Inhibition of cerebral vasospasm by intracranial delivery of ibuprofen from a controlled-release polymer in a rabbit model of subarachnoid hemorrhage

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Object. Leukocyte–endothelial cell interactions may play a role in the development of cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) because the extravasation of circulating leukocytes into the periadventitial space within 24 hours after the hemorrhage appears to be a critical event in this process. Ibuprofen is an antiinflammatory agent that inhibits the expression of specific cell adhesion molecules and, consequently, disrupts leukocyte–endothelial cell interactions. The authors investigated the efficacy of ibuprofen delivered locally from controlled-release polymers in the rabbit basilar artery (BA) model of cerebral vasospasm.

Methods. Ibuprofen was incorporated into controlled-release ethylene–vinyl acetate copolymer (EVAc) constituting 45% of the resulting polymer by weight. Fifty-four New Zealand White rabbits were randomized to 10 groups: sham operation (seven animals); SAH only (seven animals); and SAH plus either empty EVAc or ibuprofen–EVAc polymer at 30 minutes or 6, 12, or 24 hours (five animals per group; 40 total). The rabbits were killed 72 hours after induction of SAH, at the time of maximal vasospasm. The efficacy of ibuprofen in preventing vasospasm was assessed by measuring lumen patency of the rabbit’s BAs. The intracranial controlled release of ibuprofen resulted in a significant inhibition of vasospasm when treatment was initiated at 30 minutes (patency 92.3 ± 5.1% compared with 52.1 ± 5.1% in animals given empty EVAc; p < 0.001) and 6 hours (patency 69.5 ± 3.5% compared with 47.2 ± 1.5% in animals given empty EVAc; p < 0.03) after blood deposition compared with treatment with empty EVAc. No effect was observed when treatment was begun at either 12 or 24 hours.

Conclusions. Local intracranial delivery of ibuprofen accomplished using controlled-release polymers prevents vasospasm in the rabbit BA model of vasospasm when administered within 6 hours after blood exposure.

KEY WORDS • cerebral vasospasm • subarachnoid hemorrhage • ibuprofen • controlled-release polymer • intercellular adhesion molecule–1

Abbreviations used in this paper: BA = basilar artery; CAM = cell adhesion molecule; CSF = cerebrospinal fluid; ET = endothelin; EVAc = ethylene–vinyl acetate copolymer; ICAM-1 = intercellular adhesion molecule–1; IEL = internal elastic lamina; LFA-1 = lymphocyte function–associated antigen–1; mAb = monoclonal antibody; Mac-1 = macrophage antigen–1; NO = nitric oxide; SAH = subarachnoid hemorrhage; VCAM-1 = vascular CAM–1.
vasospasm in the rat femoral artery model, when treatment is initiated within 6 hours after blood exposure. That study also demonstrated that ibuprofen-induced inhibition of vasospasm correlated with a significant decrease in the number of macrophages and granulocytes in the periadventitial space.

In the present study we investigated the effect of ibuprofen delivered via a controlled-release polymer in preventing vasospasm in the rabbit BA model of vasospasm. Unlike the rat femoral artery model, in which ibuprofen was used to test its efficacy against chronic vasospasm in a peripheral blood vessel, in the rabbit SAH model an intracranial cerebral artery is used to assess vasospasm.

Materials and Methods

Animal Groups

Fifty-four male New Zealand White rabbits (Robinson Services, Inc., Winston-Salem, NC) with a mean weight of 3.4 kg were kept in standard facilities with free access to food and water. The animals were randomized to one of 10 groups: sham operation (seven animals); SAH only (seven animals); or SAH plus either empty EVAc or ibuprofen–EVAc polymer administered at 30 minutes or 6, 12, or 24 hours post-SAH (five animals per group; 40 rabbits total).

Polymer Preparation

The procedure for the preparation of EVAc has been described in our previous reports. Briefly, EVAc (40% vinyl acetate by weight; DuPont Co., Wilmington, DE) and ibuprofen (Sigma Chemical Co., St. Louis, MO) were dissolved and suspended, respectively, in high-performance liquid chromatography–grade methylene chloride (Fisher Chemicals, Fairlawn, NJ) to yield an EVAc polymer loaded with 45% (wt/wt) ibuprofen. In addition, EVAc alone was dissolved in a similar manner. The mixtures were poured into cylindrical glass molds at −70 °C. The cylindrical polymers were cut into 5-mg fragments and stored at −20 °C in sterile glass containers.

Histological Studies

All animals were killed 72 hours after treatment and received in situ perfusion fixation. The animals were transcardially perfused with 300 ml of normal saline solution and 500 ml of cold 4% paraformaldehyde in 0.1 mol/L phosphate buffer. Both the BA and the brainstem were harvested and cryoprotected in 20% sucrose in 0.1 mol/L phosphate buffer. Both the BA and the brainstem were harvested and cryoprotected in 20% sucrose in 0.1 mol/L phosphate buffer for 3 days at 4 °C. The specimens were stored at 80 °C after they had been snap-frozen in dry ice–equilibrated isopentane cooled to −60 °C. The specimens were mounted in tissue-freezing compound (Triangle Biomedical Sciences, Durham, NC) and were sectioned transversely into 10-μm slices, with the aid of a microtome cryostat (HM 500 OM; Microm GmbH, Walldorf, Germany), at 200-μm intervals beginning at the termination of the BA. The tissue slices were mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA) and stained with standard H & E (Sigma Chemical Co.); coverslips were mounted using Permound medium (Fisher Scientific).

Morphometric Analysis

Measurement of the BA circumference was performed using a computerized image-analysis system (MCID; Imaging Research, St. Catharines, ON, Canada) to quantify the vessel patency as previously described. Five sections of each vessel separated by 200 μm were measured and averaged to correct for vessel deformation and off-transverse sections. The mean percentage of lumen patency, defined as the ratio of the blood-exposed artery to the average control BA area in the sham-operated group, was obtained from the mean cross-sectional area of the blood-exposed BA to that of the mean control BA area in the sham-operated group. A statistically significant difference was achieved when treatment was initiated at 30 minutes (p < 0.001) or 6 hours (p = 0.03). Asterisks indicate a statistically significant reduction in BA vasospasm.

Animal Preparation

We have described this procedure in a previous report. Briefly, the animals were anesthetized with a 0.6-ml/kg intramuscular injection of a 5:1 mixture of ketamine HCl (Ketlar, 25 mg/ml; Parke-Davis, Morris Plains, NJ) and xylazine (Rompun, 2.5 mg/ml; Mobay Corp., Shawnee, KS). Ceftriaxone 20 mg/kg (Rocephin; Hoffmann-LaRoche, Inc., Nutley, NJ) was administered intramuscularly for prophylactic purposes. The rabbit’s neck was flexed and a midline suboccipital incision was made. The atlantooccipital membrane was exposed from the margin of the foramen magnum to the arch of the first cervical vertebra.

The animals in the sham-operated group did not receive an injection of autologous blood into the cisterna magna. The animals in the other nine groups received an injection of autologous arterial blood into the subarachnoid space. A 23-gauge butterfly needle was used to penetrate the atlantooccipital membrane and enter the cisterna magna. Approximately 3 ml of autologous nonheparinized blood was collected from the central ear artery, and an SAH was induced by the injection of 1.5 to 2 ml of this blood into the cisterna magna. The wounds were then closed in the animals in the SAH-only group. The animals with SAH that had been assigned to treatment groups were returned to the operating table at the appropriate time intervals (30 minutes or 6, 12, or 24 hours) for polymer insertion. On exposure of the atlantooccipital membrane, a 16-gauge needle was used to make a 3-mm incision in the dural and arachnoid membranes. Either 5 mg empty EVAc or ibuprofen–EVAc polymer was placed into the cisterna magna. The wound was then closed and the animals were allowed to recover before they were returned to their cages. An intramuscular injection of buprenorphine (0.04 mg/kg; Reckitt and Colman Products, Richmond, VA) was administered to minimize postoperative pain.

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Results

Treatment with ibuprofen–EV Ac polymer at either 30 minutes or 6 hours after blood deposition significantly inhibited BA vasospasm in comparison with empty EV Ac treatment. No effect was observed when the ibuprofen–EV Ac polymer treatment was initiated at either 12 or 24 hours after blood deposition (Fig. 1). Whereas insertion of ibuprofen–EV Ac polymer at 30 minutes resulted in a mean lumen patency of 92.3 ± 5.1%, insertion of empty EV Ac at 30 minutes resulted in a mean lumen patency of 52.1 ± 5.1% (p < 0.001). Similarly, although insertion of ibuprofen–EV Ac polymer at 6 hours resulted in a mean lumen patency of 69.5 ± 3.5%, insertion of empty EV Ac at 6 hours resulted in a mean lumen patency of 47.2 ± 1.5% (p = 0.03). Treatment with ibuprofen–EV Ac polymer at either 12 or 24 hours did not affect the vasospastic response in comparison with treatment with empty EV Ac (p = 0.221 and p = 0.377, respectively). There was a statistically significant reduction in mean BA cross-sectional areas in the SAH-only (p = 0.012) and SAH + empty EV Ac groups treated at 30 minutes and 6, 12, and 24 hours after blood deposition (p = 0.012), in comparison with the sham-operated group. This indicates that the presence of the polymer does not affect lumen patency. No statistically significant difference in mean BA cross-sectional areas in the SAH-only and SAH + empty EV Ac-treated groups was observed (p = 0.241).

Qualitative light microscopic examination of the BAs in the SAH-only, and the SAH + ibuprofen–EV Ac polymer–treated groups at 12 and 24 hours, and in the SAH + empty EV Ac-treated groups revealed substantial corrugation of the IEL. Corrugation of the IEL was either less prominent or absent in the groups treated with ibuprofen–EV Ac polymer at 30 minutes and 6 hours post-SAH. Figure 2 demonstrates a representative sample at the 30-minute time point.

Discussion

In this paper we demonstrate that ibuprofen delivered in the subarachnoid space by using controlled-release polymers prevents cerebral vasospasm in the rabbit BA model when treatment is initiated 30 minutes or 6 hours after SAH. This and other results indicate that leukocyte–endothelial cell interactions play an important role in chronic vasospasm.25,30,36 Although earlier studies provided indirect evidence for the role of inflammation in vasospasm, current molecular studies confirm the importance of leukocyte–endothelial cell interactions in this process. In early studies, granulocytes and mononuclear leukocytes in the tunica media and adventitia of blood vessels were associated with structural changes in the walls of vasospastic arteries.15 Current molecular studies of vasospasm show that CAMs induce leukocyte extravasation across the blood vessel wall, and that this event is associated with vasospasm.5,30

Cell adhesion molecules are grouped as selectins, integrins, or immunoglobulin superfamily members. The selectins, which include E-selectin (CD62E), P-selectin
(CD62P), and L-selectin (CD62L), mediate tethering and rolling of leukocytes on the vessel lumen in the microcirculation at the site of inflammation, and this process is a prerequisite for firm adhesion and subsequent transendothelial migration of leukocytes into tissues.32 The integrins include LFA-1 and Mac-1, which play an important role in adhesion strengthening and diapedesis across the vessel wall. Macrophage antigen-1 is expressed on granulocytes and monocytes, with the highest levels of expression on neutrophils.19 Lymphocyte function–associated antigen-1 is expressed on lymphocytes, granulocytes, and on monocyte series.32 Recently, personnel in our laboratory demonstrated that the systemic administration of anti-LFA–1 mAb prevents vasospasm and leads to a significant reduction in periadventitial leukocytes in the rat femoral artery model.6 The immunoglobulin superfamily includes ICAM-1 and VCAM-1, which are both expressed on endothelial cells. A known ligand for LFA-1, ICAM-1 appears to be involved in acute inflammation and has been implicated in vasospasm.25,30

Intercellular adhesion molecule–1 has been shown to be expressed on the endothelium of normal cerebral vessels in both humans and rodents and to be upregulated by many pathological conditions including SAH.13,18,20,31 Experimental studies have shown ICAM-1 to be upregulated in the cerebral vasculature after SAH9 and in blood-exposed vessels that subsequently develop chronic vasospasm.30 Intercellular adhesion molecule–1 has been shown to be upregulated on endothelium beginning 3 hours after SAH and this persists for up to 24 hours.30 This time period of increased ICAM-1 expression correlates with the periadventitial infiltration of macrophages and granulocytes.6,25,36 Therefore, granulocyte/macrophage infiltration occurs in the periadventitial space 3 to 24 hours after SAH, and it appears that a critical leukocyte concentration can accumulate by 6 hours.30 In addition, our laboratory has shown that endothelial ICAM-1 receptor blockade with an anti–ICAM-1 mAb results in significant inhibition of chronic vasospasm and is correlated with a reduction in the number of infiltrating macrophages and granulocytes in the periadventitial region of blood-exposed arteries.6,25 Another study has shown that the intrathecal delivery of anti–ICAM-1 antibodies decreased cerebral vasospasm in the rabbit BA model of post-hemorrhagic vasospasm.3

Clinical studies in humans have demonstrated elevated levels of soluble ICAM-1 in patients after aneurysmal SAH.18,28 Polin and colleagues28 have demonstrated elevated levels of soluble ICAM-1 in the CSF of patients soon after SAH occurred compared with corresponding levels in the CSF of healthy controls, patients with unruptured aneurysms, and patients tested months after SAH occurred. Mack and colleagues18 have shown that patients with aneurysmal SAH who had poor outcomes had significantly higher soluble ICAM-1 levels over the first 2 weeks post-SAH compared with patients who had good outcomes. Recently, Mocco and colleagues20 reported that patients with vasospasm, confirmed by both transcranial Doppler studies and cerebral angiography, displayed a significant mean rate of increase in soluble ICAM-1 levels during the peri vasospasm period, whereas admission Hunt and Hess grade–matched controls did not.

Based on current evidence for the cellular and molecular mechanisms underlying vasospasm, we have formulated a hypothesis to explain the role of leukocyte–endothelial cell interactions in cerebral vasospasm. After exposure of cerebral arteries to hemorrhage, signaling molecules in the periadventitial thrombus initiate a sequence of events, resulting in the upregulation of endothelial ICAM-1 and, possibly, other endothelial CAMs. Lymphocyte function–associated antigen–1 and Mac-1 on circulating leukocytes bind to ICAM-1 and cause transmigration of these macrophages and granulocytes across the endothelium into the periadventitial space.6,25,36 Once in the periadventitial space, these macrophages and granulocytes phagocytose and process erythrocytes, but ultimately cannot be cleared from the subarachnoid space because of the presence of the clot and the impaired CSF flow. Within 3 or 4 days, enhanced phagocytosis, cell death, and subsequent degranulation cause these leukocytes to release ETs11,25,38 and lysosomal toxins,10,22 including inflammatory mediators, which cause vasoconstriction, and inflammatory cytokines, such as interleukins 1, 6, and 8, which have been shown to be induced in the CSF during the acute stage after SAH.26 Endothelins and O2 free radicals induce transient vasoconstriction, which lasts 4 to 14 days.

Ibuprofen, an antiinflammatory agent, has been shown to inhibit the expression of ICAM-1 and thus disrupt leukocyte–endothelial cell interactions.8,23 This effect of ibuprofen occurs at high systemic concentrations, which are associated with thrombasthenia or thrombocytopenia, fluid retention, and gastric ulcers.5 To circumvent the systemic toxicity of high ibuprofen doses, controlled-release polymers can be used to administer the required high doses at the site of the SAH while avoiding exposure to tissue elsewhere. A previous study undertaken in our laboratory showed that the periadventitial, controlled release of ibuprofen from surgically implanted polymers significantly inhibited vasospasm in the rat femoral artery model when treatment was initiated within 6 hours of blood exposure.36 This study also demonstrated that vasospasm inhibition with ibuprofen correlated with a significant decrease in the number of macrophages and granulocytes in the periadventitial space. In addition, researchers at our laboratory have studied the release kinetic profile of the ibuprofen–EVAc polymer and have shown that this polymer releases approximately half its drug load within the first 24 hours and the remainder over the next 11 days.29 A more recent study undertaken at our laboratory demonstrated that ibuprofen therapy reduced striatal infarct size after focal cerebral ischemia in a middle cerebral artery occlusion model in rats.1 This effect was independent of end-ischemic cerebral blood flow and was associated with a reduction in endothelial ICAM-1 expression in the ischemic region of ibuprofen-treated animals, when compared with control animals, thus providing further evidence for the role of ibuprofen in the inhibition of ICAM-1 expression and the disruption of leukocyte–endothelial cell interactions.

In the present study, the rabbit SAH model was used to assess the efficacy of intracranially delivered ibuprofen, via the implantation of polymers into the cisterna magna, in the prevention of post-SAH cerebral vasospasm. Although Offerhaus and van Gool26 were the first to induce experimental SAH in rabbits, Baker and Chan and their colleagues4 developed the rabbit SAH model that was used in this study, in which introduction of blood into the cisterna magna resulted in a reproducible BA vasospasm over a 3- to 6-day time frame. One of the strengths of this model is that it pro-
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vides a reliable method to study vasospasm in an intracranial vessel. A disadvantage of this model, however, is that the time course of chronic vasospasm in rabbit cerebral vessels is shorter than that in human cerebral vessels. Our study shows that the intracranial administration of ibuprofen significantly inhibits vasospasm if given within 6 hours after hemorrhage. This correlates with the arrival of a critical number of leukocytes (which are needed to produce chronic vasospasm) in the subarachnoid space 3 to 24 hours after periadventitial blood deposition. A critical mass of leukocytes accumulates in the subarachnoid space by 6 to 12 hours after SAH; ibuprofen does not prevent vasospasm when administered beyond this period because it does not lead to the removal of leukocytes that have already accumulated in the subarachnoid space. If administered early enough, however, ibuprofen can prevent the extravasation of leukocytes.

Although leukocyte–endothelial cell interactions may be pivotal in the final elucidation of the molecular pathogenesis of vasospasm, an imbalance between ET-mediated vasocostriction and NO-mediated vasodilation plays a significant role. Once in the periadventitial space, leukocytes may counteract the effects of endothelium-derived NO by shifting the balance of vasomotor tone toward constriction through the release of ETs. Several experimental and clinical studies have demonstrated that NO prevents and reverses vasospasm. Recent studies in our laboratory have shown that local delivery of NO by a controlled-release polymer prevents vasospasm in the rat femoral artery and in the rabbit BA.2

Conclusions

We have described the inhibition of experimental cerebral vasospasm in the rabbit BA by the local intracranial administration of ibuprofen from controlled-release polymers. Unlike previous studies, this work demonstrates ibuprofen’s efficacy in an intracranial model. This treatment is effective when initiated within 6 hours after SAH, but no effect is observed when treatment is begun at 12 hours or later. Experimental studies have shown ICAM-1 to be upregulated in experimental cerebral vasospasm with ibuprofen and high-dose methylprednisolone. J Neurosurg 59:925–932, 1983.


References


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