Reversal and prevention of cerebral vasospasm by intracarotid infusions of nitric oxide donors in a primate model of subarachnoid hemorrhage

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More than 28,000 Americans suffer from subarachnoid hemorrhage (SAH) caused by a ruptured intracranial aneurysm each year. Severe vasospasm of the cerebral arteries, which can be seen on arteriography, often develops 4 to 9 days after SAH and is almost the exclusive cause of the late occurrence of morbidity or mortality in otherwise successful treatment of intracranial aneurysm. Despite intensive research spanning more than 40 years, the etiology of cerebral vasospasm is poorly understood, and there is no successful targeted treatment for it.

Nitric oxide (NO), or endothelium-derived relaxing factor, is a potent vasodilator. Its continuous release is required to maintain basal cerebrovascular tone. Blood breakdown products, particularly oxyhemoglobin, present in the subarachnoid space after SAH have been implicated in the pathogenesis of cerebral vasospasm. Oxyhemoglobin binds NO because NO’s high affinity for the heme iron converts NO to nitrate or nitrite. This binding or loss of NO is a potential cause of cerebral vasospasm. In support of this hypothesis, reversal of vasospasm has been demonstrated in a primate model of SAH by intracarotid infusion of NO. However, NO is an unstable compound with an in vivo half-life estimated to be between 100 msec and 5 seconds. Recently, Keefer, et al., and Maragos, et al., developed a class of NO-donating compounds with predictable kinetic behavior in vivo, called NONOates. We sought to determine whether an intracarotid infusion of a NONOate solution could acutely reverse cerebral vasospasm and whether a long-term intracarotid infusion of NONOate could prevent the occurrence of cerebral vasospasm in a primate model of SAH. We investigated three NONOates: diethylamine-NO (half-life 2.3 minutes), glucantime-NO (half-life 0.8 minutes), and proli-NO (half-life 1.8 seconds).

Key Words • nitric oxide • cerebral vasospasm • cerebral blood flow • nitric oxide donor • subarachnoid hemorrhage • Macaca cynomolgus
Materials and Methods

Animal Groups

This protocol was reviewed by the National Institute of Neurological Disorders and Stroke Animal Care and Use Committee, and met National Institutes of Health guidelines for animal care.

Acute Infusion Study. Eight cynomolgus monkeys (five males and three females), each weighing 2.3 to 4.1 kg, were studied. Four animals underwent craniotomy for whole-blood clot placement around the right middle cerebral artery (MCA) to induce vasospasm. The remaining four animals served as controls.

Long-Term Infusion Study. Thirteen cynomolgus monkeys (eight males and five females), each weighing 2.7 kg to 4.8 kg, were randomly divided into three groups: control/saline infusion (four animals), glucantime-NO infusion (three animals), and proli-NO infusion (six animals). All of the animals underwent placement of a catheter with the tip located in the cervical bifurcation of the right common carotid artery and a right frontotemporal craniectomy for clot placement around the right MCA to induce vasospasm. Intracarotid infusion was started 1 day after clot placement and was continued until additional cerebral arteriography was performed on Day 7.

Surgical Procedures

Arteriography. For arteriography, as described elsewhere, the monkeys were anesthetized by an intramuscular injection of ketamine (10 mg/kg) and xylazine (Rompun, 1 mg/kg). Cerebral arteriograms were obtained 2 to 4 days before surgery and on postoperative Day 7 to assess the degree of vasospasm. During arteriography the PCO2 concentration measured in the exhaled air remained between 38 and 42 mm Hg. A right femoral artery cutdown was performed using aseptic techniques, and a No. 3 (animals weighing < 5 kg) or No. 4 (animals weighing > 5 kg) French polyethylene catheter was advanced via the external jugular vein. The catheter was advanced under fluoroscopy and was connected to the pump. Continuous infusion (six animals) and proli-NO infusion (six animals). All of the animals underwent placement of a catheter with the tip located in the cervical bifurcation of the right common carotid artery and a right frontotemporal craniectomy for clot placement around the right MCA to induce vasospasm. Intracarotid infusion was started 1 day after clot placement and was continued until additional cerebral arteriography was performed on Day 7.

Craniecotomy for CBF Measurements. As described elsewhere, after general anesthesia had been induced in the monkeys, a right parietotemporal craniectomy was performed using aseptic techniques, and a CBF probe was placed subdurally over the right temporal lobe for continuous measurement of cortical CBF.

Transcranial Doppler Blood Velocity Measurements

Transcranial Doppler measurements of CBF velocities in the right MCA were made through an anterior temporal window at a depth of 29 to 39 mm. A 2-MHz pulsed bidirectional Doppler probe was used to measure systolic, mean, and diastolic CBF velocities.

Preparation of NO Donor Solutions

Acute Infusion Study. Sterile normal saline was buffered with phosphate-buffered saline to a pH of 6.8 to 7.2. The NONOate used was diethylamine-NO (half-life of 2.3 minutes at a pH of 7.4). A stock solution of 10 mM diethylamine-NO dissolved in 10 M NaOH was prepared. Directly before infusion, the diethylamine-NO solution was diluted to 10 μM with the sterile buffered saline that had previously been placed in a heated water bath. The control solution consisted of buffered sterile saline and diethylamine solution (10 μM) in saline.

Long-Term Infusion Study. The NONOates used in this phase were glucantime-NO (half-life of 0.8 minutes at a pH of 7.4) and proli-NO (half-life of 1.8 seconds at a pH of 7.4). Each NONOate was diluted to a final 1-μM concentration. Glucantime-NO was mixed with saline buffered to a pH of 11.1. The proli-NO was mixed with sterile saline buffered with NaOH to a pH of 10.5. After sterile filtering, the solution was placed in the D-infusor pump, which was primed for 20 minutes and then connected to the proximal end of the intracarotid catheter.

Arteriographic Measurements

Images captured on cerebral AP arteriograms were measured by using a computerized image analysis program. A 14-mm area encompassing the proximal portion of the right MCA (the region that corresponded to the part of the artery that was surgically stripped of arachnoid) was measured from its origin to the trifurcation at the insula. Preoperative arteriographic measurements in each animal were compared with measurements made on the Day 7 arteriogram. The degree of vasospasm was graded based on a comparison of the pre- and postoperative AP views of the right MCA. Vasospasm was defined by the percentage of vessel lumen narrowing: 11 to 25%, mild vasospasm; 26 to 50%, moderate vasospasm; and greater than 50%, severe vasospasm. Vasospasm is induced in 95% of animals by using this model. Typical variations in the spastic response of the animals, variations in the amount of blood that remains around the vessel, and other unknown variables. The results are presented as the mean ± standard deviation of the measurements by three researchers blinded to the animal groups.

Experimental Design of the Acute Infusion Study

After induction of anesthesia with ketamine and isoflurane, control and SAH (Day 7 post-surgery) monkeys underwent right and left femoral artery cutdowns and cannulation of both arteries with No. 3 polyethylene catheters. The right catheter was advanced under flu-
oroscopic guidance into the right ICA to facilitate administration of contrast medium for cerebral arteriography and the left catheter was used for arterial blood pressure monitoring.

For measurement of CBF a right parietotemporal burr hole was made using aseptic techniques and a subdural thermal CBF probe was inserted over the right temporal lobe. The position of the probe was verified fluoroscopically and a test bolus of intracarotid saline (1 ml), which elicited a change in CBF, confirmed that the thermal probe was located over cortex supplied by the right MCA. Following arteriography performed on Day 7 after SAH, each animal received four to six intracarotid infusions of 1) saline, 2) diethylamine, and 3) diethylamine-NO solution. Infusions were administered for 3 minutes at 1 ml per minute with a 30-minute interval between infusions to ensure stable preinfusion CBF. Cerebral arteriography was performed immediately after the cessation of the diethylamine-NO infusions in all four animals with vasospasm. Systolic CBF velocities of the right MCA were obtained before, during, and after diethylamine-NO infusions in two vasospastic animals. Cerebrovascular resistance was calculated as mean arterial blood pressure divided by CBF.

**Experimental Design of Long-Term Infusion Study**

After a control arteriogram was obtained, a right intracarotid catheter was introduced and a clot was placed around the right MCA in each monkey. Each monkey was sedated daily by intramuscular administration of ketamine (10 mg/kg) so that the delivery pump could be changed and freshly prepared drug and/or saline added; at that time the catheter was flushed with 250 IU heparin in 1 ml 0.9% saline and the animal’s blood pressure was recorded three times (the results are presented as a mean ± standard deviation of the mean arterial blood pressure). Infusions of freshly prepared glucantime-NO (1 μM, pH 11.1), proli-NO (1 μM, pH 10.5), or saline with heparin (250 IU heparin) were started 24 hours after periarterial subarachnoid clot placement. Glucantime-NO was infused via a dual pump system (two infusions of 20 ml over 24 hours with a D-infusor). One pump delivered glucantime-NO mixed with saline buffered to a pH of 11.1 and the second delivered an equal volume of acidified saline (pH 4.5–5.6). The lines came together approximately 15 cm proximal to the tip of the intracarotid catheter and the pH of the mixed solutions was approximately 7.4 at this point. Proli-NO was mixed with buffered saline (1 μM, pH 10.5) and infused via a single pump system (60 ml over 24 hours with a D-infusor). The pumps delivered 40 ± 5 ml of solution daily.

**Statistical Analysis**

The data were analyzed using analysis of variance and a paired t-test. Significance was accepted at p < 0.05.

**Sources of Supplies and Equipment**

The Conray 60% contrast medium was obtained from Mallinckrodt Medical Inc., St. Louis, MO. Fluoroscopy was performed using the C-Arm from OEC Medical Systems, Inc., Salt Lake City, UT. Radiopaque tubing (Pert-3.0) was purchased from the Cook Group Co., Bloomington, IN. The infusion pump used in the surgical procedures and the long-term infusion study was the D-infusor pump, manufactured by Disetronic Medical Systems, Minneapolis, MN; the infusion pump used in the acute infusion study was obtained from Harvard Apparatus, Millis, MA. The CBF probe used for measurement of cortical CBF was Saber Thermomonitoring, obtained from Flowtronics, Phoenix, AZ, and the bidirectional Doppler probe was Transpect TCD, from Medasonics, Mountain View, CA.
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Results

Reversal of Delayed Cerebral Vasospasm by Acute Intracarotid Infusion of an NO Donor

Delayed cerebral vasospasm was produced in four cynomolgus monkeys (three males and one female), each weighing 2.3 kg to 4.1 kg, by surgical placement of a clot of autologous blood around the intracranial right MCA,6,22 and four additional monkeys (two males and two females), each weighing 3.5 to 5.6 kg, were used as a control group. On Day 7 after SAH, a postoperative AP arteriogram revealed that the area of the proximal right MCA had decreased by 35 ± 12% (range 22–57%) compared with the area measured on the preoperative arteriogram (Fig. 1), signifying mild (11–25%) to severe (>50%) vasospasm in all animals in which clots were placed. Arteriography performed immediately after cessation of intracarotid diethylamine-NO infusion revealed that vasospasm had been reversed in all animals (Fig. 2) with the proximal right MCA decreased only by 8.4 ± 4.3% (range 6–14%; p < 0.004) compared with the preoperative values (Fig. 1). During intracarotid infusions of saline and diethylamine solution, there was no significant change in the right MCA or rCBF in control or vasospastic animals (Fig. 3 upper). However, during intracarotid diethylamine-NO infusion, rCBF increased 13 ± 1.6% (p < 0.002) in control animals and 31 ± 1.9% (p < 0.002) in vasospastic animals (Fig. 3 upper). Regional CBF returned to preinfusion values within 5 to 25 minutes after diethylamine-NO infusion. During diethylamine-NO infusions, peak systolic right MCA CBF velocity decreased from 141 to 50 cm/second in one animal and from 119 to 81 cm/second in a second animal, and returned to preinfusion velocities within 20 minutes postinfusion (Fig. 3 lower). The mean systemic arterial pressure measured in the animals during infusion dropped 5 mm Hg (p < 0.02) by the end of the 3-minute infusion. Concurrent with this drop in mean blood pressure, cerebrovascular resistance (mean blood pressure/CBF)31 in the distribution of the infused ICA decreased as well (from preinfusion value 2.02 ± 0.02 mm Hg/ml/100 g/minute to 1.45 ± 0.02 mm Hg/ml/100 g/minute; p < 0.001).

Prevention of Cerebral Vasospasm by Long-Term Intracarotid Infusion of NO Donors

Delayed cerebral vasospasm was produced in 13 cynomolgus monkeys (eight males and five females, weighing 2.7–4.8 kg) as described earlier. In four control animals that received intracarotid infusion of saline for 7 days after SAH, the area of the right MCA was reduced by 63 ± 5% compared with preoperative values (Fig. 4). After 7 days of continuous intracarotid infusion of gluntamine-NO in three animals, the area of the right MCA decreased by 15 ± 6.2%, and after proli-NO infusion in six animals, the area of the right MCA decreased by 11 ± 2.9% compared with the preoperative values (p < 0.05 between both NONOate groups and control). The mean arterial blood pressure in the control group remained unaffected by the 7-day intracarotid saline infusion (78 ± 7 mm Hg and 77 ± 9 mm Hg, before and after SAH, respectively; p = 0.8), but it decreased in the group with gluntamine-NO infusion from 75 ± 12 mm Hg (during saline infusion) to 57 ± 10 mm Hg (during gluntamine-NO infusion; p < 0.05). The mean arterial pressure remained unchanged during intracarotid proli-NO infusion (76 ± 12 mm Hg compared with 78 ± 12 mm Hg; p = 0.7). None of the animals from either treatment group developed clinical symptoms suggesting toxicity. Gross and microscopic pathological examinations revealed no toxicity to the
brain, heart, liver, kidney, lymph nodes, spleen, intestine, gonads, or skeletal muscles.

Discussion

Many vasodilators have been used to attempt the reversal and/or prevention of delayed cerebral vasospasm after SAH.20 Despite promising experimental results, none of them has proven effective in the clinical setting. One reason for their failure has been the peripheral effects of these vasodilators: systemic hypotension that is difficult to control. After SAH and during vasospasm, cerebral vessels lose their autoregulatory capacity.11,26 Therefore, any systemic hypotension produced by vasodilators unavoidably leads to a decrease in CBF20 and may produce ischemic stroke, particularly in the distribution of a spastic vessel.

Since its discovery as an endothelium-derived relaxing factor,21 it has been postulated that NO is the cause of vascular dilation. Continuous release of NO has been demonstrated to mediate cerebrovascular tone in mice,7, rats,24 and primates.24 Vascular tone is achieved by a delicate balance between two opposing endothelial cell–derived forces: NO and endothelium-derived contracting factor. Vasospasm may result from degradation of NO25 or its diminished production.22 Macdonald and Weir18 have confirmed that oxyhemoglobin is the putative spasmogen in cerebral vasospasm after SAH. Nitric oxide directly reacts with oxyhemoglobin10,16 to form methemoglobin and nitrate, and it reacts with deoxyhemoglobin to form nitrosylhemoglobin.25 This binding of NO eliminates its vasodilatory action26 on the vascular smooth muscle and, because of the imbalance in dilating and constricting influences, produces vasoconstriction. Another potential mechanism of cerebral vasospasm is reduced NO production due to loss of neuronal NO synthase in the adventitia after SAH.22

Several investigators have attempted to reverse cerebral vasospasm in animal models by using NO precursors, such as nitroglycerin and nitroprusside; however, these researchers have met with limited success.13,26,29,33,35 Possibly because of features of the metabolic conversion and kinetics of these agents as well as their vasodilatory effect on the peripheral vasculature. The rapid fluctuations in systemic arterial pressure associated with intravenous delivery of these and other NO precursors limit their use in patients with cerebral vasospasm. Our approach, in which a direct intracarotid delivery of a compound with spontaneous, nonenzymatic release of NO is used,15,19,23 overcomes these limitations. Because the pharmacokinetic advantage achieved by regional delivery of a drug is directly related to the total body clearance of the drug,4 a significant advantage is achieved by intracarotid infusion of NONOates. Furthermore, because they undergo spontaneous nonenzymatic decomposition at biological pH with a variety of decay constants, based on the nucleophilic cleaving group,15,19,23 the NONOates15 can be tailored for controlled biological release. Thus, one can target vasodilatory activity in the distribution of a specific vessel by changing the half-life of the NONOate, the infusion rate, and the drug concentration. We tested three NONOates with different half-lives by using an intracarotid infusion for assessment of their efficacy in the treatment of delayed cerebral vasospasm. During the acute, short-term infusion of diethylamine-NO, vasospasm temporarily reversed, but there was a concomitant decrease in systemic blood pressure. However, despite the decrease in blood pressure, rCBF increased as cerebrovascular resistance decreased, probably due to dilation of downstream small arteries and arterioles. Long-term infusion of glucantime-NO prevented development of cerebral vasospasm, but it also produced a decrease in mean arterial blood pressure. Although the level of reduction in blood pressure that occurred would normally not be dangerous, it could produce ischemic complications in patients with the impaired autoregulation of CBF that occurs after rupture of an intracranial aneurysm.11,26 Regional intraarterial infusion combined with the extremely short half-life of proli-NO limited systemic exposure to this strong vasodilator yet retained its activity in the region of interest, as dilation of the spastic vessel was achieved without systemic hypotension. Long-term intracarotid infusions of NONOates were achieved and prevented the development of vasospasm without any systemic toxicity. All three compounds proved to be safe even when delivered as a continuous intracarotid infusion for 7 days, confirming earlier studies on their safety.23 None of the animals from any of our treatment groups developed toxicity that was clinically evident, and pathological assessment revealed no abnormalities in any of the vital organs.

Conclusions

Intracarotid infusion of NONOates in primates reverses and prevents delayed cerebral vasospasm, significantly increases CBF, and decreases CBF velocity in the right MCA without causing systemic toxicity. These findings suggest the potential of using NONOates as a targeted therapy for treatment of delayed cerebral vasospasm. If acute intracarotid infusion of NONOates reverses vasospasm in patients after rupture of an intracranial aneurysm and if the effect is as transient in humans as it was in this study, then long-term administration will be required for sustained vasodilation. This could be accomplished with
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an indwelling infusion system that addresses the problem of intracarotid drug streaming. This result also provides evidence for a role of NO loss in the pathogenesis of cerebral vasospasm following SAH.

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References


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