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DELAYED INTRACRANIAL DELIVERY OF A NITRIC OXIDE DONOR FROM A CONTROLLED-RELEASE POLYMER PREVENTS EXPERIMENTAL CEREBRAL VASOSPASM IN RABBITS

Gustavo Pradilla, M.D.
Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland

Quoc-Anh Thai, M.D.
Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland

Federico G. Legnani, M.D.
Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, and Ospedale San Gerardo, Monza, Italy

Wesley Hsu, B.S.
Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland

Ryan M. Kretzer, B.A.
Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland

Paul P. Wang, M.D.
Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland

Rafael J. Tamargo, M.D.
Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland

Reprint requests:
Rafael J. Tamargo, M.D., Department of Neurosurgery, Cerebrovascular Surgery Division, Johns Hopkins University School of Medicine, Meyer Building 8-181, 600 N. Wolfe Street, Baltimore, MD 21287.
AQ: 1 Email: rtamarg@jhmi.edu

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OBJECTIVE: Decreased local availability of nitric oxide (NO) may mediate chronic vasospasm after aneurysmal subarachnoid hemorrhage (SAH). Previous reports have shown that early treatment with NO prevents vasospasm in animals. We evaluated the efficacy of controlled-release polymers that contain the NO donor diethylenetriamine (DETA-NO) for the delayed treatment of vasospasm in a rabbit model of SAH.

METHODS: DETA-NO 20% (wt/wt) was incorporated into ethylene-vinyl acetate (EVAc) polymers. Animals (n = 52) were randomized to two experimental groups. In the first group (n = 32), animals received SAH and implantation of either 20% DETA-NO/EVAc polymer at a dose of 0.5 mg/kg of DETA-NO (n = 16) or empty EVAc polymer (n = 16). Polymers were implanted 24 (n = 16) or 48 hours (n = 16) after SAH. In the second group (n = 20), animals received SAH and implantation of either 20% DETA-NO/EVAc polymer at a dose of 1.3 mg/kg (n = 10) or empty EVAc (n = 10). Polymers were implanted 24 (n = 10) or 48 hours (n = 10) after SAH. An additional group (n = 16) underwent either sham operation (n = 6) or SAH only (n = 10). Animals were killed 3 days after hemorrhage, and the basilar arteries were processed for morphometric measurements. Results were analyzed using Student's *t* test.

RESULTS: Treatment with 20% DETA-NO/EVAc polymers at a dose of 1.3 mg/kg significantly increased basilar artery lumen patency when administered at 24 (97 ± 6% versus 73 ± 10%; $P = 0.0396$) or 48 hours (94 ± 6% versus 71 ± 9%; $P = 0.03$) after SAH. Treatment with 20% DETA-NO/EVAc polymers at a dose of 0.5 mg/kg administered 48 hours after SAH significantly increased lumen patency (82 ± 8% versus 68 ± 12%; $P = 0.03$); a dose of 0.5 mg/kg, 24 hours after SAH, did not reach statistical significance (74 ± 7% versus 65 ± 9%; $P = 0.16$). The SAH-only group had a lumen patency of 67 ± 12%.

CONCLUSION: Delayed treatment of SAH with controlled-release DETA-NO polymers prevented experimental posthemorrhagic vasospasm in the rabbit. This inhibition was dose-dependent. This further confirms the role of NO in the pathogenesis of vasospasm.

KEY WORDS: Diethylenetriamine-nitric oxide, Polymer, Rabbit, Subarachnoid hemorrhage, Vasospasm

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Delayed cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) seems to be the result, in part, of an imbalance between the concentration of nitric oxide (NO) (15, 56) and of vasospastic molecules such as endothelin-1 (10, 15, 56) in the subarachnoid space. A decrease in NO concentration is caused by the impaired production of NO by endothelial cells (40) and by the scavenging activity of oxyhemoglobin 1(17, 50), oxygen free radicals (42), and other factors (50). Depleted NO decreases the activation of soluble guanylate cyclase within smooth muscle cells, which then decreases cyclic guanosine monophosphate levels and results in vasoconstriction (1). An increase in

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endothelin-1 concentration is caused by apoptosis and degranulation of macrophages and polymorphonuclear leukocytes (13, 20) and by increased production by endothelial cells, astrocytes, and neurons (10, 38). Endothelin-1 binds to an endothelin-A receptor located on smooth muscle cells (38), which activates a Gαs protein that operates voltage-gated calcium channels, as well as nonspecific cation channels, which results in vasoconstriction (10). Endothelin-1 also binds to an endothelin-B receptor present on endothelial cells, which mediates the release of prostacyclin, NO, and adrenomedullin and induces vasoconstriction in venous vessels (10, 38).

One strategy to restore arterial tone after SAH is to administer NO directly into the subarachnoid space. We have previously shown that local delivery of the NO donor compound diethylenetriamine-NO (DETA-NO) from controlled-release polymers implanted in the subarachnoid space prevents posthemorrhagic vasospasm in a rabbit model of SAH. In that experiment, DETA-NO polymers were placed in the subarachnoid space 30 minutes after induction of SAH (22) and significantly increased the lumen patencies of basilar arteries when compared with no treatment (93.0 ± 4.9% versus 71.4 ± 11.9%) or treatment with blank ethylene-vinyl acetate (EVAc) polymer (93.0 ± 4.9% versus 73.2 ± 6.4%).

Patients who experience an aneurysmal SAH, however, typically seek medical attention hours or even days after the hemorrhage. Furthermore, surgical or endovascular intervention may be delayed 12 to 24 hours after diagnosis. If the implantation in the subarachnoid space of controlled-release polymers with NO donors is to be useful clinically, we must first determine whether delayed treatment with DETA-NO prevents vasospasm. We have previously shown in the rat femoral artery model of chronic posthemorrhagic vasospasm that delayed treatment with DETA-NO prevents vasospasm (66). This model, however, uses a peripheral vessel, and its application to SAH is often questioned (35, 41).

In this study, we evaluated the efficacy of delayed therapy with locally delivered 20% DETA-NO incorporated into EVAc polymers in the prevention of vasospasm in a rabbit model of SAH. Peak vasospasm in this model occurs 72 hours after SAH. We evaluated treatment with two doses of DETA-NO at 24 and 48 hours after SAH, which represent one-third and two-thirds of the time course preceding the onset of peak vasospasm in the rabbit model.

MATERIALS AND METHODS

Experimental Design

Animals (n = 52) were randomized to two experimental groups. In the first group (n = 32), animals received an SAH followed by implantation of either a 20% DETA-NO/EVAc polymer at a dose of 0.5 mg/kg of DETA-NO (n = 16) or an empty EVAc polymer (n = 16). Polymer implantations were performed 24 (n = 16) or 48 hours (n = 16) after induction of SAH. In the second group (n = 20), animals received an SAH followed by implantation of either a 20% DETA-NO/EVAc polymer at a dose of 1.3 mg/kg of DETA-NO (n = 10) or an empty EVAc polymer (n = 10). Polymers were implanted at either 24 (n = 10) or 48 hours (n = 10) after SAH. To establish a baseline lumen patency of the basilar artery and a percentage of lumen patency after induction of SAH without any treatment, an additional group of animals (n = 16) underwent either a sham operation (n = 6) or induction of SAH only (n = 10).

Polymer Preparation

We have previously described the technique for incorporating DETA-NO into controlled-release EVAc polymer (61, 66). Briefly, DETA-NO (Alexis Biochemicals, Lausen, Switzerland) and EVAc polymer (40% vinyl acetate by weight; DuPont, Wilmington, DE) were dissolved in methylene chloride (Fisher Chemicals, Fair Lawn, NJ) to produce EVAc polymers loaded with 20% (wt/wt) DETA/NO. The suspensions obtained were transferred into cylindrical glass molds and maintained at −70°C for 1 hour. The resulting polymer cylinders were placed in −80°C for 48 hours and then transferred to −21°C for 1 week. The polymer rods were then placed in a vacuum desiccator at room temperature for 48 hours to extract the remaining methylene chloride. Empty EVAc polymers were synthesized similarly.

Animals

New Zealand White rabbits (*Oryctolagus cuniculus*; Robinson Co., Winston Salem, NC) (n = 68) weighing an average of 3.3 kg were used in this experiment. The animals were housed in standard animal facilities with free access to Baltimore city water and rodent chow. The Animal Care and Use Committee of the Johns Hopkins University School of Medicine approved all experimental protocols.

Surgical Technique

We have previously described the technique for induction of SAH and placement of polymers in the cisterna magna of the rabbit (22). Briefly, after induction of anesthesia, the cervical region was shaved with clippers and prepared with alcohol and povidone-iodine solution. Ceftriaxone sodium (50 mg/kg) was prophylactically administered intramuscularly. A midline incision was made in the suboccipital region, from the inion to C1; the dura mater was exposed from the foramen magnum to the lamina of C1, exposing the dura mater; and 1 ml of cerebrospinal fluid was aspirated. To induce an SAH, 1.5 to 2 ml of nonheparinized blood was aspirated from the central ear artery and immediately injected into the cisterna magna. The animals were then placed head down at 30 degrees for 30 minutes to confine the blood to the intracranial cisterns. A parasagittal linear incision was made in the dura mater, and either DETA-NO/EVAc or empty EVAc polymers were placed in the cisterna magna. A piece of Gelfoam (Pharmacia & Upjohn, Kalamazoo, MI) was placed over the insertion site, and the wound was closed.

Histological Assessment

Angiographic evidence of peak vasospasm in the rabbit model of SAH is present at 72 hours after blood injection into the cisterna magna (8, 16, 22). Therefore, animals were killed at 72 hours after induction of experimental SAH by intraperitoneal injection of sodium pentobarbital (200 mg/kg), and in situ perfusion/fixation was performed as previously described elsewhere (22). The basilar artery and the brainstem were harvested *en bloc*, cryoprotected, and frozen. Twenty-micrometer transverse sections were obtained with a microtome cryostat at 200-μm intervals beginning at the basilar termination. Tissue slices were mounted on Superfrost Plus slides (Fisher Scientific, Fair Lawn, NJ) for hematoxylin and eosin staining.

Morphometric Analysis

Histological sections of the basilar artery were digitized, luminal cross sectional areas were outlined, and the circumference of the basilar artery was measured by computerized analysis (MCID; Imaging Research, Inc., St. Catharines, ON, Canada). Sections of the basilar artery (n = 7) 20 μm thick and 200 μm apart were evaluated and averaged to control for vessel deformation and off-transverse sections. The vessel perimeter was obtained by interactive measurements of vessel sections. Estimated cross sectional areas were converted to lumen-patency percentages, and absolute values were defined by average of cross sectional areas from sham-operated animals.

Statistical Analysis

Mean vessel perimeters are expressed as mean ± standard error of the mean. Mean perimeters of basilar arteries are expressed as percentage of lumen patency (% lumen patency) obtained by dividing the mean perimeter of each group by the mean perimeter of the sham group. To determine statistical significance, the mean vessel perimeters (measured in millimeters) of the basilar arteries from the treatment and control groups for every time point and with each dose of DETA-NO were compared using Student's *t* test. A P value of <0.05 was considered significant. Statistical analysis was performed using SPSS software, version 8.0 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

Treatment with 20% DETA-NO/EVAc polymers at a dose of 1.3 mg/kg resulted in a significant increase of basilar artery lumen patency when treatment was administered at either 24 or 48 hours after SAH. At 24 hours, animals receiving implants of 20% DETA-NO/EVAc polymers had a 97 ± 6% basilar artery lumen patency, whereas animals receiving implants of empty EVAc polymers had a 73 ± 10% lumen patency (P = 0.03). Similarly, at 48 hours, animals receiving implants of 20% DETA-NO/EVAc polymers had a 94 ± 6% lumen patency, whereas animals receiving implants of empty EVAc polymers had a 71 ± 9% lumen patency (P = 0.03) (*Table 1*, *Fig. 1*). AQ: 2 T1-F1

Treatment with 20% DETA-NO/EVAc polymers at the lower dose of 0.5 mg/kg administered 48 hours after SAH resulted in a significant increase of lumen patency (82 ± 8%

TABLE 1. Lumen patency of the basilar artery[a]

	%	SEM	No.
Sham	100		6
SAH only	67	12.13	10
Blank EVAc 24 h (0.5 mg/kg)	65	9.12	8
DETA-NO 24 h (0.5 mg/kg)	74	7.46	5
Blank EVAc 48 h (0.5 mg/kg)	68	12.19	8
DETA-NO 48 h (0.5 mg/kg)	82	7.75	5
Blank EVAc 24 h (1.3 mg/kg)	73	10.25	8
DETA-NO 24 h (1.3 mg/kg)	97	5.55	5
Blank EVAc 48 h (1.3 mg/kg)	71	8.82	8
DETA-NO 48 h (1.3 mg/kg)	94	6.01	5
Total			68

[a] SEM, standard error of the mean; SAH, subarachnoid hemorrhage; EVAc, ethylene-vinyl acetate; DETA-NO, diethylenetriamine–nitric oxide.

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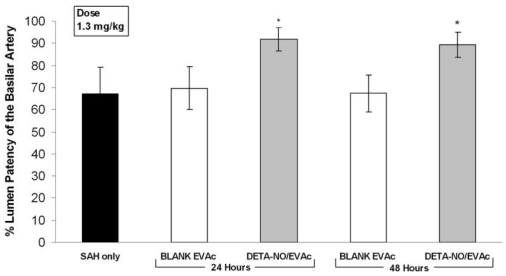

FIGURE 1. *Histogram showing the percentage of lumen patency of basilar arteries from rabbits treated with either DETA-NO/EVAc polymers at a dose of 1.3 mg/kg or blank polymers 24 and 48 hours after SAH.*

versus 68 ± 12%; $P = 0.03$). Treatment with 20% DETA-NO/EVAc polymers at the lower dose of 0.5 mg/kg administered 24 hours after SAH showed a trend of greater basilar artery lumen patency but did not reach statistical significance (74 ± 7% versus 65 ± 9%; $P = 0.16$) (*Fig. 2*). Animals in the SAH-only group had a lumen patency of 67 ± 12%.

DISCUSSION

In this study, we describe the effect of delayed treatment of cerebral vasospasm after SAH using 20% DETA-NO/EVAc polymers placed in the cisterna magna of rabbits. We found that DETA-NO doses of 1.3 mg/kg administered at 24 and 48 hours after SAH, as well as 0.5 mg/kg of DETA-NO at 48 hours (but not at 24 h), were efficacious treatments for vasospasm in rabbits (*Fig. 3*).

Previously, we demonstrated that DETA-NO delivered by controlled-release polymers at a dose of 4 mg/kg could prevent and reverse chronic vasospasm in the rat femoral artery model (66) when treatment was administered at 1, 3, or 7 days after hemorrhage. Similarly, we showed that DETA-NO delivered by controlled-release polymers at a dose of 0.5 mg/kg was effective in preventing vasospasm in the rabbit model of posthemorrhagic vasospasm (22) when treatment was initiated 30 minutes after SAH. DETA-NO therapy in patients, however, would most likely be initiated hours or days after SAH. In this study, we tested the efficacy of DETA-NO/EVAc polymers in a delayed setting, which would more closely reflect the clinical scenario.

Levels of endothelium-derived NO in the subarachnoid space tend to decrease progressively over time. This is attributed to the scavenging effect of oxyhemoglobin (17, 50) and oxygen free radicals (42) and to the disappearance of neuronal NO synthase after SAH caused by the toxic effect of oxyhemoglobin on neurons in the adventitia of cerebral vessels (28, 52). This endothelial dysfunction is perpetuated by the accumulation of vasospastic molecules such as endothelin-1 (10), which decreases cyclic guanosine monophosphate in smooth muscle cells, hence increasing vasoconstriction (15, 56). These events suggest that higher levels of exogenous NO need to be delivered to the subarachnoid space as the pathophysiological process evolves to prevent the development of cerebral vasospasm.

DETA-NO, a diazeniumdiolate-class NO donor, is a water-soluble zwitterionic triamine/NO adduct, with a half-life of approximately 20 hours (21, 26, 29, 51, 55). Diazeniumdiolates are chemical compounds that carry the [N(O)NO]− functional group (29). When dissolved in physiological solutions, DETA-NO generates molecular NO in yields that approach the theoretical 2 mol/mol. Diazeniumdiolates might be of benefit for treatment of delayed vasospasm, given their longer decomposition half-lives (which may allow intermittent therapy), high water solubility, and stability in solid form. Intra-arterial and intrathecal administration of DETA-NO has been tested experimentally for treatment of posthemorrhagic cerebral vasospasm in canine and nonhuman primate models with variable results (1, 51, 69). The vasodilatory benefits of DETA-NO have also been investigated in other pathological

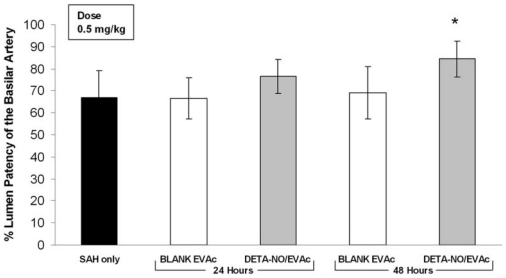

FIGURE 2. *Histogram showing the percentage of lumen patency of basilar arteries from rabbits treated with either DETA-NO/EVAc polymers at a dose of 0.5 mg/kg or blank polymers 24 and 48 hours after SAH.*

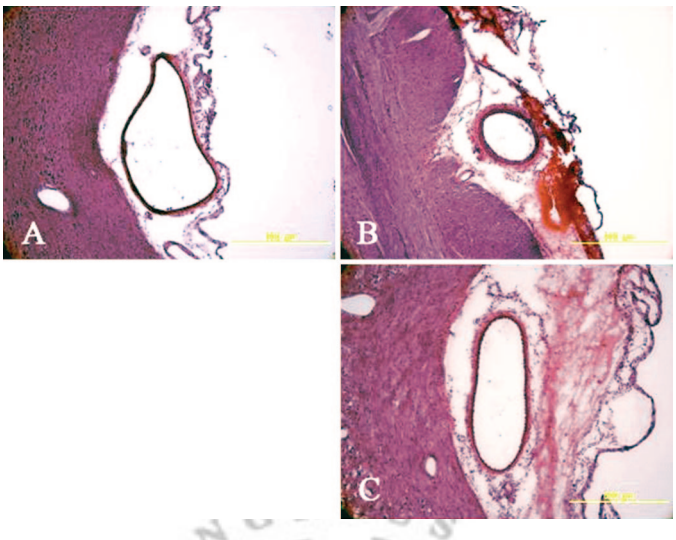

FIGURE 3. *Cross sectional views of basilar arteries.* A, *section from the basilar artery of an animal that underwent a sham operation.* B, *section from an animal that received SAH followed by implantation of an empty EVAc polymer.* C, *section from an animal that received SAH and implantation of a 20% DETA-NO/EVAc polymer 48 hours later at a dose of 1.3 mg/kg of DETA-NO* (A–C, *hematoxylin and eosin; original magnification,* ×*10).*

entities such as ischemic stroke (14, 54), pulmonary hypertension (30–32), and cardiovascular disease (68, 70), among others.

Continuous delivery of NO to the cerebral vasculature should be maintained at sufficient levels throughout the time period when vasospasm is likely to develop. Alternative ways of administration of DETA-NO have included intrathecal delivery using catheters (1) or injections (69), or repeated intra-arterial infusions (51), which increase the possibility of complications and are affected by pharmacokinetic parameters that alter the concentration and absorption of the drug.

Controlled-release polymers are devices capable of releasing high and sustained drug concentrations at the site of implantation with reproducible pharmacokinetic profiles and minimal systemic toxicity. Controlled-release polymers can be placed at the time of surgery or injected intrathecally. These devices have been shown clinically to be efficacious in the delivery of chemotherapeutic agents for the treatment of malignant brain tumors and have been used experimentally to treat cerebral edema, seizures, and vasospasm in animal models (6, 7, 33, 34, 60, 62, 63, 65, 67). Furthermore, we have previously characterized the rate and extent of diffusion of molecules loaded into controlled-release polymers, when polymers are placed in the subarachnoid space of rabbits with and without SAH (53). We have shown that agents with a molecular weight and an octanol/water partition coefficient comparable to those of DETA-NO diffuse a distance 40 cm or more when delivered via controlled-release polymers in the subarachnoid space and that the presence of SAH does not interfere with the rate and extent of diffusion in the rabbit model.

Experimental vasospasm has been reliably induced in this rabbit model by our group and others using the modified technique described by Chan et al. (8). Peak vasospasm in this model occurs at 72 hours, and adequate correlation has been demonstrated between angiographic vasospasm and perfusion-fixed cross sections of the basilar artery (35). Advantages of this model include analysis of an intracranial vessel, a standardized volume of SAH, sufficient amounts of tissue for histopathological analysis, high reproducibility, and low cost. Disadvantages of this model include the development of subacute vasospasm (Day 3 compared with human peak vasospasm, which occurs 7–10 d after SAH [48, 64]), and the absence of neurological deficits after vasospasm, a drawback shared by all animal models of posthemorrhagic vasospasm (35, 41).

The inflammatory response present after aneurysmal SAH seems to be mediated by cell adhesion molecules (CAMs), present in the endothelial surface, which regulate leukocyte-endothelial cell interactions (3, 11–13, 24, 37, 44, 45, 58, 65). One of the most important CAMs involved in this process is intercellular adhesion moelucule-1 (ICAM-1), which is upregulated through several mechanisms, including interleukin-1β. Recent reports have demonstrated that DETA-NO inhibits endothelial interleukin-1β-induced *ICAM-1* gene expression at the transcriptional level by decreasing the activity of the redox-sensitive transcription factors Sp1 and AP-1 (4). After aneurysmal SAH, erythrocytes in the subarachnoid space form a thrombus around the vessel wall (36, 49). Acute phase reactants such as interleukin-1β (25, 47), tumor necrosis factor-α (2, 39), and interferon-γ (27), among others, are produced in the thrombus and induce upregulation of CAMs, particularly selectins and ICAM-1 in the endothelial layer (56). These CAMs on the endothelial surface promote binding of macrophages and neutrophils through their β-2 integrin receptors and cause arrest of rolling leukocytes, adhesion, and diapedesis into the subarachnoid space (59), where they are attracted toward the periadventitial thrombus by released che-

moattractants. After phagocytosis of erythrocytes and debris, leukocytes die and degranulate in the subarachnoid space, releasing endothelins (5, 10, 20), oxygen free radicals (9, 18, 57), chemokines (19, 23, 43, 46), and other products (5, 20, 22, 56) that lesion the endothelium, decrease the synthesis of endothelium-derived NO, and cause chronic vasospasm. It is in this setting that the administration of exogenous NO will ameliorate or prevent vasospasm.

In conclusion, treatment with 20% DETA-NO/EVAc polymers placed in the subarachnoid space of rabbits in a delayed fashion prevents vasospasm after SAH. The effect of DETA-NO occurs in a dose-dependent manner, and a dose of 1.5 mg/kg seems to be appropriate to reverse vasospasm in a delayed fashion.

REFERENCES

1. Aihara Y, Jahromi BS, Yassari R, Sayama T, Macdonald RL: Effects of a nitric oxide donor on and correlation of changes in cyclic nucleotide levels with experimental vasospasm. **Neurosurgery** 52:661–667, 2003.
2. Aihara Y, Kasuya H, Onda H, Hori T, Takeda J: Quantitative analysis of gene expressions related to inflammation in canine spastic artery after subarachnoid hemorrhage. **Stroke** 32:212–217, 2001.
3. Bavbek M, Polin R, Kwan AL, Arthur AS, Kassell NF, Lee KS: Monoclonal antibodies against ICAM-1 and CD18 attenuate cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. **Stroke** 29:1930–1936, 1998.
4. Berendji-Grun D, Kolb-Bachofen V, Kroncke KD: Nitric oxide inhibits endothelial IL-1β-induced ICAM-1 gene expression at the transcriptional level decreasing Sp1 and AP-1 activity. **Mol Med** 7:748–754, 2001.
5. Bertsch T, Kuehl S, Muehlhauser F, Walter S, Hodapp B, Rossol S, Schmeck J, Ragoschke A, Schutt S, Fritzinger M, Horn P, Vajkoczy P, Wellner MW, Fassbender K: Source of endothelin-1 in subarachnoid hemorrhage. **Clin Chem Lab Med** 39:341–345, 2001.
6. Brem H: Polymers to treat brain tumours. **Biomaterials** 11:699–701, 1990.
7. Brem H, Gabikian P: Biodegradable polymer implants to treat brain tumors. **J Control Release** 74:63–67, 2001.
8. Chan RC, Durity FA, Thompson GB, Nugent RA, Kendall M: The role of the prostacyclin-thromboxane system in cerebral vasospasm following induced subarachnoid hemorrhage in the rabbit. **J Neurosurg** 61:1120–1128, 1984.
9. Choi JM, Kim CD, Hong KW: Involvement of NADH/NADPH oxidase-derived superoxide in experimental vasospasm induced by periarterial blood in rat femoral artery. **Life Sci** 69:1753–1763, 2001.
10. Chow M, Dumont AS, Kassell NF: Endothelin receptor antagonists and cerebral vasospasm: An update. **Neurosurgery** 51:1333–1342, 2002.
11. Chyatte D, Rusch N, Sundt TM Jr: Prevention of chronic experimental cerebral vasospasm with ibuprofen and high-dose methylprednisolone. **J Neurosurg** 59:925–932, 1983.
12. Clatterbuck RE, Gailloud P, Ogata L, Gebremariam A, Dietsch GN, Murphy KJ, Tamargo RJ: Prevention of cerebral vasospasm by a humanized anti-CD11/CD18 monoclonal antibody administered after experimental subarachnoid hemorrhage in nonhuman primates. **J Neurosurg** 99:376–382, 2003.
13. Clatterbuck RE, Oshiro EM, Hoffman PA, Dietsch GN, Pardoll DM, Tamargo RJ: Inhibition of vasospasm with lymphocyte function-associated antigen-1 monoclonal antibody in a femoral artery model in rats. **J Neurosurg** 97:676–682, 2002.
14. Coert BA, Anderson RE, Meyer FB: A comparative study of the effects of two nitric oxide synthase inhibitors and two nitric oxide donors on temporary focal cerebral ischemia in the Wistar rat. **J Neurosurg** 90:332–338, 1999.
15. Dumont AS, Dumont RJ, Chow MM, Lin CL, Calisaneller T, Ley KF, Kassell NF, Lee KS: Cerebral vasospasm after subarachnoid hemorrhage: Putative role of inflammation. **Neurosurgery** 53:123–135, 2003.
16. Endo S, Branson PJ, Alksne JF: Experimental model of symptomatic vasospasm in rabbits. **Stroke** 19:1420–1425, 1988.
17. Everse J, Hsia N: The toxicities of native and modified hemoglobins. **Free Radic Biol Med** 22:1075–1099, 1997.
18. Fabian RH, Kent TA: Superoxide anion production during reperfusion is reduced by an antineutrophil antibody after prolonged cerebral ischemia. **Free Radic Biol Med** 26:355–361, 1999.
19. Fassbender K, Hodapp B, Rossol S, Bertsch T, Schmeck J, Schutt S, Fritzinger M, Horn P, Vajkoczy P, Kreisel S, Brunner J, Schmiedek P, Hennerici M: Inflammatory cytokines in subarachnoid haemorrhage: Association with abnormal blood flow velocities in basal cerebral arteries. **J Neurol Neurosurg Psychiatry** 70:534–537, 2001.
20. Fassbender K, Hodapp B, Rossol S, Bertsch T, Schmeck J, Schutt S, Fritzinger M, Horn P, Vajkoczy P, Wendel-Wellner M, Ragoschke A, Kuehl S, Brunner J, Schurer L, Schmiedek P, Hennerici M: Endothelin-1 in subarachnoid hemorrhage: An acute-phase reactant produced by cerebrospinal fluid leukocytes. **Stroke** 31:2971–2975, 2000.
21. Fitzhugh AL, Keefer LK: Diazeniumdiolates: Pro- and antioxidant applications of the "NONOates." **Free Radic Biol Med** 28:1463–1469, 2000.
22. Gabikian P, Clatterbuck RE, Eberhart CG, Tyler BM, Tierney TS, Tamargo RJ: Prevention of experimental cerebral vasospasm by intracranial delivery of a nitric oxide donor from a controlled-release polymer: Toxicity and efficacy studies in rabbits and rats. **Stroke** 33:2681–2686, 2002.
23. Gaetani P, Tartara F, Pignatti P, Tancioni F, Rodriguez y Baena R, De Benedetti F: Cisternal CSF levels of cytokines after subarachnoid hemorrhage. **Neurol Res** 20:337–342, 1998.
24. Handa Y, Kubota T, Kaneko M, Tsuchida A, Kobayashi H, Kawano H, Kubota T: Expression of intercellular adhesion molecule 1 (ICAM-1) on the cerebral artery following subarachnoid haemorrhage in rats. **Acta Neurochir (Wien)** 132:92–97, 1995.
25. Hirashima Y, Nakamura S, Endo S, Kuwayama N, Naruse Y, Takaku A: Elevation of platelet activating factor, inflammatory cytokines, and coagulation factors in the internal jugular vein of patients with subarachnoid hemorrhage. **Neurochem Res** 22:1249–1255, 1997.
26. Hrabie JA, Keefer LK: Chemistry of the nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its oxygen-substituted derivatives. **Chem Rev** 102:1135–1154, 2002.
27. Hubbard AK, Rothlein R: Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades. **Free Radic Biol Med** 28:1379–1386, 2000.
28. Iuliano BA, Pluta RM, Jung C, Oldfield EH: Endothelial dysfunction in a primate model of cerebral vasospasm. **J Neurosurg** 100:287–294, 2004.
29. Keefer LK, Nims RW, Davies KM, Wink DA: "NONOates" (1-substituted diazen-1-ium-1,2-diolates) as nitric oxide donors: convenient nitric oxide dosage forms. **Methods Enzymol** 268:281–293, 1996.
30. Lam CF, Caterina P, Filion P, Ilett KF, van Heerden PV: The safety of aerosolized diethylenetriamine nitric oxide adduct after single-dose administration to anesthetized piglets and multiple-dose administration to conscious rats. **Toxicol Appl Pharmacol** 190:65–71, 2003.
31. Lam CF, Sviri S, Ilett KF, van Heerden PV: Inhaled diazeniumdiolates (NONOates) as selective pulmonary vasodilators. **Expert Opin Investig Drugs** 11:897–909, 2002.
32. Lam CF, van Heerden PV, Ilett KF, Caterina P, Filion P: Two aerosolized nitric oxide adducts as selective pulmonary vasodilators for acute pulmonary hypertension. **Chest** 123:869–874, 2003.
33. Legnani FG, Pradilla G, Wang PP, et al: Local delivery of antineoplastic agents using biodegradable polymers for the treatment of malignant brain tumors. **Neurotherapeutics** 3:89–102, 2003.
34. Lesniak MS, Langer R, Brem H: Drug delivery to tumors of the central nervous system. **Curr Neurol Neurosci Rep** 1:210–216, 2001.
35. Macdonald RL, Weir B: Animal models, in Macdonald RL, Weir B (eds): *Cerebral Vasospasm*. San Diego, Academic Press, 2001, pp 449–475.
36. Macdonald RL, Weir B: Hematology, in Weir B (ed): *Cerebral Vasospasm*. San Diego, Academic Press, 2001, pp 44–86.
37. Mack WJ, Mocco J, Hoh DJ, Huang J, Choudhri TF, Kreiter KT, Lozier A, Opperman M, Poisik A, Yorgason J, Solomon RA, Mayer SA, Connolly ES: Outcome prediction with serum intercellular adhesion molecule-1 levels after aneurysmal subarachnoid hemorrhage. **J Neurosurg** 96:71–75, 2002.

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38. Masaki T: The endothelin family: An overview. **J Cardiovasc Pharmacol** 35:S3–S5, 2000.
39. Mathiesen T, Edner G, Ulfarsson E, Andersson B: Cerebrospinal fluid interleukin-1 receptor antagonist and tumor necrosis factor-α following subarachnoid hemorrhage. **J Neurosurg** 87:215–220, 1997.
40. McGirt MJ, Lynch JR, Parra A, Sheng H, Pearlstein RD, Laskowitz DT, Pelligrino DA, Warner DS: Simvastatin increases endothelial nitric oxide synthase and ameliorates cerebral vasospasm resulting from subarachnoid hemorrhage. **Stroke** 33:2950–2956, 2002.
41. Megyesi JF, Vollrath B, Cook DA, Findlay JM: In vivo animal models of cerebral vasospasm: A review. **Neurosurgery** 46:448–461, 2000.
42. Mori T, Nagata K, Town T, Tan J, Matsui T, Asano T: Intracisternal increase of superoxide anion production in a canine subarachnoid hemorrhage model. **Stroke** 32:636–642, 2001.
43. Nam DH, Kim JS, Hong SC, Lee WH, Lee JI, Shin HJ, Park K, Eoh W, Han DH, Kim JH: Expression of interleukin-1β in lipopolysaccharide stimulated monocytes derived from patients with aneurysmal subarachnoid hemorrhage is correlated with cerebral vasospasm. **Neurosci Lett** 312:41–44, 2001.
44. Nissen JJ, Mantle D, Blackburn A, Barnes J, Wooldridge T, Gregson B, Mendelow AD: The selectin superfamily: The role of selectin adhesion molecules in delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage. **Acta Neurochir Suppl** 76:55–60, 2000.
45. Oshiro EM, Hoffman PA, Dietsch GN, Watts MC, Pardoll DM, Tamargo RJ: Inhibition of experimental vasospasm with anti-intercellular adhesion molecule-1 monoclonal antibody in rats. **Stroke** 28:2031–2037; discussion 2037–2038, 1997.
46. Osuka K, Suzuki Y, Tanazawa T, Hattori K, Yamamoto N, Takayasu M, Shibuya M, Yoshida J: Interleukin-6 and development of vasospasm after subarachnoid haemorrhage. **Acta Neurochir (Wien)** 140:943–951, 1998.
47. Osuka K, Suzuki Y, Watanabe Y, Takayasu M, Yoshida J: Inducible cyclooxygenase expression in canine basilar artery after experimental subarachnoid hemorrhage. **Stroke** 29:1219–1222, 1998.
48. Pakarinen S: Incidence, aetiology, and prognosis of primary subarachnoid haemorrhage: A study based on 589 cases diagnosed in a defined urban population during a defined period. **Acta Neurol Scand** 43[Suppl 29]:21–28, 1967.
49. Peterson JW, Roussos L, Kwun BD, Hackett JD, Owen CJ, Zervas NT: Evidence of the role of hemolysis in experimental cerebral vasospasm. **J Neurosurg** 72:775–781, 1990.
50. Petzold GC, Einhaupl KM, Dirnagl U, Dreier JP: Ischemia triggered by spreading neuronal activation is induced by endothelin-1 and hemoglobin in the subarachnoid space. **Ann Neurol** 54:591–598, 2003.
51. Pluta RM, Oldfield EH, Boock RJ: Reversal and prevention of cerebral vasospasm by intracarotid infusions of nitric oxide donors in a primate model of subarachnoid hemorrhage. **J Neurosurg** 87:746–751, 1997.
52. Pluta RM, Thompson BG, Dawson TM, Snyder SH, Boock RJ, Oldfield EH: Loss of nitric oxide synthase immunoreactivity in cerebral vasospasm. **J Neurosurg** 84:648–654, 1996.
53. Pradilla G, Wang PP, Legnani FG, Frazier JL, Tamargo RJ: Pharmacokinetics of controlled-release polymers in the subarachnoid space after subarachnoid hemorrhage in rabbits. **J Neurosurg** 101:99–103, 2004.
54. Salom JB, Barbera MD, Centeno JM, Orti M, Torregrosa G, Alborch E: Relaxant effects of sodium nitroprusside and NONOates in goat middle cerebral artery: Delayed impairment by global ischemia-reperfusion. **Nitric Oxide** 3:85–93, 1999.
55. Seccia M, Perugini C, Albano E, Bellomo G: Inhibition of Cu^{2+}-induced LDL oxidation by nitric oxide: A study using donors with different half-time of NO release. **Biochem Biophys Res Commun** 220:306–309, 1996.
56. Sercombe R, Dinh YR, Gomis P: Cerebrovascular inflammation following subarachnoid hemorrhage. **Jpn J Pharmacol** 88:227–249, 2002.
57. Sethi S, Sharma P, Dikshit M: Nitric oxide- and oxygen-derived free radical generation from control and lipopolysaccharide-treated rat polymorphonuclear leukocyte. **Nitric Oxide** 5:482–493, 2001.
58. Sills AK Jr, Clatterbuck RE, Thompson RC, Cohen PL, Tamargo RJ: Endothelial cell expression of intercellular adhesion molecule 1 in experimental posthemorrhagic vasospasm. **Neurosurgery** 41:453–461, 1997.
59. Springer TA: Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. **Annu Rev Physiol** 57:827–872, 1995.
60. Tamargo RJ, Epstein JI, Reinhard CS, Chasin M, Brem H: Brain biocompatibility of a biodegradable, controlled-release polymer in rats. **J Biomed Mater Res** 23:253–266, 1989.
61. Tamargo RJ, Myseros JS, Epstein JI, Yang MB, Chasin M, Brem H: Interstitial chemotherapy of the 9L gliosarcoma: Controlled release polymers for drug delivery in the brain. **Cancer Res** 53:329–333, 1993.
62. Tamargo RJ, Rossell LA, Kossoff EH, Tyler BM, Ewend MG, Aryanpur JJ: The intracerebral administration of phenytoin using controlled-release polymers reduces experimental seizures in rats. **Epilepsy Res** 48:145–155, 2002.
63. Tamargo RJ, Sills AK Jr, Reinhard CS, Pinn ML, Long DM, Brem H: Interstitial delivery of dexamethasone in the brain for the reduction of peritumoral edema. **J Neurosurg** 74:956–961, 1991.
64. Tamargo RJ, Walter KA, Oshiro EM: Aneurysmal subarachnoid hemorrhage: Prognostic features and outcomes. **New Horiz** 5:364–375, 1997.
65. Thai QA, Oshiro EM, Tamargo RJ: Inhibition of experimental vasospasm in rats with the periadventitial administration of ibuprofen using controlled-release polymers. **Stroke** 30:140–147, 1999.
66. Tierney TS, Clatterbuck RE, Lawson C, Thai QA, Rhines LD, Tamargo RJ: Prevention and reversal of experimental posthemorrhagic vasospasm by the periadventitial administration of nitric oxide from a controlled-release polymer. **Neurosurgery** 49:945–951, 2001.
67. Wang PP, Frazier J, Brem H: Local drug delivery to the brain. **Adv Drug Deliv Rev** 54:987–1013, 2002.
68. Wiley KE, Davenport AP: Novel nitric oxide donors reverse endothelin-1-mediated constriction in human blood vessels. **J Cardiovasc Pharmacol** 36[Suppl 1]:S151–S152, 2000.
69. Wolf EW, Banerjee A, Soble-Smith J, Dohan FC Jr, White RP, Robertson JT: Reversal of cerebral vasospasm using an intrathecally administered nitric oxide donor. **J Neurosurg** 89:279–288, 1998.
70. Yamamoto T, Kakar NR, Vina ER, Johnson PE, Bing RJ: The effect of aspirin and two nitric oxide donors on the infarcted heart in situ. **Life Sci** 67:839–846, 2000.

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