Reversal of cerebral vasospasm using an intrathecally administered nitric oxide donor

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Object. Intrathecal bolus administration of (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (DETA/NO), a long half-life diazeniumdiolate-class nitric oxide (NO) donor, was evaluated for safety and efficacy in the treatment of delayed cerebral vasospasm in a canine model of subarachnoid hemorrhage (SAH).

Methods. The baseline basilar artery (BA) diameter of 25 dogs was measured with the aid of angiography on Day 0. Vasospasm was then induced by intracisternal injection of autologous arterial blood on Days 0 and 2. Repeated arteriography on Day 7 revealed an average BA diameter of 58% of baseline. Each dog was then randomized to one of four groups: a pathology control group (SAH only, four animals); a treatment control group (SAH plus 2 µmol of the inactive drug carrier DETA, eight animals); a low-dose treatment group (SAH plus 0.2 µmol DETA/NO, six animals); or a high-dose treatment group (SAH plus 2 µmol DETA/NO, six animals). The drugs were administered in a 2-ml intrathecal bolus via the cisterna magna. Arterial caliber was monitored by angiography over the subsequent 4 hours. A 2-µmol dose of the drug was then given and serial arteriography continued for an additional hour to screen for tachyphylaxis. Intracranial pressure and respiratory and hemodynamic parameters were continuously monitored. Histopathological analyses of the animals’ brains were performed after the dogs were killed on Day 8.

The drug DETA/NO produced reversal of vasospasm in a dose-dependent fashion that roughly followed a double exponential time course. Doses of 2 µmol DETA/NO resulted in restoration of the angiographically monitored BA diameter to the prevasospasm size at 1.5 hours posttreatment, and this was sustained at 88% of baseline at 4 hours (p < 0.01, independent samples t-test). By contrast, the treatment control group remained on average at 54% of baseline diameter. The low-dose treatment group achieved only partial and more transitory relaxation. Histopathological analyses showed findings consistent with chronic SAH but did not demonstrate any toxicity associated with the NO donor. No adverse physiological changes were seen.

Conclusions. This study indicates that long-acting NO donors are potentially useful as agents to restore circulation in patients suffering from cerebral vasospasm.

Key Words: vasospasm • nitric oxide • cerebrospinal fluid • DETA/NO • hemoglobin • double hemorrhage model • subarachnoid hemorrhage • dog

Rupture of intracranial aneurysms and the resultant subarachnoid hemorrhage (SAH) affect nearly 27,000 people per year in North America alone and account for approximately 16 deaths per 100,000 population per year. Of those patients receiving neurosurgical care, rebleeding and spasm of the cerebral arteries are the most significant causes of morbidity and mortality. Thirty percent of patients with SAH suffer sequelae of symptomatic vasospasm; the manifestations form a continuum ranging in severity from mild reversible dysfunction, to severe permanent focal neurological deficits resulting from ischemic infarction, to death.

A poor understanding of the origins of vasospasm has hindered development of therapeutic regimens aimed specifically at dilation of cerebral arteries in spasm. Current treatment modalities include pharmacological arterial dilation using calcium channel blockers, indirect arterial dilation through volume expansion and induced arterial hypertension, and improvement in blood rheological properties by hemodilution. Unfortunately, these measures are often only palliative and are poorly tolerated in critically ill patients with dubious hemodynamic reserves. Endovascular techniques such as balloon angioplasty and intraarterial papaverine administration can be used with good results in selected settings; however, the results may be transitory and are not without attendant risks.

Delayed cerebral vasospasm may result from depletion of local nitric oxide (NO) due to the absorbefacient property of subarachnoid hemoglobin. Constitutive production of NO by endothelial NO synthase is modulated by neuronal and glial production of NO to achieve autoregulation. Nitric oxide diffuses to the vascular smooth muscle, where it combines with the heme moiety of soluble guanylate cyclase to catalyze the production of cyclic guanylate monophosphate (cGMP). Increased
cGMP causes vascular smooth muscle relaxation with resultant vasodilation. Transient reversal of vasospasm has been demonstrated in several studies after intrathecal, intravenous, or intrarterial application of short-acting NO donors. The practical utility of this treatment has been limited by the need for continuous infusion of the drug and concerns about potential neurotoxicity. The recent availability of the long-acting water-soluble NO donor, diethylenetriamine (DETA)/NO, has led us to evaluate the efficacy and safety of bolus intrathecal administration of this drug for reversal of delayed cerebral vasospasm. The vasoactive effects were monitored with the aid of serial digital subtraction angiography and surveillance for systemic effects, including monitoring of multiple physiological parameters. Finally, histopathological studies were used to screen for neurotoxicity.

Materials and Methods

Experimental Design

After a baseline transfemoral digital subtraction vertebrobasilar arteriogram was obtained, vasospasm was induced in each of 24 dogs by using the double-hemorrhage model. In accordance with this model, 5 ml of autologous arterial blood was injected into the cisterna magna on Day 0 and again on Day 2. On Day 7, a repeated arteriogram was obtained to quantify the vertebrobasilar spasm. The animals were randomized to form a pathology control group (SAH only, four dogs), a treatment control group (SAH plus the inactive vehicle DETA, eight dogs), or one of two treatment groups (SAH plus DETA/NO, six dogs apiece). The treatment groups differed in the dose amount of DETA/NO (0.2 compared with 2 μmol). One additional dog underwent arteriographic examination but not SAH. The non-SAH and SAH-only groups served as controls and were killed without further intervention.

Animals in the treatment groups were given a 2-ml intrathecal bolus injection of either 0.2 μmol or 2 μmol of DETA/NO. The treatment control group received 2 μmol of DETA. The arterial caliber was monitored using serial arteriography over the subsequent 4 hours, and physiological parameters were also monitored.

A repeated bolus injection of 2 μmol DETA/NO in both treatment groups or 2 μmol DETA in the treatment control group was given at 4 hours. Cumulative doses were 2.2 μmol (low-dose group) or 4 μmol (high-dose group) of DETA/NO, and 4 μmol DETA, respectively. The animals were killed 24 hours later and their brains were prepared for histopathological analysis.

Animal Model

The in vivo experiments described were approved by the University of Tennessee Animal Care and Use Committee and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Twenty-five unconditioned dogs of either sex, weighing between 23 and 34 kg (mean 27.86 kg) were used. General anesthesia was induced intravenously with ketamine (5 mg/kg), xylazine (1 mg/kg), and acepromazine (0.5 mg/kg). A surgical plane of anesthesia sufficient to depress respiration was maintained with 1 μg/kg/hour of pentobarbital and 20 μg/kg/hour of fentanyl administered intravenously. Penicillin G (30,000 U/kg) was given subcutaneously on preoperative Days 0 and 2. Buprenorphine (0.01–0.02 mg/kg) was administered intramuscularly for postoperative analgesia every 4 hours as needed.

Each animal was endotracheally intubated and placed on continuous mandatory mechanical ventilation (12–15 ml/kg) with room air. The respiratory rate was adjusted to maintain an end-tidal CO₂ (ETCO₂) of 40 mm Hg.

At the conclusion of each survival experiment the animal was allowed to awaken from anesthesia, underwent extubation, and then was transferred to its kennel. Each animal received appropriate dietary supplementation and was allowed to roam freely.

Death was induced by a lethal dose of intravenous, administered pentobarbital (100 mg/kg) followed by potassium chloride. The cranial vault was opened, and the brain was removed immediately postmortem.

Physiological Monitoring

The following physiological parameters were continuously monitored: heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure, intracranial pressure (ICP), cerebral perfusion pressure, ETCO₂, central venous pressure (CVP), and respiratory rate.

Each measured parameter was sampled at 100 Hz by using an analog-to-digital converter board running on commercially available software on a personal computer. Averages over 5-second epochs were continuously recorded over the duration of the Day 7 experiment.

The arterial pressure was monitored from the vertebral artery catheter, and the CVP was measured at the external jugular vein via a No. 7 French triple lumen catheter. The electrocardiogram and pressure signals were transduced using a critical care monitor. An ICP monitor with intraparenchymal bolt was used to transduce this parameter, and ETCO₂ was measured using a CO₂ monitor. Blood gas levels, cerebrospinal fluid (CSF) gases, and CSF electrolytes were measured using a blood analysis system. Spot checks of ETCO₂ and PaCO₂ consistently showed good correlation.

Arteriographic Studies

Vertebral arteriographic studies were performed using a percutaneous transfemoral approach with a Bentsen wire and a No. 5 French Berenstein catheter. Diatrizoate meglumine 60% contrast medium, diluted by 1/3 with normal saline containing heparin sulfate (2000 U/L), was injected manually at a volume of 6 ml in approximately 1 second. Digital subtraction images with 512 × 512-pixel resolution were obtained using a C-arm fluoroscope in peak opacification digital subtraction mode. Respiration was temporarily suspended during image acquisition to reduce the subtraction artifact. Steel ball bearings placed on the dorsal and ventral surfaces of the head provided dimensional reference and correction for x-ray magnification. Images were downloaded to a personal computer and archived on an optical disk.

Image Analysis

Arteriograms were analyzed using software available from the National Institutes of Health (NIH). Absolute dimensions were determined by interpolating x-ray magnification factors from the aforementioned ball bearings. The basilar artery (BA) diameter was measured at a point 5 mm proximal to the circle of Willis.

Measurements were performed in a blinded semiautomated fashion. Using the NIH software, an image-density histogram was constructed along a line perpendicular to the axis of the BA at the point of interest. These data were then imported into IgorPro and the Levenberg–Marquardt algorithm was used to fit the density histogram to a half-sine template of the form: $D(x) = \eta + \text{Asin} \left( (x - \phi + r) / (\pi/2r) \right)$ for $(x - \phi + r) \leq x \leq (x + \phi + r)$, else $D(x) = \eta$, where $D$ is the image density value at location $x$, $\eta$ is the modal (background) image density, $r$ is the vessel radius, $\phi$ is the phase, and $A$ is the density magnitude. The arterial diameter was taken as 2r. Diameter differences as small as 0.93 pixel (typically 0.12 mm) may be detected with a probability of less than 0.05 (unpublished data).

Subarachnoid Hemorrhage

After intravenous induction of anesthesia and sterile preparation of the area with povidone iodine, a 22-gauge spinal needle was introduced into the cisterna magna at the occiput–C1 junction via a midline percutaneous approach. Three milliliters of CSF was aspirated and saved for analysis, and 5 ml of autologous arterial blood that had been obtained percutaneously from the femoral artery was instilled into the cisterna magna. The spinal needle was then withdrawn and the animal placed prone in the Trendelenberg 30° position for 15 minutes.

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Analysis of CSF

The CSF samples taken on Days 0 (pre-SAH) and 7 (pre- and posttreatment) were immediately analyzed for pH, PCO₂, PO₂, Na⁺, K⁺, and Ca²⁺ by using the blood analysis system mentioned earlier.

Drug Administration

Nonsterile solutions of DETA and DETA/NO were prepared just prior to administration for which normal saline (25°C) was used as a diluent. Via the percutaneous cisterna magna puncture described previously, 2 ml of CSF was withdrawn and then replaced by a bolus injection of an equivalent volume of drug.

Histological Studies

Specimens were fixed in 10% buffered formalin for a minimum of 2 weeks. Four-millimeter-thick sections of the cerebrum were taken in the coronal plane; the cerebellum and brainstem were sectioned in the transverse plane. These were inspected for gross pathological changes. Hematoxylin and eosin slides were prepared from tissue slices taken at the level of the cerebellum, and closed medulla. When indicated, additional sections were obtained of grossly identified lesions. Special stains were used on sections in which histopathological findings of uncertain origin were seen.

All tissue slices and slides were read by the neuropathologist (F.C.D.) who was blinded to the treatment group and experimental design.

Statistical Methods

Arterial diameter measurements for each dog were normalized with respect to the pre-SAH baseline diameter for that animal. Normalized time-series data were then used to compute the mean and standard error at each discrete time point within each treatment or control group. Curve fits to the experimental data were applied using the Levenberg–Marquardt algorithm. Double exponential models were used for each of the treatment groups and a linear model was applied to the treatment control group. The chi-square test was used to evaluate goodness of fit. Independent samples t-tests were performed between homologous time points for each treatment group and the treatment control group. The probability value for each two-tailed t-test is reported. Previous work in our laboratory has found a mean pre-SAH canine BA diameter of 1.42 ± 0.06 mm. Thus, a difference in arterial diameter at the resolution of the arteriographic technique may be detected at an assigned beta risk of 0.05 with six samples.

A one-way analysis of variance (ANOVA) was used to compare CSF biochemical parameters before and after vasospasm as well as before and after treatment for vasospasm. Evaluation of the voluminous physiological data was simplified by limiting the statistical analysis to discrete time points immediately before intrathecal injection, followed by 1, 2, and 4 hours posttreatment. One-way ANOVAs were performed for each physiological parameter at each time point between treatment groups and the treatment control group.

Trend analysis was performed on time series data (including arterial diameter measurements and physiological parameters) by using the mean square successive difference method of Baines, as reported in Crow, et al. A statistically significant trend in a set of means is reported as present for the mean square successive difference/variance values of the parameter (δ²/s²) less than the critical value at the specified confidence level.

Statistical calculations were performed using commercially available software, and results were plotted with IgorPro software running on a personal computer.

Sources of Supplies and Equipment

The Harvard respirator pumps were obtained from Harvard Apparatus, Inc., S. Natick, MA. The 12-bit analog-to-digital converter board (McAdios II) and the SuperScope II software were purchased from GW Instruments, Somerville, MA. The Macintosh IIfi computer was obtained from Apple Computer, Inc., Cupertino, CA, as well all the other personal computers used in the study. The No. 7 French catheters were obtained from Arrow International, Inc., Reading, PA. The critical care monitor (Mennen Medical model 742) was purchased from Mennen Greatbatch, Inc., Clarence, NY. The ICP monitor (model 420) was acquired from Camino Laboratories, San Diego, CA, and the CO monitor (model 5200) used to assess ETCO₂ was purchased from Ohmeda, Englewood, CO. The IRMA Blood Analysis System was obtained from Diagnostics, St. Paul, MN. The fluoroscope (Series 9600) was manufactured by OEC Medical Systems, Inc., Salt Lake City, UT. A Macintosh Quadra 950 computer equipped with Image 1.59b37 software (NIH, Bethesda, MD) was used for image analysis. The IgorPro software was acquired from WaveMetrics, Lake Oswego, OR. The DETA and DETA/NO were obtained from Sigma Chemical Co., St. Louis, MO, and Alexis Biochemicals, San Diego, CA, respectively. The statistical software (Statistica 4.1) was purchased from StatSoft, Inc., Tulsa, OK.

Results

Arteriographic Studies

Representative serial arteriograms from animals in the DETA/NO and DETA groups are shown in Figs. 1 and 2, respectively. Arteriographically obtained data are summarized in Fig. 3.

The mean BA diameter was 1.56 mm before induction of SAH. The subsequent vasospasm resulted in a mean reduction of arterial caliber to 58% of baseline. The magnitude of pretreatment vasospasm was not significantly different between groups (t-test, p > 0.1). The treatment control (DETA) group maintained a relatively stable average BA diameter of approximately 54% of control. No significant trend was found (δ²/s², p > 0.05), indicating that DETA is not vasoactive. This is corroborated by in vitro work in our laboratory in which isometric force measurements were used on perfused arterial rings (unpublished observations).

In contrast, both treatment groups demonstrated dose-dependent statistically significant arterial dilation within 1 hour of drug administration. Independent samples t-tests performed for homologous time points between each treatment group and the treatment control group showed statistical significance (p < 0.05) for the DETA/NO 0.2-μmol group over the span of 45 minutes to 2 hours postinjection. Statistical significance (p < 0.01) was achieved at 45 minutes in the DETA/NO 2-μmol group as well. The BA diameter in the latter group was restored almost to normalcy at 1.5 hours and remained at almost 90% of the baseline diameter at 4 hours postinjection. The time course of the response to DETA/NO approximates a double exponential function (chi-square test, p < 0.005; δ²/s², p < 0.001) at both the 0.2- and 2-μmol dose. The half-life of the effect is approximately 5.2 hours.

Histopathological Studies

The basic histological findings are summarized in Table 1. A complex of nonspecific inflammatory changes were sporadically observed, regardless of treatment group, and did not consistently correlate with the extent of residual SAH as seen on gross examination. The most prevalent feature was a mild-to-moderate choroid plexitis manifested by infiltration of the choroid with lymphocytes, monocytes, and focal accumulations of plasma cells. A leptomeningeal inflammation was variably present, but only...
in animals with a coexistent choroid plexitis. Microglial nodules were encountered less frequently, but again, only with coexistent choroid plexitis. Neutrophilic leptomeningeal infiltration and microglial nodules were not mutually exclusive nor inclusive.

The leptomeningeal infiltrate consisted predominantly of mononuclear cells and macrophages, with occasional neutrophils present. In some instances this inflammatory process was accompanied by perivascular cuffs of similar cellular composition in the Virchow-Robin spaces. These findings are consistent with those of Sprong and Simmonds, in which they describe an initial neutrophilic infiltrate that is subsequently replaced by a preponderance of mononuclear cells during resolving SAH.

The inflammatory complex does not appear to be caused by NO as it was present in seven of 13 control animals. It was not caused by the carrier agent; two of five animals without DETA treatment demonstrated choroid plexitis.

Choroid plexitis is generally predictive, but not requisite, for the presence of diffuse microglial proliferation; when not present, there are other causes such as ischemia or infarction.

The granule cell layer of the cerebellum was the most sensitive to ischemic insult. Findings included red neurons, plasma cells, spheroids, and frank necrosis. In each case an attempt was made to classify the insult’s origin: traumatic, embolic (foreign material), thrombotic, or unknown (to include vasospasm or undetected thrombus/embolus). Traumatic infarction occurred as a result in the vicinity of intraparenchymal monitoring probes. Two cases of embolic phenomena were identified by the presence of intravascular birefringent material. In the SAH-only group, one animal had ischemic changes resulting from intraarterial thrombosis. The source of ischemia or infarction could not be identified in four of 12 animals with SAH that did not receive the NO donor. Only one of 12 animals receiving DETA/NO exhibited ischemia of unknown origin.

Tissue Gram staining and modified Gomori’s methamine-silver stains revealed an absence of bacteria and fungal elements.

**Chemistry of the CSF**

The results of the CSF electrolyte and gas analyses are shown in Table 2. A one-way ANOVA was performed to
compare pre- and postvasospasm (pretreatment) values. Two unexpected results were encountered: 1) a rise in calcium ions \( (p = 0.006) \); and 2) a rise in \( PO_2 \) \( (p = 0.01) \).

One-way ANOVAs comparing pre- and posttreatment values did not show statistical significance for any parameter, although a marked decrease in \( PO_2 \) from 111.7 to 80 mm Hg following application of the NO donor was observed.

**Behavior and Physiology**

Each animal was assigned a neurological grade according to the Cat Coma Scale of Hilton, et al.,

\( X^2 = 3.033, p < 0.005 \)

\( X^2 = 1.48, p < 0.005 \)

\( X^2 = 0.004, p < 0.005 \)

\( X^2 = 0.06 \)

\( X^2 = 1.86 \)

\( X^2 = 0.57 \)

\( X^2 = 0.88 \)

\( X^2 = 0.08 \)

\( X^2 = 0.05 \)

The graph showing posttreatment BA diameter expressed as a percentage of baseline diameter at Day 0. A straight line is fitted to the control group (eight dogs) that received DETA alone. Double exponential curves are fitted to the two experimental groups that received either 0.2 \( \mu \)mol or 2 \( \mu \)mol of DETA/NO (six dogs apiece). Animals from both experimental groups received a 2-\( \mu \)mol DETA/NO dose at 4 hours to evaluate for tachyphylaxis. Arterial diameter over the subsequent hour is plotted to the right. Vertical bars represent 1 standard error. The level of significance (p value, independent samples t-test) for both experimental groups relative to the DETA control group is plotted beneath. The solid black line depicts the limit of statistical significance at \( p = 0.05 \).

Behavior and Physiology

Each animal was assigned a neurological grade according to the Cat Coma Scale of Hilton, et al., before treatment on Day 7 and 24 hours after treatment. The results are presented in Table 3. No neurological deficit was perceptible before Day 7 of treatment. Animals with a posttreatment coma score of less than the maximum of 14 lost points exclusively on the motor component. Animals with a total coma score of eight demonstrated extensor posturing that correlated with traumatic myelopathy from multiple cisternal taps. Otherwise, lower scores were attributed to vertebrobasilar thromboembolic phenomena resulting from prolonged arteriographic studies spanning up to 6 hours.

Table 4 contains a synopsis of the mean physiological parameters as a function of time for each treatment and control group. Time 0 (baseline) immediately precedes intracisternal injection of either DETA/NO or DETA, and the time thereafter represents hours postinjection.
No significant differences between groups (ANOVA, \(\alpha = 0.05\)) were found for the following parameters: DBP, ETCO, heart rate, respiratory rate, and CVP. No trends (\(\delta /s^2, \alpha = 0.05\)) were present except in the blood pressure waveforms of the DETA/NO 2-\(\mu\)mol group as described later.

The SBP was found to be significantly elevated (ANOVA, \(p < 0.05\)) in the DETA/NO 2-\(\mu\)mol group at 0 and 1 hour posttreatment, but not thereafter. Review of the raw data revealed large exponentially decaying drifts in the SBP and DBP waveforms of two animals, indicating that hydrodynamic properties of the indwelling arterial catheter or transducer may have contributed to measurement error. Considering that the SBP was elevated before drug administration, it is probable that the DETA/NO was not responsible for this mild hypertension. The mean square successive difference analysis revealed a significant decreasing trend in the SBP (\(p < 0.01\)) and DBP (\(p < 0.05\)) waveforms of the DETA/NO 2-\(\mu\)mol group. Whether these changes in blood pressure are due to a measurement artifact or drug effect could be clarified with the use of sphygmomanometry.

The ICP was elevated (ANOVA, \(p < 0.05\)) in the DETA/NO 0.2-\(\mu\)mol group compared with the DETA group at all time points, including the pretreatment value, although no trend was present. The physiological significance of this finding is questionable.

### Discussion

Historically, the causes proposed for cerebral vasospasm have been many. White\(^6\) provided a comprehensive review of known vasoactive substances that includes various amines, polypeptides, proteins, and prostaglandins. The diverse spectra of possible causes of vasospasm have led to a similarly wide range of proposed treatments.

Recently attention has turned toward the antagonistic interaction between endothelium-derived relaxing factor and endothelin, which is a potent vasoconstrictor. Furchgott\(^3\) identified NO as the putative endothelium-derived relaxing factor and this has been confirmed in many subsequent studies (the vasoactive form may actually be a nitrosothiol\(^7\)). Intracellular calcium, calmodulin, and reduced nicotinamide-adenine dinucleotide phosphate act to modulate the production of NO from nitrosothiol, which has been confirmed in many substances. NO, reducing its availability to the heme moiety of guanylate cyclase, thereby activating the enzyme through a conformational change. This catalyzes the production of cGMP from guanylate triphosphate, which causes vascular smooth-muscle relaxation with resultant vasodilation.

Drabkin and Austin\(^12\) first demonstrated the extremely high binding affinity of NO to hemoglobin. Hemoglobin degradation products in the CSF may competitively bind NO, reducing its availability to the heme moiety of guanylate cyclase, thereby causing loss of vasodilatory tone. Sonobe and Suzuki\(^6\) provide evidence that oxyhemoglobin is largely responsible. The spasmogenic properties of blood–CSF mixtures incubated for varying periods in vitro were compared by applying the supernatant fluid to the BA of adult cats. Maximal vasoreactivity occurred at Day 7. Analysis of the supernatant fluid by means of chromatography, electrophoresis, and spectrophotometry revealed that the active chemical was oxyhemoglobin. Over time, oxyhemoglobin was spontaneously converted to methemoglobin with a corresponding loss of spasmogenicity. Conversion of oxyhemoglobin to methemoglobin using sodium nitrite also abrogated the spasmogenicity. Methemoglobin is fully oxidized and cannot act as an NO absorbent.

The greater propensity for vasospasm after aneurysm rupture compared with traumatic SAH may be explained by a difference in the oxygen tension of the blood enter-
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TABLE 3
Coma scores in each dog presented by treatment group

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Coma Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-SAH</td>
<td>14</td>
</tr>
<tr>
<td>SAH</td>
<td>12, 14, 14, 14</td>
</tr>
<tr>
<td>2 μmol DETA/NO</td>
<td>8, 12, 14, 14</td>
</tr>
<tr>
<td>0.2 μmol DETA/NO</td>
<td>12, 14, 14, 14, 14</td>
</tr>
<tr>
<td>2 μmol DETA/NO</td>
<td>8, 12, 14, 14, 14</td>
</tr>
</tbody>
</table>

* Each animal was examined posttreatment and graded according to the 14-point Cat Coma Scale of Hilton, et al. (*respiration: 1 = apneic, 2 = ataxic, 3 = normal; eye opening: 1 = no eye response, 2 = eyelids close sluggishly, 3 = normal; motor: 1 = flaccid, 2 = extensor posturing, 3 = withdraws, 4 = withdraws + has pedaling, 5 = lethargic + withdraws + lift head, 6 = lethargic + sternal recumbency, 7 = drowsy + purposeful, 8 = normal). Points were lost solely on the motor component of the scale.

TABLE 4
Physiological variables as functions of time in dogs with induced cerebral vasospasm*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>&amp; Group</th>
<th>Baseline</th>
<th>1 Hr</th>
<th>2 Hrs</th>
<th>4 Hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>control</td>
<td>86.3 ± 18.2</td>
<td>86.3 ± 24.4</td>
<td>86.1 ± 28.4</td>
<td>97.9 ± 27.9</td>
</tr>
<tr>
<td>0.2 μmol</td>
<td></td>
<td>69.3 ± 20.6</td>
<td>77.0 ± 20.3</td>
<td>77.8 ± 21.9</td>
<td>95.3 ± 31.3</td>
</tr>
<tr>
<td>2 μmol</td>
<td></td>
<td>108.8 ± 27.2</td>
<td>94.2 ± 27.8</td>
<td>92.4 ± 38.6</td>
<td>101.2 ± 39.8</td>
</tr>
<tr>
<td>RR</td>
<td>control</td>
<td>15.3 ± 2.2</td>
<td>14.9 ± 1.7</td>
<td>14.5 ± 2.1</td>
<td>15.0 ± 2.7</td>
</tr>
<tr>
<td>0.2 μmol</td>
<td></td>
<td>16.8 ± 2.6</td>
<td>16.6 ± 0.9</td>
<td>16.8 ± 1.3†</td>
<td>17.0 ± 2.9</td>
</tr>
<tr>
<td>2 μmol</td>
<td></td>
<td>13.8 ± 2.5</td>
<td>14.4 ± 2.0</td>
<td>15.4 ± 2.4</td>
<td>15.2 ± 2.3</td>
</tr>
<tr>
<td>ETCO₂</td>
<td>control</td>
<td>40.4 ± 1.7</td>
<td>40.8 ± 2.9</td>
<td>41.8 ± 1.8</td>
<td>41.1 ± 1.6</td>
</tr>
<tr>
<td>0.2 μmol</td>
<td></td>
<td>41.2 ± 2.3</td>
<td>41.4 ± 2.1</td>
<td>41.6 ± 0.9</td>
<td>39.8 ± 3.4</td>
</tr>
<tr>
<td>2 μmol</td>
<td></td>
<td>40.4 ± 2.5</td>
<td>39.8 ± 1.9</td>
<td>40.8 ± 1.1</td>
<td>41.4 ± 1.7</td>
</tr>
<tr>
<td>SBP</td>
<td>control</td>
<td>102.6 ± 9.9</td>
<td>98.4 ± 11.9</td>
<td>100.1 ± 7.8</td>
<td>100.1 ± 12.0</td>
</tr>
<tr>
<td>0.2 μmol</td>
<td></td>
<td>108.2 ± 18.1</td>
<td>122.6 ± 32.0</td>
<td>122.6 ± 32.1</td>
<td>114.4 ± 21.5</td>
</tr>
<tr>
<td>2 μmol</td>
<td></td>
<td>140.8 ± 37.3</td>
<td>129.8 ± 35.9</td>
<td>118.0 ± 22.9</td>
<td>111.6 ± 19.0</td>
</tr>
<tr>
<td>DBP</td>
<td>control</td>
<td>70.4 ± 17.5</td>
<td>60.9 ± 10.0</td>
<td>66.0 ± 4.9</td>
<td>66.4 ± 7.8</td>
</tr>
<tr>
<td>0.2 μmol</td>
<td></td>
<td>71.0 ± 19.0</td>
<td>73.8 ± 18.2</td>
<td>72.8 ± 18.2</td>
<td>67.0 ± 11.2</td>
</tr>
<tr>
<td>2 μmol</td>
<td></td>
<td>91.4 ± 35.1</td>
<td>82.4 ± 33.8</td>
<td>73.6 ± 19.4</td>
<td>70.8 ± 13.9</td>
</tr>
<tr>
<td>CVP</td>
<td>control</td>
<td>9.6 ± 11.2</td>
<td>8.8 ± 10.9</td>
<td>7.0 ± 12.5</td>
<td>7.8 ± 11.6</td>
</tr>
<tr>
<td>0.2 μmol</td>
<td></td>
<td>13.6 ± 13.4</td>
<td>13.2 ± 13.8</td>
<td>10.6 ± 16.4</td>
<td>11.0 ± 16.6</td>
</tr>
<tr>
<td>2 μmol</td>
<td></td>
<td>4.6 ± 4.3</td>
<td>6.4 ± 6.6</td>
<td>5.4 ± 7.3</td>
<td>2.6 ± 9.5</td>
</tr>
<tr>
<td>ICP</td>
<td>control</td>
<td>3.9 ± 3.2</td>
<td>2.9 ± 2.8</td>
<td>4.1 ± 3.6</td>
<td>3.9 ± 3.6</td>
</tr>
<tr>
<td>0.2 μmol</td>
<td></td>
<td>8.8 ± 4.6†</td>
<td>9.8 ± 4.3†</td>
<td>9.8 ± 3.8†</td>
<td>12.6 ± 6.7†</td>
</tr>
<tr>
<td>2 μmol</td>
<td></td>
<td>14.6 ± 3.6</td>
<td>5.8 ± 3.7</td>
<td>7.2 ± 3.8</td>
<td>8.6 ± 4.8</td>
</tr>
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</table>

* The mean ± 1 standard deviation is presented for each variable as a function of time posttreatment for each experimental group. Baseline readings (time 0) immediately precede intracarotid injection of drug. Abbreviations: HR = heart rate; RR = respiratory rate.
† Statistically significant difference from the DETA control group by ANOVA (α = 0.05). A trend was found in the SBP (SED², p < 0.01) and DBP data (SED², p < 0.01) for the DETA/NO 2-μmol group.

Conflicting experimental evidence has led to considerable debate over whether NO is cytotoxic or cytoprotective. 24,25,43,44 Certainly the redox state of the local milieu plays a role, but interestingly, so does the choice of NO donor. For example, the cyanide moiety of sodium nitroprusside, a commonly used NO donor, potentiates cell death in the presence of superoxide and hydroxyl free radicals, whereas the NO released from diazeniumdiolates can be cytoprotective. 26

Cytotoxic effects are thought to be mediated via the Fenton reaction, or alternatively, by NO combining with superoxide radicals at physiological pH to form peroxynitrite, a nonradical. In turn, peroxynitrite may be indirectly cytotoxic by decomposing to form a hydroxyl radical or directly toxic by oxidizing thiol groups.

A cytotoxic effect of NO is indicated by two in vivo observations: 1) NOS inhibitors increase cerebral cortex damage during ischemia–reperfusion; and 2) NO prevents ischemia–reperfusion injury in the brain and heart. 27

Wink and colleagues 28 have used cultured lung fibroblasts to demonstrate that NO markedly reduces superoxide- and hydrogen peroxide–mediated cytotoxicity by scavenging these free radicals and alternatively producing...
We speculate that the significance of NO in alternative redox states may prevent protective effects. Myelopathic signs were identified and a lower incidence of ischemic infarction has been demonstrated in several studies.

The efficacy of both intrathecal and intravascularly delivered NO or its precursors in mediating arterial dilation has been demonstrated in several studies. In the present study, an intrathecally administered bolus dose (0.15 μmol/kg) of DETA/NO (typical) produced arterial dilation to nearly 100% of the baseline diameter with a sustained efficacy of 88% at 4 hours. The use of a long-acting NO donor has obviated the requirement for continuous infusion, which has hindered clinical implementation of several experimental treatment modalities. Even so, the observed half-life of 5.2 hours is considerably shorter than the expected value of 20 hours for DETA/NO at physiological pH. We believe this is because of transport of the DETA adduct out of the central nervous system via nonspecific bulk transport during CSF reabsorption. The recurrent pattern of arterial dilation following the repeated dose after 4 hours (Fig. 3) indicates that, at least in the short term, tachyphylaxis does not occur.

The cause of a brief nadir in the initial phase of DETA/NO-induced arterial dilation is unclear. Possible mechanisms include release of serotonin from platelet dissolution or hemoglobin from traumatic cisternal tap or mildly undamaged feedback in the autoregulatory cascade. Drug infusion through a permanently implanted ventricular catheter could rule out the second mechanism.

The failure to dilate much beyond 100% of the baseline diameter may be a dose-dependent phenomenon, but more promising, may also indicate an inability of the drug to induce a hyperemic state. Dose escalation studies to address this issue are currently underway.

The sporadic choroid plexitis may be attributed to an interaction of ascorbate and free iron ions in the setting of SAH. The choroid plexus has a specific active transport system that concentrates ascorbic acid to 10 times that of plasma. Ascorbic acid has antioxidant properties, except when complexed with iron or other transition metals, under which circumstances it generates hydroxyl free radicals.

No histopathological sequelae resulting from DETA/NO were identified and a lower incidence of ischemic injuries in the high-dose treatment group indicates a cerebroprotective effect. Myelopathic signs were sporadically encountered regardless of treatment or control group and these were thought to result from injury secondary to repeated cisterna magna punctures. The use of an indwelling ventricular or basal cistern catheter could preclude these injuries.

The use of an intrathecal NO donor with a long half-life provides several theoretical advantages over other strategies for NO delivery (that is, continuous intrathecal, intraarterial, and intravenous infusion). Intraarterial administration of NO donors typically mandates very short-acting agents to counter adverse systemic effects, such as may be seen with intravenously administered NO donors. Relatively large quantities of NO donor must be delivered via the intravascular route, because much of the liberated NO is consumed by intravascular hemoglobin, and methemoglobinemia may ensue. Diffusion of the NO donor is limited by the blood-brain barrier such that only dissociated NO can diffuse a short distance beyond. Whereas NO is highly diffusible, its short half-life and high affinity for hemoglobin relegate it to providing only a local effect at the blood vessel wall. Unless systemic effects are tolerable, the aforementioned shortcomings necessitate continuous intrathecal infusion, which can be fraught with complications.

Bolus intrathecal administration minimizes infused volume and eliminates the requirement for a pump. If the rate of drug elimination from the CSF is slow compared with the turnover rate of CSF, then a steady-state mixing of drug may be achieved and longer diffusion distances (such as would be encountered in the human) will be consequential. The efficacy demonstrated in several experimental studies in which intrathecal NO donors were used attests that either NO or the NO donor is able to diffuse through the subarachnoid spaces to the site of the vascular smooth muscle. Thus, intrathecally administered NO donors might be expected to have a direct effect on vascular smooth muscle via cGMP. In addition, dissociation of NO adducts in the subarachnoid space would promote the conversion of oxyhemoglobin and deoxyhemoglobin to the less spasmogenic nitrosohemoglobin or methemoglobin forms. Complete conversion to the latter two forms would saturate the NO absorbent and potentially restore normal diffusion gradients for endogenously produced NO. Other potential salutary effects of intrathecally administered NO include inhibition of platelet aggregation and of smooth-muscle hyperplasia, which are concomitant features of vasospasm.

Although the etiology of vasospasm remains debatable, the dysregulation of NO metabolism is increasingly apparent. Although not a panacea, intrathecal administration of exogenous NO from an appropriately selected donor may prove to be a powerful therapeutic tool.

Conclusions

Arteriographic evidence demonstrates that intrathecal bolus injection of the long-acting water-soluble NO donor DETA/NO can effectively reverse cerebral vasospasm. Furthermore, the effect is sustained for hours and does not appear to involve tachyphylaxis.

This study did not demonstrate histopathological sequelae attributable to the doses of DETA/NO used. Concerns over the potential toxicity of exogenously intro-
Reversal of cerebral vasospasm with DETA/NO

duced intrathecal NO should be tempered by the recognition of cytoprotective effects, both from vascular perfusion and antioxidant standpoints.

The high affinity of hemoglobin for NO results in an absorbescent effect in the setting of SAH. This disrupts the normal concentration of endogenous NO that is the primary mediator of vasodilatory tone. With refinement of dosing regimens, it may be possible to titrate exogenous-ly administered NO to achieve reversal of vasospasm and a return to normal autoregulation.

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References


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