Effect of nonsteroid anti-inflammatory drugs on subarachnoid hemorrhage in dogs

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The effects of two long-acting anti-inflammatory agents on behavioral changes and cerebral vasospasm were evaluated in a canine model of chronic subarachnoid hemorrhage (SAH). The agent with the longest half-life, sudoxicam, clearly reduced both the incidence and the magnitude of the vasospasm, and prevented the usual behavior changes caused by the simulated SAH. The results obtained with the other agent, naproxen, suggested that it was better than the administration of saline. These agents were studied because of reports indicating that prostaglandins and thromboxane may play a role in the pathogenesis of the effects of SAH and because the nonsteroid anti-inflammatory agents exert pharmacological effects by reducing an excessive synthesis of these lipids. The findings suggest that some of these agents may afford an alternative treatment for the deleterious consequences of SAH.

Key Words: prostaglandin synthetase inhibitors · cerebral vasospasm · subarachnoid hemorrhage

Numerous studies suggest that prostaglandins could be responsible for the symptoms associated with subarachnoid hemorrhage (SAH). These substances produce experimental cerebral vasospasm in vivo, in situ, and in vitro. They are synthesized by blood, brain, and cerebral arteries, and the level of prostaglandins present in cerebrospinal fluid (CSF) can rise markedly with cerebrovascular and neurological disease. They also produce fever, edema, and stupor, and these might be related to similar signs reported in patients with SAH. Moreover, the synthesis of prostaglandins and their release from various tissues is increased by mechanical, neural, and chemical stimulation. In this regard, trauma to the spinal cord will cause approximately a fourfold rise in synthesis which is sustained for several hours in cats. This enhanced synthesis was abolished by indomethacin. Also, ischemia elevates synthesis tenfold in brain, and ischemic damage to myocardium is antagonized by inhibitors of such synthesis. Such results suggest that the inhibition of prostaglandin synthesis might prevent or negate various signs of SAH if these lipids are responsible for the pathogenesis of this condition.

The nonsteroid anti-inflammatory agents are known to inhibit the biosynthesis of prostaglandins, and this effect appears to be responsible for the pharmacological and clinical actions of this group of compounds. Previous studies have indicated, however, that the prostaglandin synthetase inhibitor, indomethacin, would not prevent an experimentally induced cerebral vasospasm in dogs. Nevertheless, this finding was obtained from acute experiments, and indomethacin does not uniformly affect all prostaglandin synthetase activity, and has a very short half-life in dogs. Consequently, the present study was undertaken to determine whether other nonsteroid anti-inflammatory agents with different profiles of synthetase inhibition and a longer half-life might ameliorate the signs associated with a chronic experimental model of SAH.

Materials and Methods

Mongrel dogs of either sex, weighing from 17 to 26 kg, that had been under the care of a veterinarian for at least 21 days were used for this study. The animals were housed in the same facility for at least 1 week before experimentation, and returned afterward to the same kennel until sacrificed. Surgery, arteriography, and the experimental SAH were performed under pentobarbital sodium anesthesia using procedures already described by others. The SAH was produced as follows: The animal was placed prone and a No. 22 spinal needle inserted through the atlanto-occipital
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TABLE 1

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Blood Injected (ml)</th>
<th>No. of Dogs</th>
<th>Day 0</th>
<th>Day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. Dogs with Spasm*</td>
<td>Average Constriction†</td>
</tr>
<tr>
<td>Group I</td>
<td>naproxen, 20 mg/kg</td>
<td>4</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>saline, 2 ml/kg</td>
<td>4</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p &lt; 0.1)</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>sudoxicam, 2 mg/kg</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>saline, 1 ml/kg</td>
<td>4</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p &lt; 0.05)</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>sudoxicam, 2 mg/kg</td>
<td>2</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>saline, 1 ml/kg</td>
<td>2</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p &lt; 0.1)</td>
<td></td>
</tr>
<tr>
<td>Group IV§</td>
<td>sudoxicam, 2 mg/kg</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>saline, 1 ml/kg</td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p &gt; 0.5)</td>
<td></td>
</tr>
</tbody>
</table>

*P values shown below the incidence of spasm compare drug with saline control and were obtained by Chi-square analysis.
†P values indicate significant difference.
‡Average constriction is expressed as percentage of control.
§Treatment was initiated 1 hour after the intracisternal blood injection and after the Day 0 x-ray films were obtained. In all other groups treatment was given 1 hour before the intracisternal injection of blood (see text for details).

membrane into the cisterna magna with the bevel directed rostrally. Through this needle, 2 ml of clear CSF was withdrawn and discarded, after which 2 ml or 4 ml of autogenous arterial blood was injected at a rate of 0.4 ml and 0.8 ml, respectively, per minute. The needle was then withdrawn and the table tilted 15° so that the head of the animal was positioned downward for 10 minutes.25 Arteriograms of the basilar artery were obtained by inserting a catheter cephalad into the right vertebral artery near its origin a distance of about 16 cm. Hypaque (meglumine diatrizoate, 4 ml) was injected into this cannula and its passage through cerebral arteries recorded on x-ray film to document the presence of cerebral vasospasm. Measurements were taken of the basilar artery from these x-ray films at a point 1 cm from the posterior aspect of the circle of Willis by means of a comparator.* A reduction in caliber of this artery of 10% or more was considered to be evidence of vasospasm, because this magnitude of change is generally accepted as exceeding normal biological variation, and because such changes do not occur with the procedures used in this study in the absence of subarachnoid blood or other biologically active material.20,28,47,48 In each animal the right femoral artery was cannulated so that blood pressure could be recorded by means of a Statham P23AA transducer and a Grass Polygraph,† and the rectal temperature was recorded throughout anesthesia.

For analysis of the data, Day 0 was designated as the day in which the blood was injected and Day 1 as the period 24 hours later, and so forth. After experimentation on Day 0, the vertebral cannula was filled with a 5% heparin solution and secured subcutaneously, the femoral cannula removed, all surgical wounds closed and the animal returned to the kennel for further observation.

Sudoxicam§ and naproxen‡ were selected for study because these have long half-lives of 60 and 35 hours, respectively, in dogs. These drugs were dissolved in distilled water by the addition of 1 N NaOH and back titration with 0.1 N HCl until the final pH of the solution was 7.4. The final concentration of sudoxicam used was 2 mg/ml, and for naproxen 10 mg/ml. The dose administered intravenously was 2 mg/kg for sudoxicam, and 20 mg/kg for naproxen. Both of these doses are more than adequate to have an anti-inflammatory effect in dogs; the latter was 10 times higher than the former, based on pharmacodynamic reports in humans.27,51

The experimental design required four groups of animals treated with prostaglandin synthetase inhibitors intravenously and four corresponding groups given saline for control purposes (Tables 1 and 2).

*Comparator manufactured by Edmund Scientific Company, Barrington, New Jersey.
†Statham P23AA transducer manufactured by Statham Instruments, 2230 Statham Boulevard, Oxnard, California. Grass polygraph made by Grass Instrument Company, 101 Old Colony Avenue, Quincy, Massachusetts.
TABLE 2
Effects of nonsteroid anti-inflammatory agents on behavior observed 24 hours after the intracisternal injection of blood

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Blood Injected (ml)</th>
<th>No. of Dogs</th>
<th>Behavior*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Change in Demeanor</td>
</tr>
<tr>
<td>Group I</td>
<td>naproxen, 20 mg/kg</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>saline, 2 ml/kg</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Group II</td>
<td>sudoxicam, 2 mg/kg</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>saline, 1 ml/kg</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Group III</td>
<td>sudoxicam, 2 mg/kg</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>saline, 1 ml/kg</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Group IV†</td>
<td>sudoxicam, 2 mg/kg</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>saline, 1 ml/kg</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*Number of dogs changed from control. P values shown compare the incidence of occurrence in the drug-treated animals with the saline-treated by Chi-square analysis.
†P values indicate significant difference.
‡Treatment was initiated 1 hour after the intracisternal blood injection. In all other groups treatment was given 1 hour before the intracisternal injection of blood.

saline and drugs were routinely given alternately to minimize the influence of unknown factors in the results. On Day 0, arteriograms were obtained before and 30 minutes after the infusion of the drugs (or equal volumes of saline) in most groups of animals. One hour after the administration of drugs, either 2 or 4 ml of blood was injected intracisternally, and arteriograms were taken at 15, 30, and 60 minutes thereafter. In one special group, sudoxicam was administered 1 hour after the intrathecal blood. In these, scout arteriograms were first obtained, the blood injected, and then arteriograms made at 15, 30, and 60 minutes after this injection. Immediately after the last x-ray film and 60 minutes after the injection of blood, sudoxicam was given intravenously. Blood pressure and rectal temperature were recorded throughout these procedures as described above.

All animals were permitted to recover from the anesthesia and were studied the following day (Day 1) for behavioral and other changes. On Day 1, observations of the blood pressure, rectal temperature, and arteriograms were repeated, the latter taken 15, 30, and 60 minutes after anesthesia. The behavioral condition of each animal was noted before and after the experimental procedures performed on Day 0. To simplify the analysis of the behavioral changes observed, these were subdivided into three categories as shown in Table 2. One of these, whether the animal ate on the morning of Day 1, proved to be an important index of general recovery. A second was the notation of neurological deficits, such as paresis, prostration, nystagmus, slow or staggering gait. The third was a behavioral profile in which the animals were observed for their friendliness to the investigators (whether they sought attention and wagged their tails) or unfriendliness (whether they tried to avoid handling), and alertness (their response to noise and movement of the investigator). If there were no obvious changes in these categories, the animal was deemed normal. Most of the animals were friendly in nature because they were first selected by the veterinarian staff for quarantine. These three categories proved useful because, as seen in the Results section, many animals might have deficiencies in only one category (for instance, they might have neurological deficits but eat, or vice versa).

The brains were removed after sacrifice and the distribution of the subarachnoid blood recorded. This blood was always present on the dorsal and ventral surface of the medulla, and the ventral aspects of the pons and midbrain. In about 40% of the animals some blood was present on the ventral surface of the hypothalamus and cerebrum. It was never present in the ventricles. The procedures used in these experiments (such as vertebral arteriography), in the absence of subarachnoid blood, do not noticeably affect behavior, vital signs, and cerebral arterial caliber in the dog.
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FIG. 1. Representative arteriograms of the basilar artery to illustrate the effect of saline in one animal (A, upper panels) on the vasospasm induced experimentally, as compared to sudoxicam treatment of another (B, lower panels). Sudoxicam and saline, respectively, were given intravenously immediately after the x-ray film was taken, 1 hour after the simulated subarachnoid hemorrhage (center panels). Note that the animal given sudoxicam did not manifest cerebral vasospasm the following day (B, right), whereas vasospasm persisted in the saline-treated animal (A, right). The animal treated with sudoxicam was normal in behavior, while the untreated one was paretic and failed to eat the following day: a common but not an inevitable finding (see Tables 1 and 2).

Results

It is evident in Table 1 that nearly all of the animals given saline manifested cerebral vasospasm as a result of the intracisternal administration of blood and that the drugs reduced the incidence of this vasospasm. Although this reduction was not statistically significant in all groups studied, the trend is present in each and especially on Day 1 in the animals given sudoxicam. This drug had an effect against the 4-ml as well as the 2-ml quantity of intrathecal blood, and appeared to have an inhibitory effect even when given 1 hour after the blood (Group IV). More significantly, sudoxicam protected the animals from behavioral changes produced by the intracisternal blood (Table 2). This was particularly evident in their eating behavior in that a statistically significant number of animals given sudoxicam ate 24 hours after the injection of blood in comparison with the control groups.

The overall effectiveness of sudoxicam is more evident in Table 3. This table compares the effects of saline, naproxen, and sudoxicam on the incidence of vasospasm and abnormal behavior observed 24 hours after the blood injection and treatments. Among these, the group given sudoxicam showed about a 43% less chance of exhibiting vasospasm, and was approximately 75% less likely to manifest abnormal behavior than those given saline. These differences

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Dogs</th>
<th>Dogs with Spasm (%)</th>
<th>Abnormal Behavior (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>43</td>
<td>97</td>
<td>46</td>
</tr>
<tr>
<td>naproxen</td>
<td>18</td>
<td>83</td>
<td>33</td>
</tr>
<tr>
<td>sudoxicam</td>
<td>27</td>
<td>55</td>
<td>11</td>
</tr>
</tbody>
</table>

TABLE 3
Summary of effects of naproxen, sudoxicam, and saline treatment on cerebral vasospasm and behavior observed 24 hours after intracisternal blood injection
were very significant statistically by Chi-square analysis (p < 0.001). Moreover, the vasospasm was less on Day 1 in animals treated with sudoxicam (Table 1 and Fig. 1). Naproxen seemed to have an effect also, although it was less evident (Table 3). It is also evident that the experimental procedures used are more likely to induce cerebral vasospasm than significant changes in behavior regardless of the treatment (Table 3). However, there was no absolute correlation between the degree of vasospasm and behavioral deficits (Tables 1 and 2).

Every animal that manifested notable change in behavior exhibited cerebral vasospasm, although there was no obvious correlation between the degree of spasm and the severity of the behavioral change. In this regard, two animals that might be described as “stroked out” had constrictions of 34% and 42%, while some animals that showed mild paresis, unsteady gait, or failed to eat, and some among the “normals,” exhibited comparable degrees of vasospasm. Seven animals did not survive through Day 0; three of these had received saline, two naproxen, and two sudoxicam.

There were no statistically significant changes in body temperature and blood pressure for Day 0 and Day 1 in any of the groups studied. The average temperatures for each day, respectively, for saline were 38.52°C and 38.91°C, for naproxen 38.69°C and 38.72°C, and for sudoxicam 38.83°C and 38.43°C. The average blood pressure recorded on these days for the saline group was 158.8 and 155.4 mm Hg, for naproxen 168.3 and 163.0 mm Hg, and for sudoxicam 142.1 and 153.1 mm Hg, and for sudoxicam 168.3 and 163.0 mm Hg. The body temperatures were recorded immediately after anesthesia and the blood pressure 1 hour after anesthesia. The latter recording was commonly lower on Day 1 than on Day 0 immediately following anesthesia, but soon rose and became stable within the hour. In this regard, the anesthetic may have masked or altered physiological changes that otherwise occur as the result of injecting blood intracisternally. If so, its effect on body temperature and blood pressure was similar in all of the groups of animals studied.

**Discussion**

The pathogenesis of cerebral vasospasm and the behavioral changes observed in this study are apparently complex. Several hypotheses have been proposed to account for these phenomena, although most deal specifically with the nature of vasospasm. Most studies concerning vasospasm have been in acute experiments, and the chronic studies performed were not designed to quantify behavioral changes associated with the vasospasm. Nevertheless, the results indicate that the magnitude of the responses observed are highly variable. The degree of the vasospasm, for instance, may vary more than twofold, and comments on behavioral changes are reported to correlate with this vasospasm. In our studies, the behavioral consequences caused by subarachnoid blood are quite variable, as summarized in Table 2, when only saline is used to treat the animals. Moreover, the present study, and others, clearly show that vasospasm is more easily established than are neurological or behavioral deficits. This suggests that vasospasm of the conducting arteries is only one pathological consequence of SAH, and that unless the parenchyma is affected behavioral changes may not be evident. The latter might be caused by a critically reduced blood flow in the conducting arteries, a vasoconstriction of the arterioles caused by spasmogens reaching the parenchymal vessels, or by edema due to substances diffusing to the parenchyma via the perivascular spaces. At least 25 substances are likely to be present or synthesized in varying amounts by the tissues associated with an SAH that could be deleterious in this condition. We tested prostaglandin synthetase inhibitors in an experimental model because of a number of previous reports suggesting that prostaglandins contribute significantly to the effects of an SAH. It is known that cerebral arteries synthesize prostaglandins, and these lipids will cause a prolonged cerebral vasospasm when given intracisternally. Prostaglandins are also produced by the brain and by coagulated blood or activated platelets. Hence, all of the tissues associated with an SAH synthesize prostaglandins, and the clotted blood juxtapose these as well as impede the normal flow of CSF. Normally the CSF contains little or no prostaglandins, as these are rapidly removed from this fluid, but trauma or ischemia to the central nervous system greatly increases synthesis in the parenchyma and the quantity present in spinal CSF is commonly high in neurological disorders and SAH.

Indeed, any trauma to normal tissue apparently stimulates synthesis. In addition, a large variety of compounds that are present in blood are already known to stimulate the formation of prostaglandins. These include, among others, norepinephrine, dopamine, hemoglobin, methemoglobin, hemat in platelets, and dopamine, norepinephrine, adrenochrome, and serotonin with brain. Much evidence indicates that a number of substances cause the physiological release of prostaglandins. It is possible, therefore, that the pathogenesis of SAH is initiated by such compounds, and that prostaglandins mediate or contribute to the symptoms. If so, then a prostaglandin synthetase inhibitor with a suitable pharmacological profile and distribution should ameliorate these symptoms. The present study indicates that this is possible.

Sudoxicam is similar in pharmacological actions to indomethacin: both drugs reduce edema, inflammation, pain, and fever in experimental animals. Naproxen has a similar profile of activity. Hypothetically all three drugs, and related ones,
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should be as efficacious in inhibiting the deleterious effects of an SAH as was sudoxicam. Since sudoxicam significantly reduced the incidence of cerebral vasospasm and the behavioral changes produced in an experimental model of SAH, the question arises as to why similar drugs may be less effective. The pharmacokinetics of these compounds differ greatly in laboratory animals and in man,7,25 so that the failure of naproxen or indomethacin to significantly influence the course of an SAH may be due to an inadequate regimen rather than to basic differences in the mechanism of action of the drug studied. In this regard, indomethacin has a half-life of 0.3 hour, naproxen 35 hours, and sudoxicam 60 hours in the dog.31 Moreover, the concentration of such compounds in various tissues may differ fiftyfold,97 and the enzymes that synthesize prostaglandins are different in each tissue and have a different pharmacological profile from those in any other tissue.11 Lastly, the enzymes which catabolize prostaglandins differ in concentration throughout the body and are also susceptible to inhibition by some, but not all, of the nonsteroid anti-inflammatory drugs.11 It is not surprising, therefore, that prostaglandin synthetase inhibitors may be different in effectiveness. Additional studies are necessary to establish unequivocally the mechanism(s) by which sudoxicam suppressed these effects of an experimentally induced SAH.

The fact that not all synthetase inhibitors are equally effective in decreasing synthesis in the same tissue11 raises the question for exploration as to whether such inhibitory compounds given in combination would be more efficacious in suppressing synthesis in the tissues associated with SAH. Even more important may be the combined use with drugs that are usually not considered to be prostaglandin synthetase inhibitors. However, new information suggests that these may also inhibit synthesis or activity of prostaglandins. Among these are dexamethasone,12 reserpine,13 burimamide,2 nicotinic acid,42 and the psychotropic agents.29

One aspect of such treatment that requires additional investigation is the recent report of Moretti and Abraham,31 who found an unknown factor in plasma that antagonizes prostaglandin synthetase inhibitors in a competitive manner. Thus, optimum dosages of prostaglandin synthetase inhibitors would appear important for maximum efficacy in treating SAH.

The lack of correlation between the incidence and severity of vasospasms compared to behavioral changes is not unexpected. Certainly the symptoms in SAH vary greatly in humans8-10,44 and in conscious dogs subjected to an experimental hemorrhage of the anterior cerebral artery.6 Even infarcts produced by the unilateral occlusion of the internal and middle cerebral artery in dogs may vary more than twofold in size.7 Whether behavioral deficits were correlated with the degree of infarcts is unknown, but it is interesting that pentobarbital given intramuscularly markedly reduced cerebral damage. Since all of the animals used in our study were anesthetized with pentobarbital at the time of the SAH, it is possible that this agent contributed to the behavioral recovery observed in the control group of animals. If so, then sudoxicam contributed significantly to this recovery (Table 3). On the other hand, pentobarbital apparently has no direct effect on vasospasm, as it is commonly used in experiments concerned with this phenomenon,3,9,20,28,26,46,50 and did not prevent its occurrence in this study (Table 3). In any case, the present findings demonstrate that a nonsteroid anti-inflammatory agent can materially reduce the incidence of cerebral vasospasm and the behavioral changes produced in an experimental model of SAH. The results also indicate that further study of such agents is warranted as being potentially useful in the treatment of SAH.

Acknowledgments

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