Effects of S-Nitrosoglutathione on Acute Vasoconstriction and Glutamate Release After Subarachnoid Hemorrhage

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Background and Purpose—Subarachnoid hemorrhage (SAH) causes acute vasoconstriction that contributes to ischemic brain injury shortly after the initial bleed. It has been theorized that decreased availability of nitric oxide (NO) may contribute to acute vasoconstriction. Therefore we examined the effect of the NO donor N-nitroso glutathione (GSNO) on acute vasoconstriction and early ischemic glutamate release after experimental SAH.

Methods—SAH was induced by the endovascular suture method in anesthetized rats. GSNO (1 μmol/L/kg, n=31) or saline (n=21) was injected 5 minutes after SAH. Sham-operated rats received GSNO (1 μmol/L/kg, n=5) 5 minutes after sham surgery. Arterial and intracranial pressures, cerebral blood flow (CBF), and extracellular glutamate release were measured serially for 60 minutes after SAH. SAH size was determined, and vascular measurements were made histologically.

Results—GSNO had no effect on resting blood pressure, intracranial pressure, cerebral perfusion pressure, or CBF in sham-operated animals. However, administration of GSNO after SAH was associated with significantly increased CBF (161.6±26.6%/saline 37.1±5.5%/60 minutes after SAH, P<0.05), increased blood vessel diameter (internal carotid artery [ICA] 285.0±16.5 μm versus saline 149.2±14.1 μm, P<0.01), decreased vessel wall thickness (ICA12.9±0.7 μm versus saline 25.1±1.6 μm, P<0.01), and decreased extracellular glutamate levels (3315.6±1048.3% versus saline469.7±134.3%, P<0.05). Blood pressure decreased transiently, whereas intracranial pressure, cerebral perfusion pressure, and SAH size were not affected.

Conclusions—These results suggest that GSNO can reverse acute vasoconstriction and prevent ischemic brain injury after SAH. This further implies that acute vasoconstriction contributes significantly to ischemic brain injury after SAH and is mediated in part by decreased availability of NO. (Stroke. 1999;30:1955-1961.)

Key Words: cerebral blood flow ■ glutamates ■ nitric oxide ■ subarachnoid hemorrhage ■ vasoconstriction

Cerebral arteries respond to subarachnoid hemorrhage (SAH) with a biphasic contraction that begins minutes after the bleed and delayed vasospasm more than 48 hours later.1-3 Although a great deal is known about the mechanisms and management of delayed vasospasm, less is known about the physiology, treatment, and importance of acute vasoconstriction. Different mechanisms may underlie the 2 processes,4 and a great variety have been implicated.5-11

Recently we have shown that acute vasoconstriction is associated with decreased cerebral blood flow (CBF), ischemic glutamate release, and premature mortality after experimental SAH.12 If acute vasoconstriction could be pharmacologically attenuated, then SAH-induced brain injury could potentially be reduced.

Resting cerebrovascular tone is maintained by a balance between opposing vasoconstrictive and vasodilatory forces, and both are pathologically altered in the acute phases of SAH. One of the most important of these is the resting vasodilatory influence of nitric oxide (NO). NO has been shown to induce endothelium-dependent vasodilatation via a cGMP-mediated mechanism.13 Loss of endothelium-mediated vasodilatation14,15 and decreased cGMP production16,17 have been shown to contribute to vasospasm after SAH.

Because administration of an NO donor after SAH should potentially restore c-GMP—dependent vasodilatation, a number of different NO donors have been tested for their ability to attenuate delayed vasospasm.18-21 Recent studies have suggested that sodium nitroprusside administered intrathecally22 or intra-arterially23 increases CBF acutely after SAH. In preliminary studies we have found that it also causes profound hypotension in this setting (J.B. Bederson, MD, et al, unpublished data, 1997).

The present study examines the effect of S-nitrosoglutathione (GSNO) on acute vasoconstriction after experimental SAH. GSNO, a stable nitrosothiol, is an NO donor with minimal effects on blood pressure (BP).24,25

Materials and Methods

All experimental procedures and protocols used in this study were reviewed and approved by the Animal Care Committee of the Mount Sinai Medical Center.

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Experimental SAH Model

Male Sprague-Dawley rats (300 to 400 g) underwent experimental SAH using the endovascular suture model developed in this laboratory. Briefly, rats were anesthetized with chloral hydrate (35 mg/kg IP), transorally intubated, ventilated and maintained on inspired halothane (1% to 2% in oxygen-supplemented room air). Rats were positioned in a stereotaxic frame, and the femoral artery exposed and cannulated for blood gas and BP monitoring. For measurement of intracranial pressure (ICP), the atlanto-occipital membrane was exposed and cannulated, and the cannula was affixed with methyl methacrylate cement to a stainless steel screw, implanted in the occipital bone. CBF was measured by laser-Doppler flowmetry (0.8-mm-diameter probes, model P-433, Vasamedics, Inc), advanced under stereotactic control to the epidural surface exposed by small burr holes over the middle cerebral artery territory 5 mm lateral to the midline at the coronal suture. For administration of saline or drug the left external carotid artery (ECA) was ligated distally and a catheter was placed near the ECA origin for direct injection into the internal carotid artery (ICA) without disturbing flow.

SAH was induced by advancing a suture retrograde through the ligated right ECA, and distally through the ICA until the suture perforated the intracranial bifurcation of the ICA. This event was confirmed by a sudden rise in ICP and bilateral decrease in CBF. Animals were monitored for 20 minutes before SAH and 60 minutes after SAH. At the completion of each experiment the rat was transcardially perfused with chilled 4% buffered PAF and brain was removed and stored in refrigerated PAF for histological examination. Sham-operated rats under went a similar experimental procedure except for induction of SAH.

Measurement of Extracellular Fluid Glutamate Levels

In vivo microdialysis was performed before, during, and for 60 minutes after SAH for determination of extracellular glutamate concentration. Two-millimeter-long microdialysis probes (BAS CMA/12) were inserted into the right sensorimotor cortex (from bregma: posterior 0.9 mm, lateral 4.0 mm, depth 2.0 mm). Beginning 80 minutes before SAH, probes were perfused with artificial CSF at 2 μL/min with use of a micropump (Harvard Apparatus). Dialysis samples were collected at 10-minute intervals, starting 20 minutes before SAH until 60 minutes after SAH, and stored at −70°C until analyzed. Glutamate determinations were performed with pre-column derivatization with o-phthalaldehyde dye using an auto injector program and HPLC analysis with fluorescence detector (excitation at 335 nm and emission at 455 nm). The column (Hypersil ODS C-18, 250’’ 4 mm) was eluted with buffer A (10% methanol in 0.1 mol/L sodium acetate, 50 μM EDTA, pH 5.7) for 6 minutes after the injection of sample, and then the gradient was started from buffer A to buffer B (70% methanol solution) in 20 minutes at 0.8 mL/min flow rate. Peak identification was performed using external standard (l-glutamic acid hydrochloride, amino acid calibration marker, Sigma), and concentration was calculated on the basis of peak areas. To correct for differences in animal basal neurotransmitter release and differences in probe recoveries, data are presented as percent of baseline.

Measurement of Blood Vessel Circumference, Thickness, and Hematoma Size

Brains were removed, preserving the meninges and the circle of Willis at the skull base, and sliced coronally at 3.0-mm intervals before dehydration and paraffin embedding. The slices were sectioned with a microtome at 5-μm intervals and mounted on subbed slides. Sections were stained with hematoxylin and eosin. Images were digitized (IPLab version 3.0, Signal Analytics) and analyzed for vascular measurements and hematoma size. Postfixation distortion of vessel wall shape can alter the measurement of the diameter or radius. However, these changes do not affect the measurement of circumference. Therefore, the circumference was measured and diameter was calculated using the relationship circumference/π.

Drug Treatment

Animals were randomly assigned to receive either GSNO (1 μ mol/kg, n = 31) or saline (n = 21) administered directly into ICA opposite the side of SAH induction 5 minutes after SAH. Sham-operated animals (n = 5) received GSNO 5 minutes after sham treatment.

Data Acquisition and Statistical Analysis

CBF, ICP, and mean arterial BP (MABP) data were continuously recorded starting 10 minutes before and 60 minutes after SAH with use of customized analog-to-digital conversion hardware and software (Labview version 4.0, National Instruments) and stored on a Macintosh Quadra 950 at a rate of 0.25 Hz. CBF data were normalized to an average (baseline) value obtained for 10 minutes before SAH, and expressed as a percentage of baseline. Comparisons were made at the following points: baseline (an average of values for 10 minutes before SAH), acute (the peak changes in CBF, cerebral perfusion pressure [CPP], and ICP occurring within 60 seconds after SAH), and at 10-minute intervals after GSNO or saline administration. Statistical evaluation was performed with 1-way ANOVA followed by a Bonferroni post hoc test that set significance at P<0.05.

Results

In sham-operated rats, GSNO had no effect on baseline BP, ICP, CPP, or CBF throughout the experiment (Figure 1). After SAH induction, blood was distributed primarily in the basal subarachnoid space and to a lesser extent in the interhemispheric and convexity subarachnoid space and ventricular system. Total hemorrhage size was 56.9 ± 7.3 μm² × 10⁴ in control and 64.3 ± 15.2 μm² × 10⁴ in GSNO-treated rats (NS; Figure 2).
results are in agreement with previous observations from this laboratory.12,26 Baseline ICP was 6.3 ± 0.4 mm Hg and rose immediately after SAH to 76.9 ± 8.8 mm Hg in the control group and 77.7 ± 5.5 mm Hg in the experimental group, reaching peak values 2.4 ± 0.4 minutes after SAH and declining thereafter. No significant differences were found in the peak or late values of ICP in control versus experimental group (Figure 3A). BP increased transiently after SAH in both control and experimental groups with no significant differences between groups. GSNO treatment was associated with a transient decline in BP at 10 and 20 minutes after treatment (P < 0.05, Figure 3B). CPP declined acutely after SAH, from 84.9 ± 2.7 to 34.9 ± 6.3 mm Hg in control group and from 82.5 ± 1.8 to 29.0 ± 2.0 mm Hg in experimental group, and then recovered toward baseline (Figure 3C). CPP was lower at 10 and 20 minutes after GSNO treatment, but the effect did not reach statistical significance, and it rose to 64.5 ± 2.3 mm Hg 65 minutes after SAH, with no difference between control and experimental groups.

CBF dropped acutely after SAH in both hemispheres (control values 8.4 ± 1.3% left and 6.2 ± 1.2% right), with no difference between saline- and GSNO-treated rats (Figure 4). After its initial decline, CBF rose to 37.1 ± 5.5% in left and 40.5 ± 8.9% in right hemispheres in control rats. In GSNO-treated rats, CBF increased to 93.6 ± 10.8% contralateral to the intra-arterial injection (P < 0.05 versus controls) and to 161.6 ± 26.6% ipsilaterally (P < 0.05 versus controls).

The average recovery fraction of glutamate concentration for the microdialysis probes was 34.06 ± 5.50%. The calculated pre-SAH dialysate glutamate concentration was 3.6 ± 1.7 mmol/mL in control and 4.7 ± 1.4 mmol/mL in experimental animals (P = NS). Cortical extracellular glutamate concentrations increased after SAH in control animals, reaching significance at 25 minutes (peak value 3315.6 ± 1048.3% at 45 minutes after SAH), and remained elevated for the duration of the experiment (Figure 5). GSNO treatment was associated with a significantly smaller increase in glutamate concentration compared with control treatment throughout the study, reaching a maximum of 469.7 ± 134.3% at 65 minutes after SAH (P < 0.001 versus controls).

Histological examination demonstrated a significantly increased circumference and decreased thickness in GSNO-treated rats versus controls (P < 0.05). The ICA circumference was 468.5 ± 44.2 μm in control rats, corresponding to a diameter of 149.2 ± 14.1 μm, and increased 2-fold to 955.0 ± 51.9 μm (diameter 285.0 ± 16.5 μm) in GSNO-treated rats (P < 0.05). Vessel wall thickness decreased significantly from 25.1 ± 1.6 μm in control animals to 12.9 ± 0.7 μm in experimental animals (P < 0.05). Similar changes were seen in the A1 and A2 segments (Figure 6).

Discussion

Previously, we and others have demonstrated that experimental SAH causes acute vasoconstriction, decreased CBF, and...
ischemia. In addition, we have shown that acute vasoconstriction appears to contribute directly to ischemic brain damage in this setting. The present study demonstrates that administration of the NO donor (GSNO) immediately after SAH is associated with dilation of cerebral blood vessels, increased CBF, decreased cerebral glutamate release, as well as a transient depressant effect on systemic BP.

GSNO is a stable S-nitrosothiol that has been postulated to be involved in the transport of NO in vivo. It releases NO by photolysis, catalysis by copper ions, or by transnitrosation to form S-nitrosocysteine, a less-stable compound, and is known to produce only mild hypotensive effects. NO is a potent vasodilator produced from L-arginine by NO synthase (NOS) that has been shown to mediate endothelium-dependent vasodilation and play a major role in maintaining resting cerebral vascular tone. The mechanism of the vascular effects of NO involves activation of soluble guanylate cyclase and a subsequent increase in cGMP levels. When the availability of NO is decreased by inhibition of NOS with an arginine analogue a decrease in CBF and constriction of cerebral blood vessels is seen. Similarly, administration of L-arginine, a substrate of NOS, can increase CBF by increasing the availability of NO. Involvement of NO has been implicated in SAH.

Several investigators have shown that production of cGMP in vascular smooth muscle is decreased following SAH. However, since NO donors can still produce relaxation of these blood vessels, decreased vasodilatation may be due to reduction in basal cerebral NO levels. Because SAH does not affect the ability of cerebral arteries to synthesize NO, decreased NO may occur as the result of scaveng-
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ing by hemoglobin, or free radicals. If decreased availability of NO leads to vasoconstriction, administering an NO donor could potentially reverse this effect.

Work from other laboratories has shown that nitrovasodilators can relieve delayed vasoconstriction, improve CBF, and decrease ischemic damage following SAH. These observations confirm that attenuation of the L-Arginine-NO pathway plays an important role in delayed vasospasm. However, little is known about the mechanisms, importance or treatment of acute vasospasm after SAH. We previously demonstrated that sodium nitroprusside (SNP) can increase CBF and relieve acute vasoconstriction following SAH. Similar observations have recently been made by others.

In our experiments GSNO had a transient depressant effect on BP, but this did not significantly influence CPP. The effect was smaller than that produced by other nitrovasodilators such as Sodium nitroprusside. CBF was elevated during this period of hypotension indicating that the increase was not due to hemodynamic alterations. GSNO treatment was associated with bilateral increases in CBF suggesting a systemic effect. However, the effect on ipsilateral CBF was significantly greater than on the contralateral side. This suggests that intraarterial administration produced higher local concentrations of GSNO ipsilaterally, and a corresponding increase in CBF. This apparent relationship needs to be confirmed by dose response studies. The baseline CBF in sham operated animals was not effected by GSNO treatment. This finding is not surprising as it has been previously shown that under normal conditions an additional increase in basal NO concentration by administration of NO donor or synthesis by exogenously supplied L-arginine does not effect the basal NO activities. However, when endogenous NO is inhibited or removed the vasodilatory responses of exogenously supplied or endogenously synthesized NO are potentiated.

In addition to being a potent vasodilator, NO is also an inhibitor of platelet aggregation. Intravascular thrombus related to platelet aggregation can independently produce vasoconstriction. Thus, GSNO induced increases in CBF may also involve inhibition of platelet aggregation. Studies have shown that in certain conditions GSNO can decompose to liberate the oxidative or disulfide form of glutathione along with NO. Glutathione, an antioxidant, can scavenge destructive oxidative species preventing constriction of blood vessels. At present we do not know the mechanism or the products of GSNO decomposition in our SAH model. Moreover, the kinetics of generation of reactive oxidative species and their involvement in acute vasoconstriction following SAH in our model are yet to be determined.

Extracellular brain glutamate concentrations rise rapidly during ischemia and contribute to excitotoxicity and neuronal death. Thus, extracellular glutamate concentrations can be taken as a marker of the severity of ischemic neuronal injury. In the present study GSNO administration was associated with attenuated glutamate increases produced by SAH as compared with control. GSNO may have produced this effect by attenuating the severity of SAH-induced vasoconstriction and ischemia. This concept is supported by the demonstration of vasodilatation and increased CBF in GSNO-treated rats. An alternative explanation may be a direct effect of NO on glutamate release. Studies on the influence of NO on neuronal glutamate release are controversial. L-Arginine has been shown to reverse the increase in glutamate concentration by NOS inhibitors following cerebral ischemia. Similarly, S-Nitroso-N-acetylpenicillamine can decrease Ca²⁺-dependent glutamate release in rat cerebral cortex synaptosomal preparation. Thus, a direct effect of NO in glutamate release cannot be ruled out. An additional explanation for the observed effect of GSNO on glutamate concentration after SAH could be that treated animals received a hemorrhage of lesser intensity than control animals. However, no significant differences were found between the measured SAH size in treated and control animals and the acute physiological alterations following SAH, indicating the similarity in the hemorrhage intensity among all animals.

NO may have both beneficial and deleterious effects on ischemic brain, depending on the stage of ischemia and the source of NO release. An increase in neuronal NOS expression and activity during ischemia is associated with excitotoxicity, whereas increased endothelial NOS expression and activity is believed to be neuroprotective. This concept is supported by the finding that neuronal NOS knockout mice develop smaller infarcts after MCA occlusion than endothelial NOS knockout mice. In addition, early increases in NO may be protective and later increases deleterious. Although we have demonstrated an apparent beneficial effect of NO, the question remains of whether increasing NO concentrations could contribute to later neuronal damage. Further dose-response studies and studies of the time course of histological changes are required to address this important question.

Conclusion

We have demonstrated that GSNO administered immediately after SAH attenuated the development of acute vasoconstriction, improved CBF recovery, and limited cerebral glutamate release with a transient depressant effect on systemic BP. These results support the concept that acute vasoconstriction, mediated by decreased NO availability, contributes directly to ischemic brain damage after SAH, and introduce a potentially novel type of treatment, particularly in severe SAH. The transient decrease in BP by GSNO compared with the profound decreases associated with other nitrovasodilators makes it potentially useful in the clinical setting of acute SAH. Decreased cerebral glutamate release after GSNO treatment suggests decreased ischemic damage. However, further studies are needed to determine whether this treatment would limit the development of ischemic histological changes or improve neurological outcome.

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We really do not know how clinically important acute vasospasm after SAH is: Severity? Duration? Influence on CBF? Impact on outcome? As long as there is not much we can do about acute vasospasm, the above questions are not that important, either, but this study suggests a possible treatment and at least also suggests a secondary injury (excitotoxicity) that could be prevented with GSNO treatment. Even if the findings of the authors might not be applicable to the majority of aneurysmal SAH, they are still mechanistically important and could be applied to other conditions (head injury in the first few hours?).

An interesting finding was that at 15 and 30 minutes, ICP in the GSNO group was lower (not statistically significant) despite lower blood pressure and despite vasodilation in the vessels, where diameter was measured (ICA, a1 and a2). This would indicate to me that vasospasm in the saline treatment groups led to compensatory vasodilation in the microcirculation, increased cerebral blood volume, and thus ICP.1 This chain of events was also obviously interrupted with GSNO treatment.

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