Effect of combined simvastatin and cyclosporine compared with simvastatin alone on cerebral vasospasm after subarachnoid hemorrhage in a canine model


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Object. The object of this study was to determine whether the combination of cyclosporine and simvastatin could ameliorate cerebral vasospasm after subarachnoid hemorrhage (SAH) in a canine model to a greater extent than simvastatin alone.

Methods. Animals were assigned to one of three groups: control (five dogs), simvastatin alone (four), or simvastatin and cyclosporine (four). A double SAH model was used. Baseline basilar artery (BA) angiograms were obtained. These were repeated at Days 3, 7, and 10. Measurement of the BA diameter was performed.

Decreased BA diameter was seen on Day 3 in the control and simvastatin/cyclosporine group. A return to baseline diameters was seen by Day 7. An increase from baseline diameter was seen in the simvastatin group at Day 10.

Conclusions. Cyclosporine may interfere with the vasodilatory effects of simvastatin. Vasodilation greater than baseline is seen at Day 10 in the simvastatin group. The combination of simvastatin and cyclosporine does not ameliorate cerebral vasospasm in a canine model to a greater extent than simvastatin alone.

KEY WORDS • cerebral vasospasm • subarachnoid hemorrhage • cyclosporine • simvastatin • dog

Two salient points emerge from the literature about cerebral vasospasm. The first is that SAH has been associated with histopathological damage to cerebrovascular endothelium.6,9,16 This results in impaired endothelium-dependent relaxation responses mediated by NO.6,15,19,20,40 Simvastatin upregulates NOS and has been shown to ameliorate cerebral vasospasm.24 The second point is that the breakdown of blood products leads to an intense inflammatory response that parallels the time course for cerebral vasospasm.8,32

Some studies have shown that immunosuppression with cyclosporin A ameliorates cerebral vasospasm.8,33 To our knowledge, no study has been conducted to evaluate amelioration of cerebral vasospasm during simultaneous upregulation of NO and immunosuppression. In this study, we tested the hypothesis that simultaneously upregulating NOS with simvastatin after SAH and suppressing the inflammatory reaction with cyclosporin A would ameliorate cerebral vasospasm to a greater extent than doing either one alone.

Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee at the University of Missouri. This study was conducted in collaboration with the University of Missouri College of Veterinary Medicine. Thirteen purpose-bred, intact female mongrel dogs 7 to 18 months of age, each weighing approximately 20 kg, were purchased from a laboratory animal facility (Marshall BioResources, North Rose, NY). All dogs had normal results on physical and neurological examinations, complete blood count, serum biochemistry analysis, urinalysis, and buccal mucosal bleeding times. All dogs were acclimated before the procedures for a minimum of 14 days in the controlled environment of an isolation facility.

Animal Groups

The dogs were randomly preassigned to one of three treatment protocols: Group 1 (control, five dogs) received no drug treatment; those in Group 2 (four dogs) were treated with cyclosporine (6 mg/kg/day) and simvastatin (20 mg/kg/day); and Group 3 (four dogs) received simvastatin (20 mg/kg/day) only. Drugs were administered orally for 10 days beginning 6 hours after the second autologous blood injection into the cerebellomedullary cistern. Angiograms were obtained on Days 1, 3, 7, and 10.
to evaluate chronological changes in the BA diameter. On the 10th day after the second injection, each dog was killed.

Animal Preparation Before Procedures

The dogs were not fed for 12 hours before each procedure. They were premedicated with 10 μg/kg of medetomidine and 0.01 mg/kg buprenorphine by intramuscular injection. Anesthesia was induced with propofol and the trachea was intubated. Anesthesia was maintained with 1.75 to 2.5% isoflurane mixed with 100% O2. Cardiac and respiratory rates, saturated hemoglobin, and end-tidal CO2 were regularly monitored. Respiration was assisted to maintain end-tidal CO2 between 40 and 50 mm Hg. Direct mean arterial blood pressure was monitored before and after each angiography procedure with the aid of a No. 5 French catheter inserted into the FA. Lactated Ringer solution (10 ml/kg/hr) was administered intravenously for the duration of each procedure. Normothermia was maintained using a circulating warm water blanket placed beneath each dog.

Autologous Blood Injection Into the Cerebellomedullary Cistern

The previously described double SAH model was used.33 The SAH was induced by an autologous blood injection into the cerebellomedullary cistern. The dog was placed in the right lateral recumbent position. A 22-gauge spinal needle was inserted into the cerebellomedullary cistern according to previously described landmarks. Access to the subarachnoid space was verified by observing the flow of CSF. Approximately 5 ml of CSF was removed by gravity flow, then 3 ml of blood was collected by venipuncture of the lateral saphenous vein and was immediately injected through the spinal needle into the cerebellomedullary cistern. The head of the dog was lowered below the table for 5 minutes to permit pooling and clotting of the blood around the BA. The procedure was repeated 24 hours later.

Angiography Procedure

An angiographic catheter (No. 5 French catheter) was inserted into the FA and advanced under fluoroscopic guidance into the left VA up to the level of the fourth cervical vertebra. A left VA angiogram was performed. All angiograms were obtained during manual injection of 10 ml of iopamidol contrast agent. Identical injection/exposure parameters, timing, and magnification were used throughout; magnification and exposure were kept constant throughout the experiments. Angiograms were performed in ventrodorsal and lateral views. Repeated angiograms were obtained on Days 3, 7, and 10 after induction of SAH, and these were used to evaluate chronological changes in the BA diameter.

After each FA catheterization, the dogs were kept sedated with buprenorphine (0.01 mg/kg delivered intramuscularly every 4–6 hours) for 18 hours. The sites of FA catheterizations were monitored for hematoma formation. Use of the FA in each limb was alternated for subsequent time periods. Complete neurological and physical examinations were performed before each procedure.

On Day 10 the dogs were killed using a high-dose pentobarbital solution delivered intravenously (390 mg/ml Fatal-Plus; Vortech Pharmaceuticals, Dearborn, MI) at 1 ml/5 kg body weight. The brain was immediately removed 15 minutes after death and visualized for evidence of hemorrhage. The BA was dissected and removed.

The BA was measured in a double-blinded fashion on magnified angiograms. To eliminate differences in the magnification values for the angiograms, a caliper measuring 1 cm was placed beneath the ear pinna during the angiography procedure. The angiogram was recorded in real time and the image was captured at peak arterial contrast opacification in anteroposterior and lateral orientations. The images were imported into Photoshop, and the BA was identified in the anteroposterior and lateral images. The origin of the BA was identified just proximal to the bifurcation of the VAs. Its terminus was defined just proximal to the confluence of the VAs. A separate mean average of the narrowest regions of the BA was also calculated for the 10, 40, 50, and 60% data points.

Within groups, data were analyzed using the paired t-test (when data were collected at only two time points) or one-way repeated-measures analysis of variance (when data were collected at more than two time points). Among groups, data were analyzed using the two-way repeated-measures analysis of variance, with day and group as the two factors. Descriptive statistics were calculated for all data (mean ± standard error of the mean). In all instances, significance for the coefficient alpha was set at 0.05. Analyses were conducted using SigmaStat 3.1.1 software (Systat Software, Inc., Richmond, CA).

Results

Figure 1 depicts the mean measurements of the BA. A reduction in the BA diameter was seen by Day 3. This was most pronounced in the control group; it was seen to a lesser extent in the cyclosporine group and not at all in the simvastatin group. Figure 2 is composed of representative angiographic images from Day 3. By Day 7, a return to the baseline diameter was seen. Interestingly, a vasodilation effect was seen in the simvastatin-only group at Day 10. Overall, without differentiating between the days, the vessel diameter in the control group was smaller than that in either treatment group (p = 0.029).
Discussion

Inflammation and depletion of NO after SAH have been associated with the development of cerebral vasospasm. In this study, we evaluated the benefit of simultaneous treatment with cyclosporine and simvastatin, both of which independently have been shown to ameliorate cerebral vasospasm. Our results support the suggestion that by Day 3 both cyclosporine and simvastatin decreased the extent of cerebral vasospasm. Simvastatin alone, however, was more effective. As mentioned, a vasodilation effect was seen in the simvastatin-only group by Day 10. This may be consistent with previous findings that simvastatin augments NO-mediated vasodilation and that this may persist for at least 1 month. The results of this study lead us to suggest that the combination of cyclosporine and simvastatin offers no benefit over treatment with simvastatin alone.

Interestingly, the combined treatment with simvastatin and cyclosporine may limit the efficacy of the statin treatment. Although there is no doubt that cyclosporine can effectively suppress the immune system, there is concern that it can also cause vasoconstriction. It has been shown to cause an acute release of prostanoid thromboxane in the myocardium, resulting in coronary vasospasm. Furthermore, it has been associated with endothelial injury and reversible vasospasm resulting in encephalopathy.

The origin of vasospasm may result from an imbalance between vasodilation and vasoconstriction. Vascular endothelium regulates smooth-muscle tone by generating NO and endothelium-derived constriction factors. Disruption of endothelium or its relaxing factors may alter this balance, predisposing the vessels to vasospasm. Depletion of NO accompanies these changes, supporting the suggestion that SAH-induced endothelial dysfunction con-
tributes to loss of NO.\textsuperscript{24,29} Furthermore, NO replacement reverses cerebral vasospasm in animal studies.\textsuperscript{24,29} Treatment with statins may ameliorate cerebral vasospasm in patients after SAH.\textsuperscript{34}

As the primary source of NO in vascular tone regulation, NOS metabolizes L-arginine to NO and citrulline. The endothelial isoform, eNOS, is constitutively expressed in cerebrovascular endothelium. Immunoreactivity for eNOS messenger RNA and protein has been shown to decrease after SAH.\textsuperscript{10,24} Although the mechanism of NOS reduction is not known, it likely causes reduced NO production and disruption of endothelium-dependent vasodilation after SAH.

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, also referred to as statins, are potent inhibitors of cholesterol biosynthesis.\textsuperscript{11} Statins also directly upregulate eNOS expression under cholesterol-controlled conditions.\textsuperscript{10,20} An increase in eNOS messenger RNA, protein, and enzymatic activity has been demonstrated after statin treatment, which results in increased cerebral blood flow.\textsuperscript{10} Selective upregulation of eNOS activity by statin treatment may prevent eNOS depletion or even increase its expression after SAH.

There is mounting evidence to suggest that an inflammatory response may be involved in the development of cerebral vasospasm.\textsuperscript{6,22} Intimal, medial, and subarachnoid infiltration by macrophages, neutrophils, and other inflammatory cells has been noted after SAH in autopsy studies and experimentally in dogs.\textsuperscript{7,14,25} It has also been demonstrated that severe inflammation in the perivascular space of cerebral arteries can provoke moderate to severe vasospasm.\textsuperscript{35} Concentrations of circulating serum immunocomplexes are increased during cerebral vasospasm.\textsuperscript{17,28,30} Furthermore, a postmortem study has shown increased deposition of immunoglobulin and complement protein in the walls of human cerebral vessels exposed to subarachnoid blood clots.\textsuperscript{16}

In 1978, Weir and colleagues\textsuperscript{38} established that cerebral vasospasm begins at approximately Day 3 post-SAH, is maximal at 6 to 8 days, and is gone by Day 12. This time course parallels that of a delayed-type hypersensitivity or chronic allergic reaction when lymphocyte subsets in the CSF of rats are analyzed after SAH.\textsuperscript{18} There is compelling evidence supporting the suggestion that violation of the subarachnoid space results in severe vasospasm. The mere presence of foreign bodies such as dextran or latex beads in the canine subarachnoid space results in rapid and severe vasocstriction.\textsuperscript{39} Denaturation of the subarachnoid erythrocytes results in a massive infiltration of inflammatory and immunoreactive cells.\textsuperscript{31,32,34} The delay before the onset of cerebral vasospasm in these cases is 4 to 14 days, which parallels a time frame for immunological reaction against the aging human subarachnoid erythrocyte.

Drugs that reduce inflammation, such as corticosteroid agents, improved outcome after Phase II and III trials in humans and reduced cerebral vasospasm in animal models.\textsuperscript{2,5,13} Drugs such as FK506 (tacrolimus) that suppress cell-mediated immunity do not reduce cerebral vasospasm.\textsuperscript{36} The results when using cyclosporine in human trials have been conflicting.\textsuperscript{23,39} Cyclosporin A interferes with T-helper lymphocyte interfeulin production. This limits the activation of the killer T lymphocytes, which ultimately results in immunosuppression. In both the canine and nonhuman primate models, amelioration of cerebral vasospasm after the administration of cyclosporin A has been reported.\textsuperscript{12,30} The clinical data in humans have not been so encouraging.\textsuperscript{21} It is difficult to determine if part of this is associated with the toxicity of cyclosporine. Reversible encephalopathy and vasospasm have been shown to occur in some humans treated with cyclosporin A.\textsuperscript{12,21} The results of our study support the suggestion that when using doses of cyclosporine A commonly administered for organ transplantation, some limitation to the vasodilation effects of simvastatin is noted.

### Conclusions

The results of our study lead us to suggest that combined therapy with cyclosporine and simvastatin is not as efficacious in ameliorating vasospasm as simvastatin alone. Interestingly, cyclosporin may limit the beneficial effect of simvastatin.

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### References

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