Prevention of Chronic Cerebral Vasospasm in Dogs With Ibuprofen and High-dose Methylprednisolone

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Severe chronic cerebral vasospasm was produced in dog basilar arteries by two injections, 2 days apart, of autologous blood into the cisterna magna of 25 dogs. Treatment with ibuprofen (n=8) or high-dose methylprednisolone (n=8) after the first injection of blood prevented or reduced angiographic vasospasm. Cerebrospinal fluid concentrations of prostaglandin E₂, prostaglandin F₂α, 6-ketoprostaglandin F₁α (a metabolite of prostacyclin), and thromboxane B₂ (a metabolite of thromboxane A₂) were measured in both treated and untreated (n=7) dogs. In untreated dogs, the level of prostaglandin E₂ increased 94-fold by Day 8 after the first injection of blood and was strongly and positively correlated with the degree of angiographic vasospasm. Treatment with ibuprofen and high-dose methylprednisolone prevented or significantly reduced this increase in prostaglandin E₂ concentration. Smaller increases in cerebrospinal fluid concentrations of thromboxane B₂ and 6-ketoprostaglandin F₁α occurred after experimental subarachnoid hemorrhage; the magnitude of these increases was also reduced by ibuprofen or high-dose methylprednisolone treatment. In contrast, prostaglandin F₂α levels were not significantly altered during the study. These data show that enhanced prostaglandin E₂ synthesis occurs during experimental subarachnoid hemorrhage, and the by-products generated in its synthesis may play a role in the pathogenesis of cerebral vasospasm. (Stroke 1989;20:1021-1026)

The cause of chronic cerebral vasospasm following subarachnoid hemorrhage remains poorly understood. Prompted by evidence that chronic cerebral vasospasm may be linked to an inflammatory response that occurs after subarachnoid hemorrhage, we have previously reported that two drugs with anti-inflammatory properties, ibuprofen and high-dose methylprednisolone, prevented or markedly reduced chronic cerebral vasospasm in dogs following experimental subarachnoid hemorrhage. These drugs also prevented the myonecrotic histologic changes seen in untreated vaso spas tic arteries and improved the in vitro contractile response of these basilar arteries to a variety of vasoactive agonists.

Although ibuprofen and high-dose methylprednisolone interfere with inflammation by a variety of mechanisms, inhibition of prostaglandin synthesis is one of their major actions. Prostaglandin synthesis, with the subsequent formation of free radical by-products, has been shown to damage cerebral vessels and produce histologic changes similar to those seen in chronic vasospasm. In addition, it has been suggested that cerebral vasospasm may reflect an imbalance between constrictor and dilator prostaglandins, with the former dominating in the vasospastic state.

To determine if cerebrospinal fluid (CSF) levels of prostaglandins might be involved in the pathogenesis of cerebral vasospasm, we measured prostaglandin levels in the CSF of dogs with experimental chronic cerebral vasospasm to see if 1) prostaglandin levels in the CSF change following subarachnoid hemorrhage, if 2) these changes are correlated with the degree of angiographic vasospasm, and if 3) ibuprofen and high-dose methylprednisolone affect the prostaglandin profiles.

Materials and Methods

A double-hemorrhage canine model was used, not only because it reliably produces chronic cerebral vasospasm, but also because it exhibits the pathologic and intractable pharmacologic features observed in human chronic cerebral vasospasm. Healthy adult mongrel dogs weighing 13–32 kg were anesthetized with 30 mg/kg i.v. sodium pentobarbital, intubated, and allowed to respire spontaneously. With the aid of fluoroscopic guidance,
transfemoral catheter was placed into the proximal vertebral artery. An initial vertebrobasilar angiogram was then taken. All angiograms were performed at identical magnifications and Renografin-76 (New Brunswick, New Jersey) injection parameters.

The dogs were allowed to recover and were evaluated by neurologic examination. On Day 0, those dogs that remained neurologically normal were anesthetized again. The cisterna magna was aseptically punctured with a No. 22 spinal needle, and 4 ml CSF was removed, centrifuged at 2,000g for 10 minutes, immediately frozen at —60°C, and stored for later prostaglandin assay of baseline concentration. With the dog in the 30° head-down position, 4 ml autologous venous blood was injected through the spinal needle over 2 minutes. After 30 minutes in the head-down position, the dog was returned to its cage. All dogs were evaluated daily for signs of meningeal irritation and neurologic abnormality. On Day 2, the dogs were anesthetized again, the cisterna magna was punctured, and 4 ml CSF was withdrawn, centrifuged, and frozen for later assay. Again, 4 ml autologous venous blood was introduced into the cisterna magna as on Day 0. On Day 8, a final transfemoral vertebrobasilar angiogram was taken after appropriate anesthesia. Following angiography, CSF was taken again from the cisterna magna, centrifuged, and frozen for later assay.

The dogs were divided into three groups: Group 1 dogs (n=9) received no drug treatment, Group 2 dogs (n=8) received 12.5 mg/kg i.v. ibuprofen 1 hour after the first injection of blood and every 8 hours during the study, and Group 3 dogs (n=8) received 30 mg/kg i.v. methylprednisolone (Solu-medrol, The Upjohn Co., Kalamazoo, Michigan) 1 hour after the first injection of blood and every 8 hours during the study.

Initial and final angiograms were analyzed as described elsewhere using a computer-controlled, operator-interactive, rapid videotaping system. Relative basilar artery cross-sectional areas were measured for each angiogram.

CSF samples taken on Days 0, 2, and 8 for all dogs were assayed for prostaglandin F\textsubscript{2a} (PGF\textsubscript{2a}), for prostaglandin E\textsubscript{2} (PGE\textsubscript{2}), for 6-ketoprostaglandin F\textsubscript{1a} (6-keto-PGF\textsubscript{1a}), a stable metabolite of prostacyclin, and for thromboxane B\textsubscript{2} (TxB\textsubscript{2}), a metabolite of thromboxane A\textsubscript{2}.

For the extraction procedure, CSF samples were thawed in cold tap water, their total volumes were measured, and the pH was brought to 3.0–3.4 with 1 M citric acid; 100–200-μl aliquots of [\textsuperscript{3}H]prostaglandin (12,000 cpm/ml) were added to each CSF sample to monitor individual recovery. Three prostaglandin standards (0.5, 1.0, and 2.0 ng/ml) were extracted separately to monitor extraction recovery. Chloroform-methanol (2:1, 6 ml) extraction was performed twice, and the organic phases were pooled in siliconized scintillation vials containing 0.1 ml of 1 M phosphate-buffered saline (PBS) at pH 7.4. The solvent was evaporated to dryness under nitrogen in a water bath at 30°C. The CSF samples were sealed under nitrogen and frozen at —20°C until radioimmunoassay.

For the radioimmunoassay procedure, the desicated CSF samples were diluted with distilled water until isotonic with the standard solutions. For each radioimmunoassay, all standards were freshly prepared from stock solutions by dilution with 0.1 M PBS at pH 7.4.

CSF samples and standards were assayed in duplicate. One hundred microliters of each [\textsuperscript{3}H]prostaglandin and antisera were added to a siliconized borosilicate tube, vortexed, and incubated at 4°C for 2 hours (PGE\textsubscript{2} and TxB\textsubscript{2}) or 18 hours (PGF\textsubscript{2a}, and 6-keto-PGF\textsubscript{1a}). Then a 1 ml solution of dextran (T-70)-coated charcoal (Norit A, Detroit, Michigan) (25 mg dextran/250 mg charcoal in 100 ml 0.1 M PBS for PGE\textsubscript{2} and 6-keto-PGF\textsubscript{1a}, 25 mg dextran/250 mg charcoal in 100 ml 0.05 M Tris for PGF\textsubscript{2a}, and 62.5 mg dextran/625 mg charcoal in 100 ml 0.1 M PBS for TxB\textsubscript{2}) was added to each tube. The tubes were left on ice for 10 minutes, centrifuged at 2,000g and 4°C for 15 minutes, and the supernatants were decanted into scintillation vials. Ten milliliters scintillation fluid was added, and the vials were counted using a Searle Mark II scintillation counter (Chicago, Illinois).

The logarithm of each prostaglandin level was used for statistical analysis to stabilize variance. The values of any particular prostaglandin on Days 0, 2, and 8 define a response profile for that prostaglandin in each dog. These response profiles were characterized by their mean and by their linear and quadratic trends. The trends for each prostaglandin were then simultaneously compared among the three groups using multivariate analysis of variance, which assessed treatment effects on the temporal response of each prostaglandin separately. An alternative approach, designed to assess group differences by exploiting the possible relations between the prostaglandins, considered all four prostaglandins simultaneously on each day.

Using the latter, time-independent approach, a canonical correlation analysis was performed separately for each prostaglandin to determine if their levels were associated with the degree of vasospasm induced. This analysis is a generalization of the usual correlation analysis of a pair of variables to a correlation analysis of two sets of variables (prostaglandin vs. initial and final cross-sectional areas). This analysis summarizes the (linear) associations between prostaglandins and the degree of vasospasm induced in terms of correlations between pairs of linear combinations of prostaglandin concentrations and cross-sectional areas, respectively. The linear combination determined in this method has the maximum correlation among all possible linear combinations of the variables in each set.
Results

As previously reported,1 diffuse, severe spasm of the intradural vertebral arteries and the basilar arteries developed in all Group 1 dogs; the final average basilar artery cross-sectional area was significantly reduced to only 33.7% of the initial area. Without exception, both ibuprofen and high-dose methylprednisolone prevented or markedly reduced the severity of chronic vasospasm. Final average cross-sectional areas in Group 2 and 3 dogs were 98.0% and 85.2% of the initial areas, respectively, a significant improvement over Group 1 as judged by Student’s t test.

The baseline prostaglandin levels for each group are shown in Figure 1, left. Multivariate analysis of variance indicated that baseline levels were significantly different among groups, but this difference was essentially due to TxB2 concentration, which was greater in Group 1. On Day 2, the groups were still significantly different (p<0.002), with PGE2 and 6-keto-PGF1α indicating the greatest differences (Figure 1, right). By Day 8, the groups were even more significantly different (p<0.0005), though differences in PGF2α were the smallest (Figure 1, bottom).

Analysis of the response profiles over time indicated significant differences among groups for PGE2 and 6-keto-PGF1α (p<0.05). No differences in the response profiles for PGF2β or TxB2 were detected.

In Group 1 dogs, CSF PGE2 levels rose dramatically following experimental subarachnoid hemorrhage and were even higher on Day 8 (90, 235, and 440 pg/ml for baseline, Day 2, and Day 8, respectively). Lesser increases in the concentrations of 6-keto-PGF1α (180, 1130, and 1845 pg/ml, respectively) and TxB2 (405, 560, and 2440 pg/ml, respectively) were found. PGF2β levels were only modestly altered (90, 235, and 440 pg/ml, respectively) (Figure 1). Treatment with either ibuprofen or high-dose methylprednisolone significantly changed the temporal response profiles of PGE2 but not PGF2β after subarachnoid hemorrhage compared with untreated dogs (Figure 2, top left and bottom left). Treatment with ibuprofen, and to a lesser degree treatment with high-dose methylprednisolone, prevented the marked elevation of PGE2 levels (Figures 1 and 2, top left). Treatment with ibuprofen and high-dose methylprednisolone also attenuated the increase in 6-keto-PGF1α and TxB2 levels. No significant change was noted in the PGF2β response profile when Groups 2 and 3 were compared with Group 1 (Figures 1 and 2, bottom left).

The canonical correlation analysis of the Day 8 data from all groups indicated a significant (canonical) correlation between prostaglandin values and cross-sectional areas, both initial and final (p<0.05). Figure 3 is a plot of the first linear combination of cross-sectional areas, essentially the degree of vasospasm induced (0.93×initial cross-sectional area−1.03×final cross-sectional area). The concentration of PGE2 in the CSF was strongly and positively correlated with the degree of vasospasm (i.e., dogs with higher concentrations of PGE2 had worse vasospasm). Concentrations of both 6-keto-PGF1α and TxB2 were less closely related to the degree of vasospasm, and in a negative fashion (i.e., increasing concentrations were associated with lessened vasospasm). Finally, PGF2β had a weak correlation, indicating that it was not associated with the degree of vasospasm.

Discussion

Efforts to relate changes in central nervous system prostaglandin levels to chronic cerebral vasospasm...
spasm following subarachnoid hemorrhage remain speculative despite a decade of research. The discovery in our laboratory that two drugs that inhibit prostaglandin synthesis, ibuprofen and high-dose methylprednisolone, can prevent or markedly reduce experimental cerebral vasospasm in dogs prompted us to reexamine this relation.

In 1975, Pickard et al. studied the site of central nervous system prostaglandin synthesis and concluded that cerebral arteries can produce large quantities of prostaglandins. More recent evidence suggests that under normal conditions cerebral blood vessels are an important source of intracranial prostaglandins, with the exception of thromboxane. Prostaglandin synthesis in cerebral vessels appears to be oriented toward prostacyclin production, while that in nonvascular intracranial tissue tends to be oriented toward the synthesis of other eicosanoids.

**Figure 2.** Response profile of prostaglandins in cisternal spinal fluid in dogs with experimental subarachnoid hemorrhage. Top left: Prostaglandin $E_2$. Top right: 6-Ketoprostaglandin $F_{1\alpha}$. Bottom left: Prostaglandin $F_{2\alpha}$. Bottom right: Thromboxane $B_2$.

**Figure 3.** Canonical correlation showing relation between angiographic vasospasm and cisternal spinal fluid prostaglandin levels in dogs with experimental subarachnoid hemorrhage. $PGE_2$, prostaglandin $E_2$; 6-KETO-$F_{1\alpha}$, 6-ketoprostaglandin $F_{1\alpha}$; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; $TxB_2$, thromboxane $B_2$.

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Prost_1 = 1.36 \text{ (log } PGE_2) - 0.41 \text{ (log } 6\text{-KETO}-F_{1\alpha}) + 0.52 \text{ (log } PGF_{2\alpha}) - 0.43 \text{ (TxB}_2)\]

Area 1 = 0.93 (initial) - 1.03 (final)
depending on species.12,19 Following synthesis, stable prostaglandins are rapidly cleared from the cerebral extracellular space by facilitated transport rather than by metabolic degradation. Transport from the brain to the blood stream occurs so rapidly that lumbar CSF levels bear little relation to bulk cisternal levels.20

Maeda et al21 reported that dog basilar arteries increase the rate of PGE2 synthesis following an intracisternal injection of blood. In our study, the 94-fold increase in PGE2 and the smaller increases in 6-keto-PGF1α and TxB2 levels measured on Day 8 after the initial intracisternal injection of blood must reflect, at least in part, enhanced prostaglandin synthesis since ibuprofen and high-dose methylprednisolone, which inhibit prostaglandin synthesis, markedly attenuated this posthemorrhagic rise. Whether subarachnoid hemorrhage also alters the clearance of prostaglandins from the CSF is not known. Thus, the role, if any, that an altered clearance rate played in determining the prostaglandin levels found in this study is uncertain.

A number of authors have suggested that chronic cerebral vasospasm after subarachnoid hemorrhage represents an imbalance between vasoconstrictor and vasodilator prostaglandins, with the former predominating during vasospasm.7,9,12 In my study, however, the level of PGE2, a classical cerebrovascular contractile agonist, did not increase after subarachnoid hemorrhage. Furthermore, TxB2, the stable metabolite of another contractile agent, although elevated in untreated compared with treated dogs, was found to be negatively correlated with the degree of angiographic vasospasm. Conversely, Boullin et al10 and later Maeda et al21 suggested that decreased synthesis of prostacyclin, a vasodilator, after subarachnoid hemorrhage causes vasospasm. My data do not support this concept since the concentration of 6-keto-PGF1α, the stable metabolite of prostacyclin, increased rather than fell in untreated vasospastic dogs. In addition, dogs with minimal angiographic vasospasm because of treatment with ibuprofen or high-dose methylprednisolone had lower levels of 6-keto-PGF1α than untreated vasospastic controls.

My study draws attention to the relation between PGE2 and posthemorrhagic vasospasm. Cisternal PGE2 levels rose precipitously following experimental subarachnoid hemorrhage and were correlated closely with the degree of angiographic vasospasm. Ibuprofen and high-dose methylprednisolone, which prevented or markedly reduced vasospasm, also reduced PGE2 levels. PGE2 is synthesized from arachidonic acid via the intermediates PGG2 and PGH2. Evidence indicates that when PGD2 is converted to PGH2, free oxygen radicals are released.5 Cerebral vasospasm could occur then, at least in part, because a flood of free radical species released as by-products of accelerated prostaglandin synthesis damage cerebral vessels13 after subarachnoid hemorrhage. Evidence from our laboratory1 and elsewhere14,15 suggests that chronic “vasospasm” is a vasculopathy characterized by structural narrowing of the arterial lumen, rather than a prolonged state of contraction by a relatively normal artery. Consequently, it is not likely that chronic vasoconstriction induced by PGE2 explains chronic vasospasm. However, whether accelerated PGE2 synthesis is causally related to vasospasm or is in fact a sequela of vasospasm needs to be resolved by future experimentation.

My study provides further evidence that ibuprofen and high-dose methylprednisolone may be useful in the management of cerebral vasospasm after subarachnoid hemorrhage. Both drugs must reach therapeutic levels in the pharmacological compartment(s) responsible for prostaglandin synthesis since the CSF of treated dogs had lower levels of PGE2, 6-keto-PGF1α, and TxB2 than that of untreated dogs. The finding that lower levels of prostaglandins were correlated with the prevention or reduction of angiographic vasospasm suggests that chronic cerebral vasospasm may be related to elevated prostaglandin levels in the CSF and that the efficacy of ibuprofen and high-dose methylprednisolone to reduce vasospasm may depend on their ability to inhibit prostaglandin synthesis.

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