Prevention of chronic experimental cerebral vasospasm with ibuprofen and high-dose methylprednisolone

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Severe chronic cerebral vasospasm was reliably induced in dogs by two injections, 2 days apart, of autologous blood into the cisterna magna. Treatment with ibuprofen or high-dose methylprednisolone after the first injection prevented or reduced vasospasm. Both drugs reduced meningismus and accelerated the rate of neurological recovery. Compared with specimens from normal dogs, rings of basilar arteries obtained from untreated dogs contracted weakly in response to 5-hydroxytryptamine, prostaglandin F2α, potassium chloride, and barium chloride. Rings of arteries from dogs who received ibuprofen or methylprednisolone contracted more strongly. Electron micrographs of basilar arteries from untreated dogs showed degeneration of smooth muscle, whereas those from treated dogs did not. Thus, what is termed “chronic cerebral vasospasm” probably represents a structural derangement of the blood vessel wall leading to its narrowing, rather than a sustained contraction of the vascular smooth muscle. Administration of high-dose methylprednisolone and ibuprofen can prevent its occurrence.

KEY WORDS: cerebral arterial spasm · subarachnoid hemorrhage · vascular smooth muscle · cerebral vascular pathology · methylprednisolone · ibuprofen

The refractoriness of chronic cerebral vasospasm to modern pharmacological interventions contributes to the high rates of morbidity and mortality associated with subarachnoid hemorrhage (SAH). Since the etiology of the vasospasm remains unknown, treatment in the past has varied from attempts to reduce the chronic spasm with vasodilating agents, to efforts directed at enhancing cardiac output and perfusion pressure to promote cerebral blood flow. However, despite various therapeutic attempts, an effective program of drug therapy for this condition remains elusive.

Currently, a growing amount of circumstantial evidence suggests that chronic cerebral vasospasm may be linked with an inflammatory response induced by the presence of blood in the subarachnoid space. The time course of chronic vasospasm is consistent with that of an inflammatory reaction, appearing several days after SAH, and lasting for days to weeks. Ultrastructural examination of chronically spastic cerebral arteries obtained from humans and experimental animals shows signs of cell inflammation and damage, most notably necrotic changes in the arterial media and an inflammatory cell infiltration of the arterial wall. Neutrophils, the predominant cell seen in the infiltrate, are capable of producing vascular damage when activated by the complement cascade. The failure of vasodilating agents to alleviate the spasm may indicate that the narrowing of the arterial lumen is due to inflammatory changes of the vascular wall, rather than an active constriction of the smooth-muscle cells. In addition, in vitro experiments indicate that breakdown products of blood, in particular oxyhemoglobin, can induce the formation of prostaglandin-like substances in cerebral blood vessels. Several recent reports have focused on the role that these chemicals might play in the pathogenesis of chronic cerebral vasospasm.

The present study was performed to determine if drugs that interrupt the inflammatory process could prevent chronic cerebral vasospasm from occurring after experimentally induced SAH. High-dose methylprednisolone and ibuprofen were selected for this study, since these drugs have proven steroidal and nonsteroidal anti-inflammatory actions, respectively, and are commonly used in current drug therapy in patients.

Materials and Methods

Animal Model

A “double hemorrhage” canine model was used in this study not only because it reliably produces chronic cerebral vasospasm, but because it exhibits the patho-
Healthy adult mongrel dogs (each weighing 13 to 32 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg), intubated, and allowed to respire spontaneously. With the aid of fluoroscopic guidance, a transfemoral catheter was placed into the dominant vertebral artery and advanced several centimeters past its origin. Either vertebral artery was used when neither was dominant. A baseline vertebrobasilar angiogram was then taken. All angiograms were performed at identical magnification and Renografin-76 injection parameters (volume, pressure, and duration).

The animals were allowed to recover and evaluated by neurological examination. On Day 1 of the study, those animals that remained neurologically normal were anesthetized again. The cisterna magna was aseptically punctured with a No. 22 spinal needle and 4 cc of cerebrospinal fluid (CSF) removed. With the animal in the 30° head-down position, 4 cc of autologous venous blood was injected through the spinal needle over a 2-minute period. After 30 minutes in the head-down position, the animal was returned to its cage. All animals were evaluated daily for signs of meningeal irritation and neurological abnormalities.

On the 3rd day of the study, the animals were reanesthetized. Again, 4 cc of autologous venous blood was introduced into the cisterna magna after CSF had been withdrawn as on Day 1. On Day 8 of the study, a final transfemoral vertebrobasilar angiogram was taken after appropriate anesthesia.

The dogs were divided into three experimental groups. Group 1 dogs received no drug treatment; Group 2 dogs received ibuprofen, 12.5 mg/kg intravenously, 1 hour after the initial injection of blood and every 8 hours during the study; and Group 3 dogs received methylprednisolone (Solu-Medrol), 30 mg/kg intravenously, 1 hour after the initial injection of blood and every 8 hours during the study. In addition, five dogs received both ibuprofen and methylprednisolone as described for Groups 2 and 3. Reference to this group will be made in the discussion.

Analysis of Angiograms

Angiograms were analyzed using a computer-controlled, operator-interactive, rapid videodigitizing system. Final digital images represented a matrix of 500 lines with 2000 samples on each line using 256 gray levels. Background brightness was measured within a rectangular mask immediately adjacent to each basilar artery image. The threshold of background brightness was defined as two standard deviations above average background brightness and was calculated for each mask. Another rectangular mask was constructed over a segment of the basilar artery image and the number of pixels above the threshold measured. Baseline angiograms were compared to final angiograms using identical segments of the basilar artery image and identical mask sizes. The number of pixels above background brightness threshold is proportional to vessel cross-sectional area and correlates closely with vessel dimensions measured directly in pathological specimens (unpublished data). The percent of original vessel cross-sectional area was calculated for each angiographic set (number of pixels above threshold on the final angiogram/number of pixels above threshold on the baseline angiogram × 100). This technique provides an accurate, reproducible (5% coefficient of variation) index of vessel size over an arterial segment.

Student's t-test for unpaired observations was used to compare values between treatment groups, and Student's t-test for paired observations was used to compare final vessel size to original vessel size within each group (p values less than 0.05 were considered to be statistically significant).

Organ Baths

Brains were removed from dogs after completion of the angiogram on Day 8. For control preparations, brains were removed from mongrel dogs that had been anesthetized with intravenous sodium pentobarbital (30 mg/kg). All dogs were sacrificed by rapid exsanguination from the common carotid arteries.

After removal, the brains were placed immediately in a dissecting dish filled with Krebs-Ringer bicarbonate solution and the distribution of subarachnoid blood was noted. With the aid of magnification, the basilar arteries were excised, cleaned of connective tissue, and cut into rings 3 mm long. The rings were mounted in organ chambers and attached to a strain gauge* for continuous isometric tension recording. The preparations were studied in Krebs-Ringer bicarbonate solution (composition, mM: NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, Ca EDTA 0.026, 0.026

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* No. Uc2 strain gauge manufactured by Statham Laboratories, Inc., P.O. Box 1178, Hato Rey, Puerto Rico.
Prevention of chronic experimental vasospasm and glucose 11.1) aerated with a 95% O2-5% CO2 gas mixture, and maintained at 37°C.

Resting tension was adjusted to 500 mg. After equilibration for 1 hour, tension was increased to 3 gm and another hour of equilibration was permitted. Resting tension was then maintained at 3 gm throughout the experiment. This has been shown to be the optimal point of the length-active tension relationship for this preparation.1

The drugs used were (in chronological order): KCl, 40 mM; BaCl2, 2.5 x 10^-3M; prostaglandin F2a (PGF2a), 5 x 10^-6M; and 5-hydroxytryptamine (5HT:creatinine sulfate complex), 10^-4M. Drugs were added directly to the bath solution, and concentrations are expressed as final bath concentrations. For each agonist, the concentration used causes maximal activation of the preparation. A 20-minute equilibration period was allowed after complete relaxation from the previous response, before the next agonist was added.

For statistical analysis, Student’s t-test for unpaired observations was used. Each observation represented the mean results of two rings for control preparations, and the mean results of three rings for experimental preparations (p values less than 0.05 were considered to be statistically significant).

Transmission Electron Microscopy

After sacrifice, representative segments of basilar arteries were placed immediately in Trump’s fixative21 containing 4% formaldehyde and 1% glutaraldehyde in 200 mOsm sodium phosphate buffer (pH 7.2). The samples were rinsed three times in 0.1 M sodium phosphate buffer and then postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer. Samples were then rinsed three times in distilled water, stained en bloc in 2% aqueous uranyl acetate (60°C) for 30 minutes, dehydrated in a graded series of ethanol, and rinsed twice in propylene oxide. The arteries were subsequently infiltrated and embedded in Spurr’s low-viscosity embedding resin.32 Thin sections were cut with an LKB Ultrotome III, poststained with a 0.3% aqueous lead citrate, and examined with a Philips 400 or a Philips 201 transmission electron microscope.†

Results

Dogs Eliminated From Study

Eleven dogs were eliminated from the study. Five dogs died as a result of the initial anesthesia, and three died immediately after the initial injection of blood. Three dogs were eliminated because of complications from the initial angiogram: one died of large vessel dissection, another died suddenly after the procedure, and the third suffered a nonfatal brain-stem infarction. No dogs died as a delayed consequence of SAH.

Meningeal Signs

Meningeal signs were considered to be present if the dog held his head below its shoulders in a characteristic fashion, and resisted passive movement of its neck. All nine dogs in the untreated group developed meningeal signs that persisted nearly the duration of the study. Dogs treated with ibuprofen or methylprednisolone had significantly less (p < 0.005, unpaired t-test) meningeismus (Fig. 1). Of the eight dogs treated with ibuprofen, only two developed transient meningeal signs. Similarly, temporary meningeal signs were noted in two of the eight dogs treated with methylprednisolone.

Neurological Deficits

At least one dog in each group developed a neurological deficit, which was evident immediately after the initial injection of blood (Fig. 2). In each case, the neurological abnormality either remained static or improved during the course of the study. No deficit became progressively worse during the 8-day course of the experiment, although one dog in the untreated group improved briefly, only to return to its previous neurological status. Although the numbers are small, it appeared that the neurological deficits of dogs in the treatment groups improved or subsided more often than those receiving no treatment.

Distribution of Subarachnoid Blood

Brains removed on Day 8 of the experiment from untreated animals uniformly showed a dense blood clot encasing the basilar artery and anterior brain stem. Dogs that were treated with methylprednisolone had a similar distribution of blood. Animals treated with ibuprofen, however, had much less blood around the basilar artery and brain stem. Within each treatment group, the amount of subarachnoid blood noted at autopsy did not correlate with the severity of angiographic spasm, or with the isolated arterial contractile responses.

† Ultrotome III manufactured by LKB Instruments, Inc., Bromma, Sweden. Philips 400 and Philips 201 transmission electron microscopes manufactured by Philips Electronic Instruments, Mount Vernon, New York.

Fig. 2. Nature and evolution of neurological deficits after experimental subarachnoid hemorrhage.

and the third suffered a nonfatal brain-stem infarction. No dogs died as a delayed consequence of SAH.
FIG. 3. Angiograms in an untreated dog. A: Subtraction angiogram showing the baseline appearance of the basilar artery. B: Final subtraction angiogram of the basilar artery showing severe diffuse spasm on Day 8.

ANGIOGRAPHIC SPASM

Diffuse, severe spasm of the intradural vertebral arteries and the basilar artery developed in all nine untreated dogs (Fig. 3). In this group, the average basilar artery cross-sectional area was significantly reduced, measuring only 33.7% of the baseline area (Fig. 6). Without exception, both ibuprofen and methylprednisolone prevented or markedly reduced the severity of chronic vasospasm (Figs. 4 and 5). Average cross-sectional areas were 98.0% and 85.2% of the baseline area, respectively, and represented significant improvement over the untreated group (Fig. 6). Unlike the untreated group, ibuprofen prevented statistically significant vasospasm from occurring, while methylprednisolone appeared to be somewhat less effective.

RESPONSE TO VASOCONSTRICTOR AGENTS

The contractions induced by KCl, BaCl2, PGF2α, and 5HT were compared in basilar arteries removed from control dogs, and untreated and treated experimental dogs (Fig. 7). Arteries obtained from untreated dogs showed significantly depressed contractile responses to all four agonists used, averaging only 25.7% (KCl), 25.9% (BaCl2), 29.0% (PGF2α), and 35.3% (5HT) of the control response. Although the contractions of arteries obtained from dogs treated with ibuprofen or methylprednisolone were also smaller than control responses, these arteries had a significantly greater response to all four agonists than vessels removed from untreated animals. Arteries from dogs treated with ibuprofen or methylprednisolone averaged, respectively: 51.1% and 52.2% (KCl), 58.1% and 63.0% (BaCl2), 67.3% and 67.9% (PGF2α), and 73.9% and 76.8% (5HT) of the control response. No difference was found between the two groups of treated vessels.
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Transmission Electron Microscopy

Cerebral vascular ultrastructure was compared in basilar arteries taken from two control, untreated, ibuprofen-treated, and methylprednisolone-treated dogs. Electron micrographs of arteries from untreated animals showed striking necrosis and degeneration of the smooth-muscle cells, as compared to control basilar arteries (Fig. 8 upper). In addition, portions of the endothelial cell layer were lifted off the underlying elastic lamina. Disruption and infiltration with cellular debris were apparent throughout the elastic lamina.

Arteries taken from either ibuprofen- or methylprednisolone-treated dogs failed to show degeneration or necrosis of smooth-muscle cells. Disruption of the elastic lamina and endothelial alterations were still present (Fig. 8 lower).

Discussion

In the present study, a “double hemorrhage” canine model was used which reliably caused severe diffuse spasm of the intradural vertebral and basilar arteries 7 days after the first injection of blood. This model was used because it produces the same pathological arterial changes and refractoriness to pharmacological therapy exhibited by chronic vasospasm in humans. Treatment with ibuprofen or high-dose methylprednisolone, started shortly after the initial injection of blood and continued every 8 hours throughout the course of the experiment, prevented or markedly reduced the severity of chronic vasospasm. In addition, these agents significantly reduced the frequency, severity, and duration of meningismus and appeared to hasten the recovery from neurological deficits.

Ibuprofen and methylprednisolone, when used alone, had no apparent side effects; however, in five dogs we attempted to use both drugs in a combined-treatment program. Of the five animals, one died of hemorrhagic pneumonitis on Day 3 of the study, and another died of a perforated duodenal ulcer on Day 7. Of the three remaining dogs that survived until sacrificed on Day 8, two had massive upper gastrointestinal bleeding from antral and duodenal ulcers, and in the third dog, lesser signs of gastrointestinal hemorrhage were noted at autopsy. Thus, while comparable angiographic improvement was noted in the three surviving animals, the toxic side effects of the combined treatment program precluded further investigation.

Earlier studies have indicated that anti-inflammatory agents relieve the early reversible phase of cerebral vasospasm. Fox and Yaşargil10 reported that topically applied methylprednisolone and cortisol successfully relieved acute vasospasm induced by topical barium chloride, PGF2α, serotonin, or arterial puncture in monkey basilar arteries. Ohmoto, et al.,23 reported similar findings in a cat model. In addition, intravenous hydrocortisone is known to dilate normal cerebral vessels.14,15 Although Hashi, et al.,14,15 reported that a single high dose of hydrocortisone can relieve chronic vasospasm in dogs, their experiments were performed only 48 hours after SAH during a period when spasm is still reversible. This point is emphasized by the uniformly poor results they obtained when they tried to treat humans with high-dose hydrocortisone alone after chronic cerebral vasospasm was present and had become symptomatic. White, et al.,40 reported that suboxicum but not naproxen (two long-acting anti-inflammatory agents) relieved early vasospasm in dogs following SAH, but they did not extend their experiments longer than 24 hours after SAH. Chapleau, et al.,3 reported that low concentrations of meclofenamate and indomethacin prevented contraction of isolated basilar artery rings induced by arachidonic acid, but not by PGF2α or 5HT. Higher concentrations of these drugs inhibited contractions caused by all three agonists. Okwuasaba, et al.,24 however, reported that indomethacin did not significantly diminish the contractions of isolated canine cerebral arteries induced by blood-containing CSF.

To our knowledge, this study is the first to demonstrate that anti-inflammatory drugs can reduce the severity of cerebral vasospasm in a chronic animal model.
FIG. 8. Electron micrographs of sections of canine basilar artery. Upper Left: Section from a control dog showing the appearance of the endothelial cells (E) adjacent to the vessel lumen (L), the elastic lamina (EL), and smooth-muscle cells (S). × 1667. Upper Right: Section from an untreated dog showing striking necrosis of the smooth-muscle cells (S). Ultrastructural abnormalities involving the endothelium (E) and the elastic lamina (EL) are also apparent. × 1085. Lower Left: Section from a dog treated with ibuprofen. The appearance of the smooth-muscle cells (S) is indistinguishable from that seen in normal dogs. The endothelium (E) and elastic lamina (EL) still appear abnormal. × 1100. Lower Right: Specimen from a dog treated with methylprednisolone. The appearance of the smooth-muscle cells (S) is indistinguishable from that seen in normal dogs. The endothelium (E) and elastic lamina (EL) still appear abnormal. × 1100.
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Our data also indirectly support the concept that chronic spasm is related to an inflammatory response induced by the presence of blood in the subarachnoid space. This study does not define the mechanism by which the anti-inflammatory agents act to inhibit the induction of cerebral vasospasm. However, steroid and nonsteroid anti-inflammatory drugs interfere with prostaglandin synthesis, inhibit complement activation, depress leukocyte migration and function, and inhibit lymphocyte function. Many of these events are known to cause vascular damage. Thus, the possibility exists that the drugs administered in this study reduced the severity of vasospasm by interrupting the inflammatory response and preserving the integrity of the arterial wall.

Toda, et al., reported that middle cerebral arteries taken from dogs 7 days after experimentally-induced SAH showed a significantly depressed response to KCl, 5HT, norepinephrine, and histamine. They suggested that this non-specific depression could be due to a reduction in Ca ++ influx, mobilization, and availability, or to a metabolic or histological impairment of the arterial smooth-muscle cells. Our data obtained from basilar arteries from untreated dogs concur with their results. The attenuated contractile responses in these arteries cannot be explained by the destruction or desensitization of membrane receptors, since both receptor-mediated and nonreceptor-mediated responses were depressed. Nor is it likely that an isolated defect in Ca ++ influx or intracellular mobilization could account for the depressed responsiveness. Although the contraction induced by KCl is dependent upon the entry of extracellular Ca ++, it has recently been demonstrated that 5HT causes contraction in bovine basilar arteries by the mobilization of intracellular Ca ++, and BaCl2 is thought, at least in part, to induce contraction both by the release of intracellular Ca ++ and by direct activation of the contractile proteins. Therefore, our data suggest that a functional or histological derangement of the smooth-muscle cell contractile elements is a probable explanation for the inability of these arteries to contract. The fact that, in our study, anti-inflammatory drugs improved the ability of the arteries to respond to vasoactive agonists and prevented the myonecrotic changes noted in histological examination of chronically spastic cerebral arteries, led us to favor the latter explanation. Thus, what is termed "chronic cerebral vasospasm" appears to represent a structural derangement of the blood vessel wall leading to its narrowing, rather than a sustained contraction of vascular smooth muscle.

Clearly, both ibuprofen and high-dose methylprednisolone, when given prophylactically, can prevent severe chronic cerebral vasospasm from occurring in dogs. A review of past experiences with these classes of drugs suggests that they may also have some activity against the early phase of vasospasm as well. The spectrum of pharmacological activity of both drugs is consistent with the speculation that chronic cerebral vasospasm represents a vasculopathy of inflammatory origin. Similarly, the pharmacological properties of these drugs suggest how they should be used in human trials. Ibuprofen, because of its transient antiplatelet effect, should be reserved for postoperative patients in whom the risk of aneurysmal rebleeding has been eliminated. Methylprednisolone has significantly less anti-hemostatic effect, but causes more metabolic disturbance and would be more appropriate for short-term preoperative use.

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