LOCAL DELIVERY OF IBUPROFEN VIA CONTROLLED-RELEASE POLYMERS PREVENTS ANGIOGRAPHIC VASOSPASM IN A MONKEY MODEL OF SUBARACHNOID HEMORRHAGE

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OBJECTIVE: Adhesion and migration of leukocytes into the periadventitial space play a role in the pathophysiology of vasospasm after subarachnoid hemorrhage (SAH). Intercellular adhesion molecule-1 is a determinant cell adhesion molecule involved in this process. Ibuprofen has been shown to inhibit intercellular adhesion molecule-1 upregulation and prevent vasospasm in animal models of SAH. In this study, we report the toxicity and efficacy of locally delivered ibuprofen incorporated into controlled-release polymers to prevent vasospasm in a monkey model of SAH.

METHODS: Ibuprofen was incorporated into ethylene-vinyl acetate (EVAc) polymers at 45% loading (wt:wt). For the toxicity study, cynomolgus monkeys (n = 5) underwent surgical implantation of either blank/EVAc polymers (n = 3) or 45% ibuprofen/EVAc polymers (n = 2) in the subarachnoid space, were followed up for 13 weeks, and were killed for histopathological analysis. For the efficacy study, cynomolgus monkeys (n = 14) underwent cerebral angiography 7 days before and 7 days after surgery and SAH and were randomized to receive either a 45% ibuprofen/EVAc polymer (n = 7; mean dose of ibuprofen, 6 mg/kg) or blank EVAc polymers (n = 7) in the subarachnoid space. Angiographic vasospasm was determined by digital image analysis. Student's *t* test was used for analysis.

RESULTS: Animals implanted with ibuprofen polymers showed no signs of local or systemic toxicity. Animals treated with ibuprofen polymers had 91 ± 9% lumen patency of the middle cerebral artery, compared with 53 ± 11% of animals treated with blank/EVAc polymers ($P < 0.001$).

CONCLUSION: Ibuprofen polymers are safe and prevent angiographic vasospasm after SAH in the monkey model. These findings support the role of cell adhesion molecules and inflammation in the pathophysiology of vasospasm.

KEY WORDS: Controlled release, Ibuprofen, Monkey, Polymer, Vasospasm

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Adhesion and migration of leukocytes into the periadventitial space may be determinant events in the pathophysiology of vasospasm after aneurysmal subarachnoid hemorrhage (SAH) (11–14, 32, 36–38, 44, 47). Leukocyte adhesion is mediated by the interaction between cell adhesion molecules expressed on the endothelium, such as intercellular adhesion molecule-1 (ICAM-1, CD54) and vascular cell adhesion molecule-1 (VCAM-1, CD106), and integrins expressed on the membrane of leukocytes, such as lymphocyte function antigen-1 (CD11a/CD18) and macrophage antigen-1 (CD11b/CD18), which are present on the surface of neutrophils and macrophages (39, 40). Marked upregulation of ICAM-1 has been observed by our group and others after periadventitial hemorrhage in animal models (1, 20, 38), and elevated levels of these proteins have been found in the cerebrospinal fluid and serum of patients with SAH who develop vasospasm (28, 30, 34).

Upregulation of ICAM-1 specifically occurs between 3 and 24 hours after periadventitial hemorrhage in animal models (38). Increased expression of ICAM-1 induces leukocytes to adhere to the endothelium and enter the subarachnoid space to perform phagocytosis of

senescent erythrocytes. Subsequent death and degranulation of these leukocytes releases oxygen free radicals, endothelin-1, cytokines, and other molecules that lesion the endothelium, impair normal vasodilatory mechanisms, and induce sustained vasoconstriction (1–3, 7, 13, 15, 37).

Disruption of the interaction between ICAM-1 and lymphocyte function antigen-1/macrophage antigen-1 after SAH greatly reduces leukocyte trafficking and prevents cerebral vasospasm in rat, rabbit, and monkey models (11, 12, 32, 36). Prevention of vasospasm has been specifically achieved by systemic administration of monoclonal antibodies targeted to ICAM-1 (32) and lymphocyte function antigen-1 (12) in the rat femoral artery model and to CD11/CD18 in the rabbit (36) and monkey SAH models (11). Similarly, intracisternal administration of anti-ICAM-1 and anti-CD18 monoclonal antibodies also prevented vasospasm in a rabbit model (5).

Ibuprofen is an anti-inflammatory agent that inhibits ICAM-1 and VCAM-1 upregulation and has been shown to prevent vasospasm in animal models (9, 10, 18, 21, 31, 44). The doses of ibuprofen required to prevent ICAM-1/VCAM-1 upregulation are significantly higher compared with the doses required for prostaglandin inhibition (18, 44); therefore, systemic administration of ibuprofen at these doses may increase the likelihood of severe side effects such as gastric erosion, thrombasthenia or thrombocytopenia, and fluid retention (9, 10). To obtain elevated ibuprofen levels on the affected endothelium with minimum systemic toxicity, a local drug delivery system that incorporates ibuprofen into a controlled-release polymer has been developed with reproducible pharmacokinetic properties (18, 44).

Local administration of drugs using controlled-release polymers is a safe and effective strategy for drug delivery to the brain that allows increased concentrations of agents on targeted sites and minimizes systemic toxicity. 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. They can be inserted at the time of surgery or injected intrathecally and have been shown clinically to be efficacious in the delivery of chemotherapy for the treatment of malignant brain tumors and have been used experimentally to treat cerebral edema, seizures, and vasospasm in animal models (23, 24, 41–44).

In this study, we determined the safety of locally delivered ibuprofen incorporated into controlled-release polymers placed in the subarachnoid space of monkeys and the efficacy of ibuprofen/polymer formulations to prevent vasospasm in a monkey model of SAH.

MATERIALS AND METHODS

Experimental Design

Toxicity Study

Male cynomolgus monkeys (*Macaca fascicularis*) (n = 5) were randomized to two experimental groups. In the first group (n = 2), 45% ibuprofen/ethylene-vinyl acetate (EVAc) polymers were implanted in the subarachnoid space. In the second group (n = 3), blank EVAc polymers were implanted. Neurological examinations were conducted on the day before surgery and twice daily for the duration of the experiment. Blood samples were drawn for standard chemistries, complete blood cell counts, and clotting times immediately before surgery, 1 week after surgery, and subsequently every 2 weeks for the duration of the experiment. All animals were allowed to survive 13 postsurgical weeks. The animals were killed at the conclusion of the experiment, and full autopsies were performed. Brain samples were processed for comparison.

Efficacy Study

Male cynomolgus monkeys (n = 14) underwent transfemoral cerebral angiography 7 days before surgery and were randomized to two experimental groups. In the first group (n = 7), animals underwent induction of SAH followed by placement in the subarachnoid space of a 45% ibuprofen/EVAc polymer at an average dose of 6 mg/kg of ibuprofen 10 minutes after SAH. In the second group (n = 7), animals underwent induction of SAH followed by placement in the subarachnoid space of blank EVAc polymers with a weight equivalent to that of the ibuprofen/EVAc polymers. Seven days after SAH, cerebral angiography was repeated, and the animals were subsequently killed. Middle cerebral artery (MCA) areas were measured and compared.

Animals

Cynomolgus monkeys (*Macaca fascicularis*) (n = 19) (Charles River Laboratories, Houston, TX) weighing an average of 3.4 kg were used in this study. The animals were housed in standard animal facilities with free access to Baltimore city water and 5037 Monkey LabDiet (PMI Nutrition International, Brentwood, MO) supplemented with primate enrichment diet. The Animal Care and Use Committee of The Johns Hopkins University School of Medicine approved all experimental protocols.

Polymer Preparation

We have previously described the technique for incorporating ibuprofen into controlled-release EVAc polymer (44). Briefly, ibuprofen (Sigma-Aldrich Corp., St. Louis, MO) 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 45% (wt:wt) ibuprofen. The suspensions obtained were transferred into cylindrical glass molds and maintained at −70°C for 1 hour. The resulting polymer cylinders were kept at −80°C for 48 hours and then transferred to −21°C conditions for 1 week. The polymer rods were then placed in a vacuum desiccator at room temperature for 48 hours. Empty EVAc polymers were prepared in a similar manner.

Anesthesia: Toxicity and Efficacy Studies

Anesthesia was induced with intramuscular ketamine (100 mg/ml; Abbot Laboratories, Chicago, IL) 10 to 20 mg/kg, the animals were subsequently intubated, and anesthesia was maintained with isoflurane (0.7–1.5 vol %). For angiograms, anesthesia was administered with intramuscular ketamine (10 to 20 mg/kg), intravenous atropine (0.05 mg/kg), and intravenous pentobarbital sodium (12.5 mg/kg).

Cerebral Angiography

Animals underwent cerebral angiography 7 days before and 7 days after surgery (*Figs. 1* and *2*). Intravenous access was obtained with a 22-gauge angiocatheter in the saphenous vein. Additional doses of pentobarbital were given as needed to keep the animals anesthetized. Heart rate and oxygen saturation were monitored throughout the procedure. An intravenous infusion of normal saline solution was maintained at a minimal rate to keep the vein open. The right groin was prepared and draped in a sterile manner. A 21-gauge butterfly and a 4-French micropuncture set were used to access the femoral artery. Heparinization was achieved, and a 0.035-cm glidewire was then used to pass a 4-French pigtail catheter into the ascending thoracic aorta for arch injections. Omnipaque 300 boluses (5–10 ml) (Amersham Health, Princeton, NJ) were injected by hand. Images were acquired with a Philips Integris biplane fluoroscope at 1 frame per second. The anteroposterior and lateral distances were 102 and 106 cm, respectively.

Surgical Technique

We have previously described the technique for SAH and polymer placement in the monkeys (11). Briefly, animals underwent surgery on Day 0. Before surgery, the animals had received cefazolin (25 mg/kg), dexamethasone (0.1 mg/kg), furosemide (0.5 mg/kg), and mannitol (0.5 mg/kg), all delivered intravenously. For animals undergoing induction of SAH, 3 ml of peripheral blood was withdrawn and placed in a clot activator tube. Heart rate, temperature, respiratory rate, and oxygen saturation were monitored continuously. Lactated Ringer's solution was infused at a rate of 10 ml/h. Under microscopic visualization, the cisterns containing the internal carotid artery, the MCA origin, and the anterior cerebral artery origin were exposed and opened, the arachnoid was dissected, and the autologous clot was placed around the exposed vessels in the basal and sylvian cisterns. A 45% ibuprofen/EVAc polymer or a blank EVAc polymer was placed inside the clot, and the dura mater, temporalis muscle, and skin were closed as previously described.

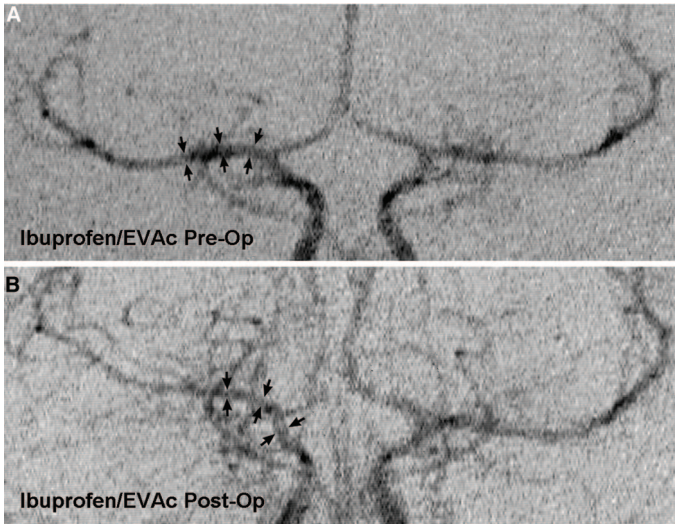

FIGURE 1. *Angiograms of a monkey treated with a 45% ibuprofen/EVAc polymer after undergoing SAH.* A, *preoperative angiogram.* B, *postoperative angiogram taken 7 days after surgery.*

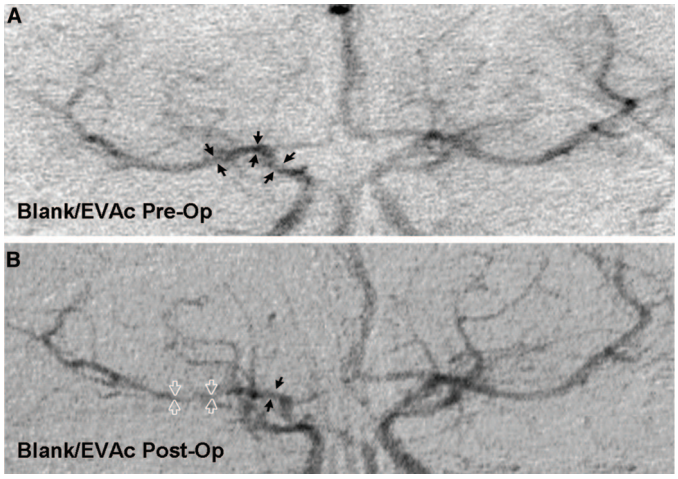

FIGURE 2. *Angiograms of a monkey treated with a blank EVAc polymer after undergoing SAH.* A, *preoperative angiogram.* B, *postoperative angiogram taken 7 days after surgery.*

Pathological Analysis (Toxicity Study)

At autopsy, the calvaria was removed, the dura was opened circumferentially, and the brain with intact basal vessels was removed and placed in 10% formalin. In all cases, polymer placement was confirmed, and the polymer was retrieved. After postfixing for 4 weeks, the brains were grossly examined, and tissue adjacent to the polymer deposition site was harvested and sent for routine hematoxylin and eosin staining. Tissue samples from the eye, tongue, thyroid, thymus, heart, lungs, tracheobronchial and mesenteric lymph nodes, liver, spleen, stomach, duodenum, jejunum, ileum, pancreas, kidneys, adrenal glands, proximal colon, urinary bladder, urethra, testicles, and skeletal muscle were collected and placed in 10% formalin. This tissue was sent for hematoxylin and eosin staining and was microscopically examined by a veterinary pathologist.

Angiographic Analysis

Vasospasm was determined by digital imaging analysis using Image J software (NIH, Bethesda, MD), according to the methodology previously described by Pluta et al. (33). Briefly, angiograms were digitized by use of an Epson scanner (Epson America, Inc., Long Beach, CA), and identification information was removed from all images. The proximal MCA luminal areas were measured by two blinded observers and used for comparison between groups. Lumen patency was established by comparing a ratio of the luminal area between the right and left MCAs on the preoperative angiogram with the ratio of the luminal area between the right and left MCAs on the postoperative angiogram.

Statistical Analysis

In the efficacy study, vessel luminal areas are expressed as mean percentage ± standard error of the mean. Mean vessel ratios were used to establish percent luminal areas, and the mean percentages of luminal areas were compared between groups by use of Student's t test. A value of $P < 0.05$ was considered significant. Statistical analysis was performed with SPSS version 8.0 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

Toxicity Study

Animals implanted with 45% ibuprofen/EVAc or blank EVAc polymers did not show signs of local or systemic toxicity. No differences were noted in the neurological status of the animals in either group. The brain parenchyma surrounding areas of polymer implantation did not show signs of inflammation or hemorrhage. Analysis of peripheral blood samples revealed normal hematological profiles, with serum chemistry and coagulation times within normal limits for all animals. Full autopsies did not reveal signs of systemic toxicity.

Efficacy Study

Local delivery of ibuprofen via controlled-release polymers prevented angiographic vasospasm 7 days after SAH in this model. Animals treated with 45% ibuprofen/EVAc polymers had 91 ± 9% lumen patency of the MCA, compared with 53 ± 11% lumen patency of the MCA of animals treated with blank/EVAc polymers ($P < 0.001$) (*Fig. 3*).

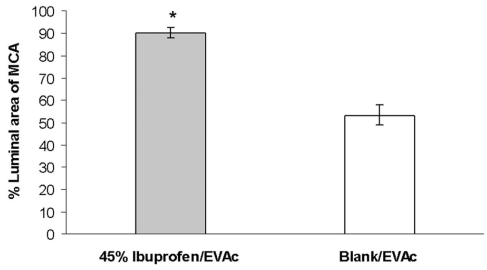

FIGURE 3. *Histogram comparing the mean percentage of luminal area of the MCA in animals treated with 45% ibuprofen/EVAc polymers with that of animals treated with blank EVAc polymers. *, P < 0.001.*

DISCUSSION

In this study, we report the safety of 45% ibuprofen/EVAc polymers placed in the subarachnoid space of monkeys and the efficacy of this ibuprofen polymer formulation in the prevention of experimental cerebral vasospasm after SAH in monkeys. We found that 45% ibuprofen/EVAc polymers do not have local or systemic toxicity and that a dose of approximately 6 mg/kg of ibuprofen administered 10 minutes after SAH prevents angiographic vasospasm in monkeys evaluated 7 days after hemorrhage.

As previously reported, ibuprofen is continuously released in a controlled manner from EVAc polymers in vitro (44). Ibuprofen polymers have been efficacious in preventing posthemorrhagic vasospasm in the rat femoral artery model (44) and in the basilar artery model in rabbits (18). The efficacy of this therapy is related to the effect that ibuprofen has on the overexpression of cell adhesion molecules on the endothelium and the subsequent decrease in leukocyte migration into the subarachnoid space. Ibuprofen specifically inhibits interleukin-1α- and tumor necrosis factor-α-induced expression of endothelial VCAM-1 and ICAM-1 and thus specifically inhibits leukocyte-endothelial cell interactions (21, 31). We have previously shown that ibuprofen decreases the expression of ICAM-1 in the rat brain after MCA occlusion (4) and that locally delivered ibuprofen via controlled-release polymers decreases the number of periadventitial neutrophils and macrophages after hemorrhage (44). This effect of ibuprofen and of other nonsteroidal anti-inflammatory drugs seems to be unrelated to their cyclooxygenase inhibition and occurs at higher drug concentrations. Furthermore, ibuprofen has been shown to decrease oxidative-stress damage through several mechanisms, hence decreasing endothelial damage (48).

Locally delivered ibuprofen prevents vasospasm when administered within the first 12 hours after SAH in animal models (18, 44). We have shown, however, that the benefits of ibuprofen therapy are lost if ibuprofen polymers are placed in the subarachnoid space later than 12 hours after SAH in the rat and rabbit models, and we are aware of the clinical limitations of this approach. Therefore, we have concluded that ibuprofen can be used to prevent, but not to reverse, vasospasm under experimental settings in these animal models.

The monkey model of vasospasm popularized in previous publications (8, 16, 17, 25, 27, 46) has been characterized as the best animal model for posthemorrhagic cerebral vasospasm (25), has proved to be highly reproducible, and has been used extensively (26, 29, 33). Although the peak onset of angiographic vasospasm occurs 7 days after SAH, neurological deficits fail to develop. Advantages of the model include a course of onset similar to the human disease, highly preserved protein structures, the presence of controls for every animal

because SAH is induced in only one side by use of clotted blood, sufficient arterial tissue for analysis, similar vascular histology, homologous mechanisms for maintenance of vascular tone, reproducible vasospasm, and adequate angiographic techniques. Disadvantages of the model include elevated costs and the absence of neurological deficits.

Local drug delivery via controlled-release polymers constitutes an ideal mechanism to achieve adequate ibuprofen levels in the subarachnoid space. We have previously studied the pharmacokinetic properties of controlled-release polymers placed in the subarachnoid space for treatment of posthemorrhagic vasospasm in a rabbit model (35). Agents loaded into the polymers travel a distance of 40 cm or more, and the rate and extent of diffusion are not altered by the presence of blood in the subarachnoid space.

The combination of down-regulation of expression of cell adhesion molecules after SAH with the restoration of the periadventitial levels of nitric oxide (NO) might be the ideal therapeutic approach for this condition. Periadventitial NO levels can be raised when controlled-release polymers loaded with an NO donor such as diethylene-triamine-NO (DETA-NO) are placed in the subarachnoid space (19, 45). DETA-NO polymers provide sustained release of DETA-NO and have been effective in preventing vasospasm in rat, rabbit, and monkey models of vasospasm (19, 45). Furthermore, DETA-NO could potentially provide a synergistic effect with ibuprofen, because DETA-NO has been shown experimentally to down-regulate ICAM-1 expression (6, 22).

In conclusion, 45% ibuprofen/EVAc polymers are safe for implantation in the subarachnoid space, and a dose of 6 mg/kg of ibuprofen prevents angiographic vasospasm after SAH in monkeys. These findings support the role of cell adhesion molecules and inflammation in the pathophysiology of vasospasm.

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COMMENTS

The authors suggest that ibuprofen prevents vasospasm after subarachnoid hemorrhage (SAH) in monkeys. In my extensive experience with this model, 3 ml of blood would produce at most 2 ml of clot, which is not enough to cause much vasospasm and certainly not enough to cause severe vasospasm with accompanying structural changes. Therefore, it is likely that the authors are studying a very mild form of vasospasm that would be easier to reverse. All animals received dexamethasone before surgery. This might have had some effect on inflammation. The authors also missed the opportunity to gather some information about the effects of ibuprofen among the animals with SAH because no immunohistochemistry or other pathological assessments were performed.

The problems with vasospasm research are that we are hampered greatly by the inability to accurately reproduce the disease in rodents, specifically mice. This leads to an inability, or at least difficulty, in conducting definitive scientific experiments. Pharmacological approaches leave alternate explanations in most cases. In addition, manipulation of protein expression in the intact arterial wall is also very difficult and leads to the same issue. Partly because of these issues, there is no scientific evidence to support the idea that inflammation causes vasospasm. Undoubtedly, it contributes in some ways.

Investigators should always use blinding and randomization in such studies. Delivery of drugs by polymers is potentially an excellent way to treat vasospasm in humans, although it could be very problematic in animal studies because the physical proximity of the clot to the artery is so important to the development of vasospasm that simply adding polymers to separate the two could reduce vasospasm in control animals. Up-regulation of ICAM-1 3 to 24 hours after SAH cannot be an important causative factor in vasospasm because removing the clot after this prevents vasospasm. Although one could argue with the effects of clot removal in humans, it is well established that removing a clot surgically or by using a tissue plasminogen activator completely prevents vasospasm in the very model that is tested in this study (1–3). Other types of studies are required for a better understanding of the role of the complex inflammatory system in vasospasm.

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The authors of this article performed a safety and efficacy study of locally delivered ibuprofen via controlled-release polymers in the subarachnoid space for the prevention of angiographic vasospasm

after SAH in a monkey model. They found no local or systemic toxicity at the doses used (average 6 mg/kg). The compound was highly effective in preventing angiographic vasospasm compared with placebo. This is another excellent contribution from a group with profound expertise on the topic of leukocyte-endothelial cell interaction in the pathophysiology of cerebral vasospasm after SAH (2). Among other things, their research is focused on 1) ibuprofen, which inhibits up-regulation of certain cell adhesion molecules, thereby preventing vasospasm; and 2) substance delivery from controlled-release polymers. This combination is, of course, a highly attractive and promising concept, which was tested step-by-step in a systematic and convincing manner in previous articles (1, 3) and the present article. Efficacy for prevention of vasospasm has thus been shown for extracranial arteries and already intracranially in a rabbit model of SAH. In the present article, the "ideal animal model" was used to demonstrate safety and efficacy. In the Discussion section, the authors allude to their next step, a combination of locally delivered ibuprofen with a nitric oxide-donor. This is excellent work performed in a convincing and systematic manner on a subject of high clinical relevance.

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1. Frazier JL, Pradilla G, Wang PP, Tamargo RJ: Inhibition of cerebral vasospasm by intracranial delivery of ibuprofen from a controlled-release polymer in a rabbit model of subarachnoid hemorrhage. **J Neurosurg** 101:93–98, 2004.
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Using the best animal model of SAH available, the authors of this controlled study report that the administration of the anti-inflammatory agent ibuprofen (in slow-release implantable capsules) into the subarachnoid space of monkeys at the time of clot placement almost entirely prevents cerebral vasospasm, without any apparent toxicity. The number of study animals was understandably small, cerebrospinal fluid drug levels were not measured, and proposed mechanisms of vasospasm prevention (ICAM-1 up-regulation, leukocyte adhesion, and infiltration) were not measured, but the results of this straightforward study are nevertheless informative and compelling. The "inflammatory" hypothesis of cerebral vasospasm after aneurysmal SAH has been given new life in the primate model.

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Anti-inflammatory therapeutic strategies have long been sought in cerebral vasospasm, and the authors describe a creative approach by applying polymer-based local drug delivery. The model is appropriate, and the controls and observational parameters are well planned. Dosage, pharmacokinetics, and technical issues had been worked out in preliminary studies in rabbits and in other studies on the same model in primates. The results are positive, convincing, and self explanatory, obviously calling for consideration of this therapeutic approach to prevent vasospasm in patients. These would start with Phase I studies on the polymer alone, and then with drug delivery, perhaps initially on patients with high Fisher grade and serious risk of spasm.

Before human studies are undertaken, one would want to compare this treatment with systemic anti-inflammatory agents in the same model, thus avoiding invasive treatment when a less invasive route could produce the same results. It is also not clear whether currently used "best practice" management—nimodipine, volume monitoring, and resuscitation, etc.—would accomplish a similar or additive benefit in the same model. If indeed the local delivery of this agent or others is proven to be beneficial, vascular neurosurgeons might resurrect another argument for open surgical intervention—to deliver therapeutic agents that may positively alter the course of the disease more than clip versus coil. Maybe we will be able to spare patients numerous invasive catheter procedures for the diagnosis and treatment of aneurysm and spasm, which could be accomplished most effectively in a single surgical session.

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