture</i>
<i>Aneurysmal etiology of SAH confirmed by CT angiography or conventional angiography, or during surgical intervention</i>
<i>Ability of patient to give consent</i>
Exclusion Criteria
<i>Known hypersensitivity to penicillamine</i>
<i>Creatinine level >1.5 mg/dL on admission</i>
<i>Platelet count <100,000/μL on admission</i>
<i>White blood cell count <3500/mm³ on admission</i>
<i>AST or ALT >60 U/L on admission, or history of liver failure</i>
<i>Pregnancy</i>
<i>History of systemic lupus erythematosus, Goopasture syndrome, myasthenia gravis, pemphigus vulgaris, nephrotic syndrome, glomerulonephritis, or renal failure</i>

^a SAH, subarachnoid hemorrhage; AST, aspartate amino transferase; ALT, alanine amino transferase.

TABLE 2Dosing Guidelines based on “3 + 3” Dose-escalation Study Design^a

1. If none of a cohort of 3 pts experiences SE, the dose will be increased by 1 g for the next cohort.
2. If one of a cohort of 3 pts experiences SE, the dose will remain the same for the next cohort.
3. If 2 or 3 pts experience SE, the dose will be reduced by 500 mg for the next cohort.
4. If 2 out of 6 consecutive pts (2 cohorts of 3 pts each, all at the same dose) experience SE, the dose will be reduced by 500 mg.
5. If, at the dose of 500 mg, 2 or more pts of a cohort of 3, or 2 or more out of 6 consecutive pts experience SE, the study will be terminated.
6. If 2 or more out of a cohort of 3, or 2 or more out of 6 consecutive pts experience SE at the prior dose, then the highest dose at which 0 out of 3 or 1 out of 6 consecutive pts experienced SE will be used to execute the study outlined below. The exception to this is guideline #5.
7. If 0 of 3 or 1 of 6 consecutive pts experience significant side effects at a dose that had just previously been reduced (ie, 2/3 or 2/6 patients experienced SE at the prior dose), then that dose will be used in Phase II.
8. If a dose of 3 g/d is reached, the dose escalation will be stopped and this dose will be used in Phase II.

^a pts, patients; SE, side effect

TABLE 3Significant Side Effects Considered to be “Drop-out Criteria” ^a

Based on laboratory results	
Leukopenia	2 consecutive white blood cell counts <3500/mm ³
Thrombocytopenia	2 consecutive platelet counts <100 000/mm ³
Renal failure	2 consecutive serum creatinine levels >50% rise above admission creatinine levels
Proteinuria	2 consecutive urinalyses showing >2+ protein followed by a 24-hour urine protein >200 mg/d ^b
Based on clinical signs	
Laryngeal edema	
Hemoptysis	
Diagnosis of myasthenic syndrome	
Persistent diarrhea that cannot be attributed to any other source	
Fever that cannot be attributed to any other source	
Pemphigus-type reaction	
Based on clinical signs followed by laboratory results	
Liver failure: jaundice and 2 consecutive serum AST and ALT levels demonstrating >50% rise above admission levels	
Lupus-like drug reaction: fever, lymphadenopathy, positive antinuclear antibody test	

^a AST, aspartate amino transferase; ALT, alanine amino transferase.^b 2+ corresponds to an abnormal value approximately above 100 mg.

Patient Demographics, Hospital Complications, and Functional Outcomes^a

TABLE 4

Patient	Age, y	Sex	H&H Grade	Fisher Grade	Operation	Clinical CVS	Angio CVS	TCD CVS	CVS Vessel	Infarct From CVS	Other Complications	D/C or 14-day mRS	3-Month mRS	12-Month mRS	Tiopronin Dose, g/d
1	47	F	1	1	Coil	N	N	N	N/A	No	CSW, UTI	0	2	__ <i>b</i>	1
2	74	F	3	3	Clip	N	N	N	N/A	No	Ventriculitis	5	5	6	1
3	43	F	1	2	Coil	Y	Y	Y	R MCA	No	CSW, UTI	3	1	1	1
4	45	F	3	2	Coil	N	N	N	N/A	No	None	1	0	0	2
5	46	M	1	3	Clip	N	N	N	N/A	No	UTI	2	0	0	2
6	43	F	2	3	Clip	N	N	Y	L MCA	No	UTI	1	2	__ <i>b</i>	2
7	44	F	3	4	Clip	Y	N	Y	R MCA	No	UTI, PNA, sepsis	1	1	1	3
8	45	F	1	2	Clip	N	Y	N	Diffuse	No	UTI	1	__ <i>b</i>	__ <i>b</i>	3
9	43	F	3	3	Coil	Y	Y	Y	Diffuse	No	CSW, HCP, UTI, fever, rebleed	4	1	1	3

^aH&H, Hunt-Hess; CVS, cerebral vasospasm; TCD, transcranial Doppler ultrasonography; R MCA, right middle cerebral artery; L MCA, left middle cerebral artery; CSW, cerebral salt-wasting; UTI, urinary tract infection; PNA, pneumonia; HCP, hydrocephalus; D/C, discharge; mRS, modified Rankin Scale score.

^bFollow-up data not available.