Inhibition of Experimental Vasospasm in Rats With the Periadventitial Administration of Ibuprofen Using Controlled-Release Polymers

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Background and Purpose—The chronic phase of vasospasm after an aneurysmal subarachnoid hemorrhage may be mediated in part by early leukocyte-endothelial cell interactions. Ibuprofen is an anti-inflammatory agent that inhibits expression of certain cell adhesion molecules and therefore disrupts leukocyte-endothelial cell interactions. Its systemic administration, however, has dose-limiting side effects. We evaluated the effect of the periadventitial delivery of ibuprofen using controlled-release polymers in the rat femoral artery model of chronic posthemorrhagic vasospasm.

Methods—Before the animal studies, the release pharmacokinetics of the ibuprofen-loaded ethylene–vinyl acetate polymers were determined in vitro. Subsequently, the femoral arteries (n=266) of Fischer 344 rats (n=133) were enclosed in latex pouches bilaterally. In the toxicity study (n=15 rats), the animals were randomized into 5 dose groups in which 0%--, 10%--, 20%--, 30%--, or 50%-loaded ibuprofen polymers were evaluated. In the efficacy study, the animals were randomized into 5 time groups in which 50%-loaded ibuprofen polymers were inserted at 0 (n=58 rats), 6 (n=16), 12 (n=13), 24 (n=11), or 48 hours (n=12) after blood injection into the pouch. The rats were killed 12 days after blood exposure, at the time of maximal vasospasm in this model. Vasospasm was expressed as percent lumen patency. To evaluate the effect of ibuprofen on leukocyte migration, 8 rats were randomized into 2 groups. Macrophages and granulocytes were stained by immunohistochemistry with the use of a mouse OX-41 monoclonal antibody and counted in the periadventitial space 24 hours after blood exposure.

Results—In vitro pharmacokinetics showed that the 50%-loaded ibuprofen polymer released its total drug load over a 12-day period. In the toxicity study, a nonsignificant arterial vasodilatation with ibuprofen treatment was seen at higher doses, and no deleterious effects were noted on the vessel wall histologically. In the efficacy study, ibuprofen treatment resulted in significant vasospasm inhibition when treatment was initiated at 0 hour (73.7±4.9% versus 94.5±3.3% [mean±SEM percent lumen patency]; P<0.001) and 6 hours (69.2±5.7% versus 98.0±3.9%; P=0.002) after blood exposure, but not at 12, 24, or 48 hours. Leukocyte immunohistochemistry showed that ibuprofen treatment resulted in significantly lower periadventitial macrophage and granulocyte counts of 25.0±3.9 cells per high-powered field compared with counts of 140.5±18.2 cells per high-powered field in the untreated vessels (P<0.001).

Conclusions—The periadventitial, controlled release of ibuprofen from surgically implanted polymers significantly inhibits chronic posthemorrhagic vasospasm in this model when treatment is initiated within 6 hours of blood exposure. Vasospasm inhibition with ibuprofen correlates with a significant decrease in the number of macrophages and granulocytes in the periadventitial space. This study supports the hypothesis that inflammation mediates in part the chronic phase of posthemorrhagic vasospasm and suggests a potential alternative treatment for this condition. (Stroke. 1999;30:140-147.)

Key Words: cell adhesion molecules ■ ibuprofen ■ inflammation ■ polymers ■ vasospasm

Leukocyte–endothelial cell interactions may play a role in the etiology of chronic vasospasm after an intracranial aneurysmal subarachnoid hemorrhage (SAH). Chronic vasospasm is the delayed narrowing of the cerebral vasculature after SAH that often results in ischemic injury and death after aneurysmal rupture.1 Although the etiology of chronic vasospasm remains unclear, recent work in our laboratory and by others has implicated cell adhesion molecules (CAMs) in...
experimental chronic vasospasm and suggested that leukocyte–endothelial cell interactions may be important in this process. Specifically, we have previously demonstrated that the increased expression of intercellular adhesion molecule-1 (ICAM-1, CD54) on endothelial cells of blood-exposed vessels is correlated with the subsequent development of chronic vasospasm and that chronic vasospasm can be prevented by the systemic administration of an anti-ICAM-1 monoclonal antibody (Mab). In addition, we have documented a correlation between the early migration of macrophages and granulocytes across the vessel wall after the hemorrhage and the subsequent development of chronic vasospasm.

Ibuprofen is an anti-inflammatory agent that has been recently shown to be an inhibitor of both ICAM-1 expression and also of vascular cell adhesion molecule-1 (VCAM-1, CD106) expression. Ibuprofen’s inhibition of leukocyte–endothelial cell binding and of ICAM-1 and VCAM-1 expression appears to be unrelated to its well-described cyclooxygenase inhibition, in which prostaglandin synthesis from arachidonic acid is disrupted. This prostaglandin-independent effect of ibuprofen on leukocyte-endothelial cell interactions is becoming increasingly recognized as a shared property of several NSAIDs. This observation may also explain why higher NSAID doses are needed to inhibit inflammation than to disrupt prostaglandin synthesis. At higher doses, however, the systemic side effects of NSAIDs, such as gastric erosion, thrombasthenia or thrombocytopenia, and fluid retention, may become severe.

Therefore, since posthemorrhagic chronic vasospasm is correlated with ICAM-1 expression and leukocyte migration, and since ibuprofen is a specific inhibitor of ICAM-1 expression and leukocyte–endothelial cell interaction, we hypothesized that ibuprofen could be used to treat chronic vasospasm. Indeed, in the canine double hemorrhage model of chronic vasospasm, the systemic administration of high doses of ibuprofen (37.5 mg/kg per day) and methylprednisolone (90 mg/kg per day) starting 1 hour after the initial hemorrhage was reported to inhibit chronic vasospasm.

The systemic administration of these agents, however, resulted in serious complications such as hemorrhagic pneumonia and duodenal ulcer perforation in some animals.

The toxicity associated with the systemic administration of high doses of ibuprofen could be avoided by delivering the drug in high concentration only at the site of the hemorrhage with the use of controlled-release polymers. Controlled-release polymers are biologically inert matrices capable of releasing extremely high drug concentrations locally (thus minimizing systemic drug exposure and side effects) with highly reproducible release kinetic profiles. We have previously demonstrated that controlled-release polymers have several specific advantages for drug delivery in the brain. In this study we examine the effect of the local, periadventitial delivery of ibuprofen, with the use of controlled-release polymers surgically implanted at the site of blood deposition, on chronic posthemorrhagic vasospasm in the rat femoral artery model. We correlate the inhibition of chronic vasospasm with the decreased migration of macrophages and granulocytes across the vessel wall in the presence of ibuprofen. In addition, we describe an ibuprofen controlled-release polymer that releases its complete drug load over a 12-day period and document the lack of vascular toxicity associated with this form of ibuprofen administration.

Materials and Methods

Experimental Design

Three experiments are described in this report. In the first experiment, the pharmacokinetics of the drug-polymer formulation were evaluated by measuring the controlled release of ibuprofen from the polymer in vitro. In the second experiment, the potential toxicity of the controlled release of high doses of ibuprofen adjacent to the femoral artery was evaluated in a dose-escalation study in which the histology and lumen diameter of the drug-exposed vessels were compared. In the third experiment, the efficacy of the controlled release of ibuprofen in preventing chronic vasospasm of the femoral vessels exposed to blood was evaluated. The polymers were implanted next to the vessels at 0, 6, 12, 24, or 48 hours after the initial blood deposition. At each time point, 2 cohorts were studied: a control cohort that received empty polymers and a treatment cohort that received polymers loaded with ibuprofen. In this experiment, granulocytes and macrophages in the periadventitial space were identified immunohistochemically 24 hours after blood deposition, and their counts around ibuprofen-treated vessels were compared with those in the control group.

Polymer Preparation

Ibuprofen was incorporated into controlled-release polymers by a previously described technique. Briefly, ibuprofen (Sigma Chemical Co) and ethylene-vinyl acetate copolymer (EVAc; 40% vinyl acetate by weight, DuPont Co) in 5 different weight ratios were dissolved in methylene chloride (1:9 [wt/vol] ratio of EVAc to methylene chloride), resulting in ibuprofen-EVAc–methylene chloride suspensions that yielded dry polymers 0%, 10%, 20%, 30%, and 50% loaded (wt/wt) with ibuprofen. The suspension was poured into cylindrical glass molds at −70°C. The frozen polymer cylinders were transferred to glass plates at −30°C, and the solvent methylene chloride was allowed to evaporate over 6 days. Residual methylene chloride was extracted in a vacuum desiccator over another 2 days. The polymers were cut into 5.0-mm cylindrical fragments (diameter=1 mm, height=6 mm) and exposed to ultraviolet irradiation for 1 to 2 hours before implantation to reduce the risk of infection.

Femoral Artery Isolation and Polymer Implantation

Adult male Fischer 344 rats (n=133; Harlan Sprague Dawley, Inc, Indianapolis, Ind) weighing 200 to 250 g were used in 3 different experiments: (1) toxicity study (n=15), (2) efficacy study (n=110), and (3) immunohistochemistry study (n=8). The animals were anesthetized (3 mL/kg IP) with a stock solution containing ketamine hydrochloride (25 mg/mL), xylazine (2.5 mg/mL), and 14.25% ethyl alcohol in normal saline. Each animal received a dose of ampicillin (25 mg/kg IP) at the time of surgery. The inguinal regions were shaved and prepared in sterile fashion with alcohol and povidone-iodine. With the use of microsurgical technique, the femoral artery was dissected out and wrapped in an inert latex pouch measuring 8×8 mm, as previously described. The EVAc polymers were inserted into the latex pouch either at the time of blood deposition or at later time points during reoperation. The latex pouches were sealed with cyanacrylate (Borden, Inc). Autologous venous blood was drawn from the adjacent femoral vein and allowed to clot spontaneously. Approximately 0.1 mL of clotted blood was injected into the reference pouch. The incisions were closed with surgical staples. For reoperations at 6, 12, 24,
and 48 hours, the animals were reanesthetized as described above, the incisions were reopened, and the polymers were inserted into the latex pouches through a small incision in the pouch. The wounds were closed as described above, and the animals were allowed to recover. Given that the implanted polymers weighed on average 5.0 mg, that they were 50% loaded with ibuprofen, and that the smallest animals used in the experiment weighed 200 g, then the maximum ibuprofen dose used in this experiment was 12.5 mg/kg over the 12-day period. All procedures were performed in accordance with guidelines established by the Animal Care and Use Committee of The Johns Hopkins University School of Medicine.

Vessel Histology and Cross-Sectional Area Measurements

We have previously shown that the time of peak chronic vasospasm in the rat femoral artery model is 12 days after blood exposure. Therefore, at 12 days the animals were anesthetized as described above, and the abdominal aorta was exposed in preparation for in situ perfusion-fixation. The aorta was isolated and cannulated with polyethylene tubing (Intramedic PE-90, Clay Adams). The inferior vena cava was pierced for drainage. The lower extremities were then perfused with 40 mL of heparinized normal saline followed by 40 mL of ice-cold freshly depolymerized 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4). The femoral arteries were dissected out of the pouch and placed in 4% paraformaldehyde for 1 hour. For cryoprotection, the vessels were then placed in a solution of 20% sucrose in PBS for 24 hours at 4°C. The vessels were then frozen in dry ice–equilibrated isopentane at −50°C and stored at −40°C until sectioning. Vessel cross-sectional slices (14 μm) in a transverse orientation were sectioned in a cryostat (Microm) resulting in 10-μm slices taken at 200-μm intervals, thaw-mounted onto Superfrost Plus–pretreated microscope slides, and allowed to air dry. Standard staining with hematoxylin and eosin was then performed.

Lumen cross-sectional areas were calculated by computerized image analysis on an Apple Macintosh 8100/80 AV computer using the public domain NIH Image Program (developed at the US National Institutes of Health and available from the Internet by anonymous file transfer protocol from zippy.nimh.nih.gov). The areas were calculated by measuring the actual circumference of the vessel lumen and then calculating the area of a generalized circle (πr², where r=radius) based on the calculated r value from the circumference measurement (r=measured circumference/2π), thus correcting for vessel deformation and off-transverse sections. For each vessel, 3 separate sections at least 200 μm apart were measured and averaged. Results of the cross-sectional area measurements were expressed as percent lumen patency, defined as the ratio of the area of the blood-exposed or ibuprofen-exposed vessel to the area of the contralateral saline-exposed vessel and expressed as a percentage. According to this protocol, each animal served as its own control, and vessel size variability was thus taken into account. All ratios are presented as mean±SEM. Statistical analysis was performed with the Student’s t test; P<0.05 was considered significant.

Controlled-Release Pharmacokinetics of Ibuprofen

The release pharmacokinetics of the 50%-loaded ibuprofen-EVAc polymers were evaluated in vitro as previously described.16 Ibuprofen-EVAc polymers (n=3) weighing 35 mg were suspended in individual glass vials containing 3 mL of PBS at pH=7.4. The polymers were sequentially transplanted to new vials each with 3 mL of fresh PBS at 1, 3, 6, 12, and 24 hours and then daily for a total of 12 days. Ibuprofen release in the PBS aliquots was quantified spectrophotometrically by comparison against standardized ibuprofen concentration curves.

Controlled-Release Toxicity of Ibuprofen on the Femoral Artery

The potential toxicity on the femoral vessels of the periadventitial, controlled-release of ibuprofen was evaluated. With the use of a Fibonacci dose escalation, ibuprofen-EVAc polymers (n=15), 0%, 10%, 20%, 30%, and 50% loaded with ibuprofen, were inserted in a latex pouch encasing the femoral artery (n=15 rats, 30 vessels). Normal saline (0.1 mL) was injected into the pouch. The contralateral vessel was encased in a latex pouch with normal saline. Empty polymers were inserted in the control pouch. The animals were killed, and the vessels were harvested 12 days after surgery. The vessels were processed as described above and examined histologically. Vessel histology was evaluated, and cross-sectional area ratios were calculated as described above.

Effect of Ibuprofen on Lumen Patency of Blood-Exposed Vessels

To determine the effect of ibuprofen on lumen patency, a total of 110 animals (220 femoral vessels) were randomized into 5 groups. Each of these 5 groups in turn contained 2 cohorts, one in which vessels were exposed to empty polymer (n=54 rats) and the other to ibuprofen polymer (n=56). Ibuprofen polymers 50% loaded with the drug were used in the treatment cohorts. After implantation of femoral latex pouches bilaterally and subsequent injection of normal saline into the control pouch and of autologous venous blood into the other, controlled-release polymers were inserted in the pouches during reoperation at the following time points after initial blood exposure: 0 (ie, at the time of blood injection, n=58 rats), 6 (n=16), 12 (n=13), 24 (n=11), and 48 hours (n=12). In the empty polymer (control) cohort, polymers without drug were inserted into both the blood-containing femoral pouch and the contralateral saline-containing pouch. In the ibuprofen polymer (treatment) cohort, empty polymers were inserted in the saline-containing femoral pouch, and 50%-loaded ibuprofen polymers were inserted in the blood-containing femoral pouch. The animals were killed on day 12 after initial blood deposition, previously determined to be the time of maximum vasospasm in this model,3,4 and the vessels were processed as described above.

Immunohistochemical Staining for Granulocytes and Macrophages

Immunohistochemistry was used to quantitatively assess the migration of granulocytes and macrophages into the periadventitial space. According to this protocol, each animal served as its own control, and vessel size variability was thus taken into account. All ratios are presented as mean±SEM. Statistical analysis was performed with the Student’s t test; P<0.05 was considered significant.

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Immunohistochemical Staining for Granulocytes and Macrophages

Immunohistochemistry was used to quantitatively assess the migration of granulocytes and macrophages into the periadventitial space. For this purpose, rats were randomized into 2 groups, one exposed to empty polymer (n=4 rats, 8 vessels) and the other exposed to 50%-loaded ibuprofen polymers (n=4 rats, 8 vessels). We have previously shown that 24 hours after blood deposition is the peak time for leucocyte migration into the periadventitial space.3 Therefore, the animals were killed 24 hours after blood deposition, and the vessels were harvested, sectioned, and mounted as described above. Slides were hydrated in PBS for 5 minutes then permeabilized in Triton X-100 (0.2% in PBS) for 15 minutes at room temperature. After they were washed in PBS for 5 minutes, slides were incubated in 3% normal horse serum for 20 minutes. After the slides were washed again in PBS for 5 minutes, the mouse OX-41 MAb (MAS 369, Harlan Sera Laboratory, Ltd, Sussex, England) was applied to each section and incubated for 1 hour in a humidified chamber at 37°C. The mouse OX-41 MAb was diluted to 1:200 in PBS with 1.0% BSA and 3% normal horse serum. OX-41 detects a molecular complex (ABC Elite solution; Vectastain Elite, Vector Laboratories, Burlingame, Calif) diluted 1:100 in PBS with 1.0% BSA and 3% normal horse serum for 30 minutes. After they were washed again in PBS for 5 minutes, then incubated in avidin and biotinylated horseradish peroxidase macro-molecular complex (ABC Elite solution; Vectastain Elite, Vector Laboratories) for 30 minutes. After they were washed again in PBS for 5 minutes, then incubated in avidin and biotinylated horseradish peroxidase substrate (Vector VIP, Vector Laboratories). Slides were then washed in tap water for 5 minutes, then counterstained with nuclear fast red for 30 seconds. Slides were finally dried mounted.

Positively stained macrophages and granulocytes were counted by light microscopy (Olympus BH-2, Optical Elements Corp) with the
The controlled release of ibuprofen next to the adventitial surface of the femoral artery resulted in no histologically identifiable deleterious changes of the vessel wall. Vessel cross-sectional area measurements showed vasodilatation that peaked at the 30% loading (Figure 2). The effect was not statistically significant.

**Effect of Ibuprofen on Lumen Patency of Blood-Exposed Vessels**

The controlled release of ibuprofen resulted in a significant inhibition of chronic vasospasm when treatment was initiated at 0 and 6 hours after blood exposure. When treatment was initiated 12 hours after blood exposure, a similar trend was observed, but the difference did not reach statistical significance ($P=0.131$). Microscopic examination of the untreated blood-exposed vessels revealed the characteristic histological changes of chronic vasospasm in the form of corrugation of the internal elastic lamina, thickening of the vessel wall in general and the tunica media in particular, and intimal proliferation. At the 0-hour time point in which the polymer was introduced at the time of blood deposition, whereas the control empty polymer group ($n=27$) had a lumen patency of 73.7±4.9% (mean±SEM), the treated ibuprofen polymer group ($n=31$) had a lumen patency of 94.5±3.3%. This difference was significant (Student’s $t$ test, $P<0.001$) and represents a 79% inhibition of vasospasm. At the 6-hour time point, whereas the control empty polymer group ($n=9$) had a lumen patency of 69.2±5.7%, the treated ibuprofen polymer group ($n=7$) had a lumen patency of 98.0±3.9%. This difference was also significant ($P=0.002$). At the 12-hour time point, however, the difference between the 2 groups showed a similar trend but was not statistically significant ($P=0.131$). At the 24- and 48-hour time points, no difference was observed between lumen patencies in the 2 groups (Figure 3). Therefore, vasospasm inhibition by ibuprofen was not effective if the treatment was initiated beyond 6 hours after blood exposure.

**Immunohistochemical Staining for Granulocytes and Macrophages**

The controlled release of ibuprofen resulted in significant reduction of periadventitial macrophage and granulocyte infiltration around blood-exposed vessels at 24 hours (Figure 4). Whereas the control vessels exposed to empty polymers
Discussion

Inflammation has long been suspected to play a role in chronic posthemorrhagic vasospasm, mostly on the basis of indirect evidence. In clinical studies, this evidence has consisted of the correlation of significantly higher systemic temperatures in patients with severe vasospasm after aneurysmal SAH, the accumulation of macrophages and granulocytes in the wall of arteries exposed to blood after SAH, and increased levels of immunoglobulins and complement fractions in the serum and vessel walls of patients in vasospasm.

In experimental studies, this evidence has consisted of the inhibition of chronic vasospasm with the administration of methylprednisolone, ibuprofen, cyclosporin A, or complement depletion and the reproduction of chronic vasospasm with the introduction in the subarachnoid space of inflammatory agents such as latex beads or talc powder.

Recent experimental work provides direct evidence that leukocyte–endothelial cell interactions may play a role in the etiology of chronic posthemorrhagic vasospasm. It has been shown that ICAM-1 expression is increased in blood-exposed vessels that subsequently develop chronic vasospasm, that endothelial ICAM-1 receptor blockade with an anti–ICAM-1 MAB prevents chronic vasospasm, that periadventitial migration of macrophages and granulocytes correlates with the subsequent development of chronic vasospasm, and that ICAM-1 expression is increased in the cerebral vasculature in ischemia and after SAH. On the basis of these studies, we have concluded that ICAM-1 may play an important role in the development of chronic posthemorrhagic vasospasm.

Although the anti-inflammatory properties of ibuprofen have been known for a long time, only recently has it been recognized that ibuprofen 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. This effect of ibuprofen and of other NSAIDs appears to be unrelated to their cyclooxygenase inhibition and requires higher drug concentrations. The decreased expression of these endothelial CAMs disrupts the interactions between endothelial cells and leukocyte integrins such as lymphocyte function–associated antigen-1 (LFA-1, CD11a) and macrophage antigen-1 (Mac-1, complement receptor 3, CD11b). This prevents the irreversible adhesion of leukocytes to the endothelial membrane, which is a required step before diapedesis and extravasation, and thus inhibits the inflammatory cascade.

In addition to inhibiting leukocyte–endothelial cell interactions, ibuprofen may have a direct vasodilatory effect in the femoral artery. We observed a trend toward vasodilation at higher ibuprofen doses in the toxicity experiment (Figure 2). Although the effect of ibuprofen on vascular smooth muscle has been shown to be variable and to depend on the species and organ system under study, a vasodilatory effect of ibuprofen has been reported in some models and has been attributed its cyclooxygenase inhibition. We do not think, however, that the direct vasodilatory effect of ibuprofen observed in this study was primarily responsible for the inhibition of chronic vasospasm, since we saw no effect in the groups in which treatment was initiated 24 and 48 hours after blood deposition (Figure 3).

Controlled-release polymers such as EVAc can be implanted surgically at the site of the hemorrhage, thus delivering high doses of ibuprofen at this site but avoiding the systemic toxicity of high doses of ibuprofen. Selective surgical implantation of ibuprofen polymers in the subarachnoid space would expose the arteries in this compartment to high doses of ibuprofen but spare tissues elsewhere, thus avoiding unwanted systemic side effects. Previous studies in the canine double hemorrhage model of chronic vasospasm reported that the systemic administration of high doses of ibuprofen (37.5 mg/kg per day) starting 1 hour after the initial hemorrhage inhibited chronic vasospasm. The systemic administration of this agent, however, resulted in serious complications such as hemorrhagic pneumonitis and duodenal ulcer perforation in the animals. The ibuprofen-EVAc preparation (50% loading) used in this study resulted in a polymer that releases approximately half its drug load within the first 24 hours and the remainder over the next 11 days. This release profile is ideal for the treatment of chronic posthemorrhagic vasospasm in patients, since the process typically spans a 4- to 14-day period and since theoretically the highest ibuprofen doses are required within the first 24 hours of the hemorrhage at the time of peak ICAM-1 upregulation and leukocyte extravasation.

Although the rat femoral artery is different from a cerebral vessel in its endothelial permeability, response to vasoactive agents, and structure of its adventitial matrix, the inflammatory mechanisms considered in this study appear to be conserved in both the peripheral and central nervous system vasculature. This model has been validated by Okada and colleagues, who documented that the rat femoral artery shares many morphological and physiological features of cerebral arteries. In our laboratory, we have confirmed that...
this vessel, in response to periadventitial blood deposition, displays acute vasospasm within the first 24 hours and then goes on to gradually develop corrugation of the internal elastic lamina, medial thickening, and endothelial proliferation that starts 4 days and peaks 10 to 12 days after blood deposition and results in significant luminal narrowing, similar to that seen angiographically in patients with chronic vasospasm of the cerebral arteries.\(^3\) We recognize, however, that the results of this study must be replicated in an intracranial model of chronic posthemorrhagic vasospasm before generalizing them to chronic vasospasm after aneurysmal SAH.

In this study, ibuprofen therapy was effective when initiated within 6 hours after blood deposition. Vasospasm inhibition was significant only when treatment was initiated at 0 and 6 hours after blood exposure (\(P<0.001\) and \(P=0.002\), respectively). A nonsignificant but suggestive trend was seen when treatment was initiated at 12 hours after blood exposure (\(P=0.131\)). It is likely that initiating treatment between 6 and 12 hours after blood exposure may be similarly effective. We hypothesize that since the extravasation of macrophages and granulocytes occurs primarily 3 to 24 hours after blood deposition during the period of peak ICAM-1 upregulation,\(^3\) a treatment aimed at disrupting leukocyte–endothelial cell binding but initiated after a significant portion of these leukocytes have extravasated may be ineffective. In this study, no inhibition was seen when treatment was started 24 hours after blood exposure. These results suggest that in chronic posthemorrhagic vasospasm there may be a therapeutic window of at least 6 hours for the initiation of anti-inflammatory therapy. Although this limited time window is a potential clinical obstacle to the treatment of vasospasm using ibuprofen, it is less restrictive than the 3-hour therapeutic window for thrombolytic therapy in ischemic stroke.\(^38\)

A therapeutic window for the treatment of chronic vasospasm has been similarly observed in the monkey intracranial model of SAH and chronic vasospasm. In studies of clot evacuation in this model, Handa et al\(^39\) and Nosko et al\(^40\) demonstrated that removal of the clot from the subarachnoid space followed by copious irrigation with normal saline prevented chronic vasospasm only when clot evacuation was performed within 48 hours of blood deposition. In a similar study of clot fibrinolysis in this model, Findlay and colleagues\(^41\) demonstrated that intrathecal administration of recombinant tissue plasminogen activator prevented chronic vasospasm only when it was administered within 48 hours of blood deposition. The therapeutic window for initiating ibuprofen treatment in our study, however, was only 6 hours. The difference between our 6-hour therapeutic window and the 48-hour therapeutic window in these other studies could be explained as follows. We hypothesize that chronic posthemorrhagic vasospasm is primarily a result of the increased expression of endothelial CAMs of the cerebral arteries exposed to the hemorrhage. The increased expression of endothelial CAMs results in the extravasation of macrophages and granulocytes that participate in erythrocyte phagocytosis but cannot be cleared from the subarachnoid space because of the presence of the clot and impaired cerebrospinal fluid flow. These extravasated leukocytes are then primarily responsible for the subsequent events leading to chronic vasospasm because they release endothelins\(^42,43\) and lysosomal toxins\(^44,45\) during enhanced phagocytosis and after they die. Ibuprofen therapy prevents leukocyte extravasation but does not clear the leukocytes that are already present in the subarachnoid space. By contrast, clot evacuation followed by irrigation removes not only the clot but also the extravasated leukocytes. Similarly, intrathecal fibrinolysis dissolves the clot and allows for more effective clearance from the subarachnoid space by bulk flow of both erythrocytes and leukocytes. Therefore, in order to be effective, ibuprofen therapy has to be started earlier to prevent the accumulation in the subarachnoid space of a critical number of leukocytes that will then cause chronic vasospasm. By contrast, clot removal or fibrinolytic therapy can be initiated later since both will clear the subarachnoid space of extravasated leukocytes. When considered together, the results of these studies suggest that in SAH a critical number of leukocytes reaches the subarachnoid space 6 to 12 hours after the hemorrhage and that these leukocytes start to release the toxic products that eventually injure the vessel wall and lead to chronic vasospasm by 48 hours after the hemorrhage.

There are many toxic products of macrophages and granulocytes that can cause chronic vasospasm. It is apparent that nitric oxide and endothelins play a major role in chronic posthemorrhagic vasospasm.\(^46–48\) The balance in vascular tone exerted by endothelial cells may be altered by the exogenous endothelin production from macrophages\(^42\) and neutrophils.\(^43\) In addition, activated neutrophils may directly inhibit endothelium-dependent relaxation.\(^49,50\) Furthermore, activated macrophages during enhanced phagocytosis can secrete other toxic products that have been implicated in chronic vasospasm, such as hydrogen peroxide, superoxide anion, hydroxyl radical, perhydroxyl radical, and singlet oxygen.\(^44,45,48\)

In summary, we describe in this report the inhibition of experimental chronic posthemorrhagic vasospasm with the periadventitial administration of ibuprofen using controlled-release polymers when therapy is initiated within 6 hours of blood deposition. We correlate this inhibition with a decreased concentration of extravasated macrophages and granulocytes in the periadventitial space of ibuprofen-treated vessels. In addition, we describe an ibuprofen-EVAc polymer preparation that delivers ibuprofen in a controlled fashion over a 12-day period with an initial 24-hour burst, which may be ideally suited for the treatment of chronic vasospasm in aneurysmal SAH.

Acknowledgments

This work was supported in part by the Cerebrovascular Scholarship from the American Heart Association Stroke Council as well as by the Dr Harold Lampart Award from the Johns Hopkins School of Medicine (Q-A.T.) and by the Richard S. Ross Clinician Scientist Award from the Johns Hopkins University School of Medicine (R.J.T.). The authors thank Betty Tyler and Michael Pinn for technical assistance.

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Editorial Comment

Controlled-release polymers will undoubtedly become the “darlings” of physicians treating a wide variety of central nervous system afflictions, and the present work is the first suggesting they may one day have a use in the prevention of cerebral aneurysmal vasospasm. I cannot help but congratulate the authors on a beautiful piece of all-encompassing work: pharmacokinetic studies, dose-response toxicity studies, efficacy studies, and explanatory studies, all compressed into one, easy-to-read paper! I only wish that similar studies had been done with the carmustine-containing polymers for the treatment of brain tumors, so that we would not now have the low-dose (<4%) carmustine-containing polymers when higher doses might have been more effective. As the authors point out, intracranial experimental studies with this technique need to be done before it can be applied to humans, and one can only hope that before embarking on time- and resources-consuming human studies, the best drug and dose for maximal clinical safety and effect can be chosen with confidence. The present work demonstrates ways to reach such decisions.

**J. Paul Muizelaar, MD, PhD, Guest Editor**
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*Stroke.* 1999;30:140-147
doi: 10.1161/01.STR.30.1.140

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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