Cyclosporine A Reduces Cerebral Vasospasm After Subarachnoid Hemorrhage in Dogs

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The double subarachnoid hemorrhage canine model was used to test the prophylactic value of immunosuppression in the prevention of cerebral vasospasm after subarachnoid hemorrhage. Dogs treated with cyclosporine A following the regimen prescribed for organ transplant procedures in patients showed a significant reduction in the severity of angiographic constriction of cerebral arteries. While basilar artery diameter after double experimental subarachnoid hemorrhage in a series of untreated dogs (n=34) averaged 65% of baseline diameter, arterial diameter in dogs treated prophylactically (n=18) with 6 mg/kg/day cyclosporine A and adjunct low-dose steroid averaged 80% of baseline diameter, for a mean reduction in the severity of chronic arterial constriction of 42%. More important than the average effect, however, is the statistical observation that this mean improvement was obtained primarily by a dramatic reduction in the incidence of severe cerebral vasospasm, the situation most likely to account for morbidity and mortality after aneurysmal rupture. (Stroke 1990;21:133-137)

The double subarachnoid hemorrhage (SAH) canine model has been accepted by many laboratories as a suitable model of human cerebral vasospasm after SAH in which to test therapeutic or prophylactic interventions. Using this model, we have attempted to determine the extent to which an immunologic1'2 or a rejection reaction against the aging subarachnoid blood clot plays a role in the development of delayed chronic cerebral vasospasm. These studies were prompted by clinical reports 3'4 that concentrations of circulating serum immunocomplexes are increased during cerebral vasospasm and may well be predictive of its occurrence after aneurysmal rupture. A postmortem study has shown increased deposition of immunoglobulin and complement protein in the walls of human cerebral vessels exposed to subarachnoid blood clot.7

We chose to investigate the prophylactic value of cyclosporine A, in particular, since it is a highly effective clinical immunosuppressant (for review see Reference 8) and quite rapid in its action, being administered only 4–12 hours before solid organ transplant.9 This means that any prophylactic action could be evaluated in a context more relevant to the clinical situation, that is, starting some 24–48 hours after initial subarachnoid bleeding. In the interest of possible direct application to the clinical situation, we initiated prophylactic trials in dogs with a constant drug regimen, using dosages suggested for intravenous administration in patients,9 although variable disposition of the drug has been implicated in cases of treatment failure.10,11

Materials and Methods

Dogs weighing 18–26 kg were anesthetized with 15–25 mg/kg i.v. thiamylal sodium, and hydration was maintained with intravenous infusion of lactated Ringer's solution. The dogs were ventilated mechanically to maintain an expired gas Pcc>2 of 38-42 mm Hg (End-TIdL 200, Instrumentation Laboratory, Lexington, Massachusetts). No significant changes in blood cell counts or electrolyte concentrations were noted during the experiment in untreated dogs.

Using aseptic technique, either femoral artery was exposed and a 100-cm 6.5-F Torcon Blue angiographic catheter (Cook Co., Bloomington, Indiana) was advanced under fluoroscopy to the level of C4 in the left vertebral artery; arterial blood pressure was recorded continuously. The dog’s head secured in a stereotactic frame, cerebral angiography was performed by manual injection of 7–9 ml Renografin 76 (E. R. Squibb Co., Princeton, New Jersey) over 2–3 seconds. With the head still elevated, the dog’s nose was held flexed downward and the cisterna magna was punctured by a 19-gauge needle. After withdrawal of 3.0 ml clear cerebrospinal fluid, 3.0 ml
freshly drawn venous blood was injected over 2 minutes. The dog's head was immediately released and the table was tilted 15° head-down for 20 minutes to promote pooling and coagulation of blood in the basal cistern. The catheter was then withdrawn, the femoral artery was ligated, and the incision was sutured. Cerebral angiography was repeated as above 72 hours later in seven untreated and in all cyclosporine A-treated dogs before administration of a second experimental SAH. Final cerebral angiography then was performed 72 hours later. Basilar artery diameter was determined at 1-cm intervals and is expressed as a percentage of the baseline diameter at each of four or five points and averaged over the length of the vessel. Three independent readings were averaged and SEM was calculated for the data presentation.

Treated dogs followed protocols of experimental SAH and cerebral angiography exactly as described above. Eighteen dogs entered an immunosuppressive regimen (excluding four that died; only one death was likely associated with the drug treatment). In 13 dogs, treatment was not begun until 14±4 (mean±SEM) hours before the second experimental SAH. At that time, 6.0±0.5 mg/kg cyclosporine A (Sandimmune i.v., Sandoz Inc., East Hanover, New Jersey) with 0.3±0.05 mg/kg dexamethasone sodium phosphate as adjunct low-dose steroid was infused intravenously over 2 hours in 100 ml lactated Ringer's solution, which often produced vomiting and an apparent taste-aversion reaction to the drug vehicle (polyoxyethylated castor oil), requiring sedation with minimal intravenous thiamylal sodium. The next morning, cerebral angiography was performed and the second experimental SAH was administered. One hour later, a second priming dose of cyclosporine A/steroid was given as above, and in six treated dogs, angiography repeated. No acute change in cerebrovascular dimensions, mean arterial blood pressure, or other systemic parameters were noted in any of the 18 treated dogs.

Because of the long half-life of cyclosporine A in dogs, a single daily intravenous maintenance dose was used. Each morning, 6.0 mg/kg cyclosporine A and 0.15 mg/kg dexamethasone was delivered by intravenous bolus during 5–10 minutes, supplemented by 0.15 mg/kg i.m. dexamethasone each evening. Final angiography was performed 72 hours after the second experimental SAH to assess the efficacy of cyclosporine A prophylaxis against cerebral vasospasm. The prophylactic regimen induced no significant (p>0.05) changes in blood electrolyte concentrations, hematocrit, or cell counts, except that the leukocyte count after 72 hours of cyclosporine A treatment was significantly increased (from 8,200 to 12,800/mm², as determined in seven dogs).

In an attempt to improve prophylaxis, five treated dogs were prepared exactly as above, except that the prophylactic regimen was initiated 2 hours after the first experimental SAH. In another five treated dogs, methylprednisolone at 4.0±0.2 mg/kg/day was substituted for dexamethasone. Since neither change in protocol caused any significant effect, all data for treated dogs has been averaged together.

In five treated dogs, the basilar arteries were excised and portions were mounted in a double-cannulated, flow-through system for in vitro study. Artery segments 1.5 cm long were superfused and perfused intraluminally at 120 mm Hg distending pressure with warmed, gassed physiological saline. Contractile responses were recorded continuously using a television camera and an electronic circuit that calculated average vessel diameter. The membrane potentials of vascular smooth muscle cells were measured by direct cellular puncture using glass microelectrodes (tip diameter approximately 0.3 μm) connected to a Duo 773 electrometer (World Precision Instruments, Inc., New Haven, Connecticut).

Results are reported as mean±SEM. Differences between basilar artery diameters in untreated and cyclosporine A-treated groups were evaluated using Student's t test.

Results
Basilar artery diameters determined 72 hours after the first and second experimental SAHs are shown in Figure 1 for untreated and treated groups. On day 3, basilar artery diameters in the treated and untreated groups did not differ significantly (p=0.40). Since basilar artery diameter on day 3 for the five dogs treated prophylactically from day 0 (85.1±4.8%) is identical to that for the 13 dogs treated only 14 hours before the second SAH (86.6±3.7%), the reported value includes both data sets. Cyclosporine A prophylaxis for 14–72 hours did not alter the severity of the vascular reaction to a single experimental SAH. It is clear from Figure 1, however, that the prophylactic regimen of cyclosporine A significantly reduced the severity of cerebral vasospasm after a second experimental SAH. Basilar artery diameter in the treated group (79.8±3.3%, n=18) differs significantly (p=0.001) from that of the untreated group (65.0±1.9%, n=34). This difference in the prophylactic value of cyclosporine A on days 3 and 6 of the model suggests that different processes dominate the vascular response observed at those times. This is further indicated by the data of Table 1, which considers the progression of vasospasm in response to the second experimental SAH by calculating the pairwise ratio of basilar artery diameters observed on days 6 and 3 for the treated and untreated groups. While basilar artery constriction increased sharply (22%) in the untreated group, only a marginally significant increase in constriction (7%) was found with cyclosporine A prophylaxis, which was therefore approximately 70% effective in inhibiting those processes that increase the vascular reaction after a second experimental SAH.

At 72 hours after the first experimental SAH, both treated and untreated groups show not only the same basilar artery diameter, but also essentially identical frequency distributions of the cerebrovascular reac-
tions. In both groups, the majority of the dogs (approximately 70%) showed very little constriction (average diameter 92% of baseline), while a minority (approximately 30%) showed considerable reaction (average diameter 70% of baseline).

The data for the untreated and treated groups on day 6 are sufficiently large to allow consideration of the distribution of the varying degrees of severity of cerebral vasospasm. By day 6 the double-SAH canine model produces three characteristic degrees of basilar artery constriction in untreated dogs: severe, moderate, and little-to-no reaction (<60%, 60-80%, and >80% of baseline diameter, respectively). Data from treated dogs, when similarly characterized, showed a visible change in distribution relative to the untreated group (Figure 2). The increase in basilar artery diameter with cyclosporine A prophylaxis shown in Figure 1 arises largely from a sharp reduction in the frequency of severe cerebral vasospasm. We cannot determine with this protocol whether the increased occurrence of little-to-no reaction is due to improvement of some dogs with moderate reaction or due to a more dramatic shift of dogs directly from severe to little-or-no reaction, although such a distinction could be of mechanistic importance.

The resting membrane potential in vascular smooth muscle cells was measured in basilar artery segments from five cyclosporine A–treated dogs, as well as in numerous vessels from untreated dogs with experimental SAH and control dogs without SAH (Table 2). Earlier studies have shown that cerebral vessels excised from animals with experimental SAH show substantial depolarization of the membrane potential relative to normal vessels. While cyclosporine A prophylaxis reduced the severity of vasospasm by nearly half, membrane depolarization in the basilar artery

table 1. Decrease in Angiographic Basilar Artery Diameter Due to Second Subarachnoid Hemorrhage in Dogs Treated With Cyclosporine A

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Progression ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>7</td>
<td>0.78±0.04</td>
</tr>
<tr>
<td>Treated</td>
<td>18</td>
<td>0.93±0.03</td>
</tr>
</tbody>
</table>

Data are mean±SEM progression ratio (diameter on day 6+diameter on day 3).


**TABLE 2. Vascular Smooth Muscle Cell Membrane Potential in Basilar Artery of Dogs in Double SAH Model**

<table>
<thead>
<tr>
<th>Group</th>
<th>E&lt;sub&gt;m&lt;/sub&gt;</th>
<th>No. vessels</th>
<th>No. cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-40.6±3.1</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>-36.0±3.9</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>-34.9±3.9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>-32.6±2.5</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>-39.2±3.8</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>-36.7±1.4</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>Untreated</td>
<td>-38.2±0.4</td>
<td>24</td>
<td>166</td>
</tr>
<tr>
<td>Control</td>
<td>-43.0±0.9</td>
<td>32</td>
<td>99</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Treated, cyclosporine plus two subarachnoid hemorrhages (SAHs); Untreated, no cyclosporine plus two SAHs; Control, no cyclosporine and no SAH.

The improvement in basilar artery diameter results primarily from a notable reduction in the frequency of occurrence of severe vasospasm, from approximately one in three untreated dogs to about one in 10 dogs treated with cyclosporine A. These results may be of clinical significance in the treatment of patients with ruptured aneurysms since severe cerebral vasospasm is expected to be the greatest source of morbidity and mortality. We know of no earlier reports evaluating the efficacy of immunosuppressive agents in preventing cerebral vasospasm after SAH (see Reference 15 for review), although it is possible that animal trials with other prophylactic agents may have unknowingly produced some level of immunosuppression (for example, high-dose steroid therapy). Further, there is controversy regarding the possibility that the mechanism of action of cyclosporine A is through its activity as a calmodulin antagonist. In another study in the canine model, however, specific efforts at calmodulin antagonism provided only slight prophylaxis against cerebral vasospasm after SAH, raising the possibility that prophylaxis in that study was achieved by inadvertent and low-level immunosuppression rather than by direct inhibition of calmodulin in the cerebral vessel itself.

It is further interesting to note that cyclosporine A prophylaxis had no significant effect on the small degree of vasospasm developed in response to a single experimental SAH at the time (72 hours) when vascular reaction is maximal. This is highly suggestive that the processes initiated by the second experimental SAH, which lead to severe chronic vasoconstriction and make the double-SAH canine model most applicable to the human situation, may not be the same as those processes involved in the initial reaction. The fact that cyclosporine A is approximately 70% effective in inhibiting those processes suggests an immunologic basis for these second-phase reactions.

Lastly, our in vitro studies of basilar arteries with significantly reduced levels of vasospasm due to cyclosporine A treatment showed that certain properties of the vasospastic arteries frequently hypothe-

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**FIGURE 3. Contractile response (downward deflection, in mm) to application of serotonin (X) shows great difference in sensitivity to exposure to stimulator of endothelium-dependent relaxing factor (o, substance P) between control vessels and untreated vessels excised from dogs subjected to double subarachnoid hemorrhage model. Although cyclosporine A (CsA) prophylaxis reduced severity of cerebral vasospasm, loss of endothelium-dependent relaxation is nonetheless evident. Time bar indicates 15 minutes.
sized to play some role in the basic mechanism of vasospasm (specifically, smooth muscle cell membrane depolarization and impairment of endothelium-dependent relaxing mechanisms), were not significantly different from those in untreated vasospastic arteries. These observations suggest that it is unlikely that such mechanisms play a crucial role in the processes that lead to chronic cerebral arterial constriction after SAH.

References

KEY WORDS • cerebral vasospasm • immunosuppression • cyclosporins • dogs
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