Cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) has been an issue of major clinical significance in the field of neurosurgery. Although intensive efforts have been made to investigate the pathogenesis of this phenomenon, the issue remains unsolved. The accumulated data, however, have gradually unmasked the enigma of the phenomenon. Recently we have focused our studies on the endothelial injury initiated by superoxide anions. From these two different phenomena, it was learned that the common pathway to producing endothelial injury triggers an imbalance in endothelium-derived relaxing factors (EDRF; nitric oxide and prostacyclin) and endothelium-derived constriction factor (EDCF; endothelin-1) and that vasoconstriction occurs because of the action of EDCF. According to this theory, the factor responsible for vasoconstriction is endothelial injury. Thus, we sought to study the cause of endothelial injury in SAH. During the past decade, oxyhemoglobin was found to be a most potent trigger of cerebral vasospasm because of its direct vasoconstrictive effect or its release of superoxide anion as it is autoxidized to methemoglobin. Many experimental studies have proven that erythrocytes are essential for causing vasospasm and that endothelial injury is always associated with the occurrence of vasospasm.

In the present study we studied the ability of human recombinant copper-zinc superoxide dismutase (h-r SOD) to prevent cerebral vasospasm in the experimental rabbit SAH model.

Materials and Methods

Forty-five male Japanese White rabbits weighing 2.5 to 3.0 kg were anesthetized intravenously with 20 mg/kg pentobarbital and allowed to respire spontaneously. They were placed in the supine position, and a 22F catheter was inserted selectively into their left vertebral artery via a femoral artery by the Seldinger method. Baseline vertebral angiograms were obtained by manual injection of 0.5 mL containing 306 mg iopamidol (Iopamiron 300, Schering AG) for 2 seconds. During these procedures, arterial blood gases were checked, and it was ascertained that PO2, PCO2, and pH were normal. Twenty minutes after the first angiogram, the major cistern was injected and treated by multiple intracisternal injections of 0.5 mL saline (saline group, n=10); and (4) blood injected and treated by multiple intracisternal injections of 0.5 mL saline (saline group, n=10). Serial angiograms were performed after the blood injection, and the diameter of the basilar artery was measured. Three animals from the control group and five animals from the SAH and SOD groups each were killed 2 days after SAH, and their basilar arteries were processed for transmission electron microscopic observations.

Conclusions We determined that h-r SOD prevents the occurrence of vasospasm, possibly as a result of preventing endothelial injury initiated by superoxide anions. (Stroke. 1994;25:864-868.)

Key Words • cerebral vasospasm • endothelium • free radicals • subarachnoid hemorrhage • superoxide dismutase

The Role of Superoxide Anions in the Pathogenesis of Cerebral Vasospasm

Tsuneo Shishido, MD; Ryuta Suzuki, MD; Liang Qian, MD; Kimiyoshi Hirakawa, MD

Background and Purpose To determine the role of superoxide anions in the pathogenesis of cerebral vasospasm after aneurysmal subarachnoid hemorrhage, we studied the preventive effect of human recombinant copper-zinc superoxide dismutase (h-r SOD) in a rabbit subarachnoid hemorrhage (SAH) model.

Methods Forty-five rabbits receiving intracisternal injection of 3 mL autologous nonheparinized blood or 3 mL saline were divided into four groups as follows: (1) saline injected and no treatment (control group, n=6); (2) blood injected and no treatment (SAH group, n=20); (3) blood injected and treated by multiple intracisternal injections of 30 000 U of h-r SOD in 0.5 mL saline (SOD group, n=9); and (4) blood injected and treated by multiple intracisternal injections of 0.5 mL saline (saline group, n=10). Serial angiograms were performed after the blood injection, and the diameter of the basilar artery was measured. Three animals from the control group and five animals from the SAH and SOD groups each were killed 2 days after SAH, and their basilar arteries were processed for transmission electron microscopic observations. Results In the SAH and saline groups, the diameter of the basilar arteries was significantly reduced (28±14% and 27±9%, respectively) at 2 days after the blood injection, then recovered to pre-SA H levels until 11 days. In the SOD group, the diameter of the basilar artery was only minimally changed during the follow-up period. Transmission electron microscopy revealed endothelial injury in all basilar arteries in the SAH group, whereas endothelial injury was minimal in the SOD group.
TABLE 1. Protocol and Animal Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Cisternal Injection</th>
<th>Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>Saline 3 mL</td>
<td>Angiography</td>
</tr>
<tr>
<td>3</td>
<td>Saline 3 mL</td>
<td>TEM</td>
<td></td>
</tr>
<tr>
<td>SAH</td>
<td>20</td>
<td>Blood 3 mL</td>
<td>Angiography</td>
</tr>
<tr>
<td>5</td>
<td>Blood 3 mL</td>
<td>TEM</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>9</td>
<td>Blood 3 mL followed by 30 000 U SOD b.i.d. for 72 h</td>
<td>Angiography</td>
</tr>
<tr>
<td>5</td>
<td>Blood 3 mL followed by 30 000 U SOD b.i.d. for 72 h</td>
<td>TEM</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>10</td>
<td>Blood 3 mL followed by 0.5 mL saline b.i.d. for 72 h</td>
<td>Angiography</td>
</tr>
</tbody>
</table>

SAH indicates subarachnoid hemorrhage; SOD, superoxide dismutase; and TEM, transmission electron microscopy.

The other 39 animals received in their major cistern 3 mL autologous nonheparinized arterial blood that was obtained from the femoral artery (SAH animals). Those SAH animals were grouped into three categories (Table 1). Twenty SAH animals were examined to assess the occurrence of angiographic cerebral vasospasm in their basilar artery (SAH group). Nine SAH animals were injected with 30 000 U of h-r SOD (Nippon Kayaku Co, Ltd) in 0.5 mL saline solution into their major cistern every 12 hours for 72 hours after receiving the autologous blood in their major cistern (SOD group). The other 10 SAH animals received intracisternal injection of 0.5 mL saline every 12 hours for 72 hours (saline group).

Serial vertebral angiograms were obtained at 1 hour and 1, 2, 4, 7, and 11 days after injecting the intracisternal blood in each group. The angiograms obtained were transferred to an analytic processing system, and the diameter of the basilar artery was measured at five points (at the midpoint of the basilar artery, at 1 mm central and peripheral to the midpoint, and at 2 mm central and peripheral to the midpoint). Then the mean diameter at these five points was determined.

Additionally, 3 animals from the control group and 5 animals from the SAH and SOD groups each were killed at 2 days after initiation of SAH by transcardial perfusion with 2.5% glutaraldehyde. The basilar arteries were removed and processed for transmission electron microscopy (TEM).

Results

A total of 106 angiograms (excluding baseline angiograms) were obtained from 45 rabbits. The mortality rate associated with each angiographic procedure was 13%. There was no significant difference in mortality rate among the groups. In the control group the diameter of the basilar artery did not change throughout the period of observation, although in the SAH group the diameter of the basilar artery was significantly reduced (28±14%) at 2 days after the intracisternal blood injection (day 2) and then gradually recovered to the pre-SAH level within 11 days (Fig 1). In the SOD group the basilar artery diameter was only minimally changed during the 11-day period. In the saline group, however, the diameter of the basilar artery was decreased (27±9%), as it was in the SAH group on the serial angiograms (Fig 2).

From observations on TEM, the endothelia in the basilar arteries of almost all day 2 rabbits in the SAH group (n=5) were severely damaged, and fragmentations of the internal elastic lamina were also seen. In day 2 rabbits of the SOD group (n=5), however, the morphology was similar compared with the control group (n=3) (Table 2, Fig 3).

Discussion

Experimental Model

To investigate the pathogenesis of the vasospasm that occurs after SAH, a wide variety of laboratory experiments have been performed. Several experiments in SAH models using rabbits have been reported. In our rabbit SAH model, the diameter of the basilar artery was reduced significantly within 2 days after intracisternal blood injection and then increased gradually to near the baseline within 7 days after blood injection in almost every animal. This sequential change in arterial narrowing is similar to that seen in other rabbit SAH models.14-16 In our rabbit model, endothelial injury to the basilar artery was seen on TEM, as reported recently in dog and monkey SAH models.12

Endothelial Injury

Recently some experiments have demonstrated endothelial injury, barrier disruption in the cerebral arteries...
after SAH, and loss of EDRF activity after SAH. Impairment of endothelium-dependent relaxation in human basilar arteries after SAH has also been reported. Kim et al performed a series of experiments that proved that in spastic arteries there is no endothelium-dependent relaxation; however, the release of EDRF was maintained in basilar arteries of dogs 8 days after they received two intracisternal blood injections. They hypothesized that reduced production of cyclic GMP (cGMP) in smooth muscle cells impairs the responsiveness of smooth muscle cells to EDRF. The results of Edwards et al were controversial in that cGMP levels in isolated spastic cerebral arteries in pigs were not reduced after in vivo exposure to hemoglobin or to whole blood for 2 days. Hatake et al studied the vasodilatory responses to various drugs by human basilar arteries obtained from SAH patients who died within 1 day of SAH onset. They revealed that endothelium-dependent relaxation responses to thrombin, bradykinin, and calcium ionophore A23187 were im-

**TABLE 2. Transmission Electron Microscopic Observation of the Endothelia**

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal No.</th>
<th>Vacuolatlon of Endothelia</th>
<th>Fragmentation of Internal Elastic Lamina</th>
<th>Loss of Tight Junction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SAH</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>SOD</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

SAH indicates subarachnoid hemorrhage; SOD, superoxide dismutase.
paired; however, the endothelium-independent response to sodium nitroprusside did not differ from the control. In contrast to the findings of Kim et al, their findings suggested that cGMP in the wall of human cerebral arteries is not altered after SAH. Considering these diverse results together, it may be speculated that morphological endothelial injury causes functional disturbances of endothelium and that it has an important role in the genesis of cerebral vasospasm.

Oxyhemoglobin is postulated to be the most potent trigger of vasospasm in SAH, and oxyhemoglobin administration causes vasospasm. On the other hand, inflammation associated with SAH alone may induce persistent and severe cerebroarterial constriction. According to these two different theories, the superoxide anion is speculated to be the common mediator of endothelial injury.

**Hypothesis**

The superoxide anion likely mediates endothelial injury as follows. Oxyhemoglobin from a subarachnoid clot releases superoxide anion as it is autoxidized to methemoglobin. Then the superoxide anion is converted to hydrogen peroxide by SOD. The iron-catalyzed interaction of hydrogen peroxide and superoxide anion generates more active radicals such as hydroxyl radical and singlet oxygen by the Haber-Weiss reaction.

Superoxide anion and hydroxyl radical are generated as follows:

$2\text{O}_2^{-}\text{superoxide dismutase} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$

$\text{O}_2^{-} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \cdot \text{OH} + \text{OH}^{-}$

$\text{Fe}^{2+} + \text{O}_2^{-} \rightarrow \text{Fe}^{3+} + \text{OH}^{-}$

$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \text{OH}^{-}$

Interactions between these free radicals and phospholipids in the surrounding membranes may produce lipid peroxidation. Then a chain reaction of lipid peroxidation in the arterial wall is initiated and finally causes endothelial damage. During inflammation, free radicals such as superoxide or hydroxyl radicals generated in the arterial wall may induce endothelial injury and breakdown of the physical blood-brain barrier.

Once endothelial injury occurs, endothelin production and breakdown of the physical blood-brain barrier. The imbalance between EDCF and EDRF induces more active radicals such as hydroxyl radical and singlet oxygen by the Haber-Weiss reaction.

**Conclusions**

We conclude that (1) vasospasm after SAH is thought to be initiated by superoxide anions and other free radicals from oxyhemoglobin; (2) h-r SOD prevents the occurrence of vasospasm in vivo; (3) morphological endothelial injury was also prevented in SOD-treated animals; and (4) endothelial injury may have an important role in the occurrence of vasospasm.

**Acknowledgments**

The authors would like to thank Shizuko Ichinose for technical assistance. We would also like to thank Nippon Kayaku Co, Ltd for providing h-r SOD for this study.
In the accompanying article it is rather convincingly shown that six intracisternal injections (the first 12 hours after the blood injection) of copper-zinc superoxide dismutase (SOD) prevent moderate vasospasm in a single blood injection subarachnoid hemorrhage (SAH) rabbit model. The difference in effectiveness between this model and that of Macdonald et al is not wholly satisfactorily explained. Possibly it is the very high dose of SOD used in the present model; possibly it is the stability of the copper-zinc SOD. It is even conceivable that the copper-zinc combination has a beneficial effect, and therefore it would have been better to include a group treated with inactivated enzyme. That would also have strengthened the case made for a role of superoxide anions in vasospasm, although the earlier work of this model and the literature data are, again, rather convincing.

Further work remains to be done before clinical trials could be initiated. First, there is anecdotal evidence that intracisternal irrigation with nimodipine prevents or alleviates vasospasm after aneurysmal SAH, yet this has not been widely adopted or even tried, probably because neurosurgeons are reluctant to leave cisternal catheters. Thus, we will have to determine whether one, or possibly two, cisternal injections might suffice, or whether it would be necessary to apply intracisternal SOD during the whole period of maximum vasospasm in humans. Second, what is the time window within which the SOD should be applied? And third, considering the important role the authors ascribe to endothelium, one wonders whether intravenous administration of this copper-zinc combination has a beneficial effect, and therefore it would have been better to include a group treated with inactivated enzyme. That would also have strengthened the case made for a role of superoxide anions in vasospasm, although the earlier work of this model and the literature data are, again, rather convincing.

Further work remains to be done before clinical trials could be initiated. First, there is anecdotal evidence that intracisternal irrigation with nimodipine prevents or alleviates vasospasm after aneurysmal SAH, yet this has not been widely adopted or even tried, probably because neurosurgeons are reluctant to leave cisternal catheters. Thus, we will have to determine whether one, or possibly two, cisternal injections might suffice, or whether it would be necessary to apply intracisternal SOD during the whole period of maximum vasospasm in humans. Second, what is the time window within which the SOD should be applied? And third, considering the important role the authors ascribe to endothelium, one wonders whether intravenous administration of this copper-zinc human recombinant SOD would be effective.

J. Paul Muizelaar, MD, PhD, Guest Editor
Department of Surgery
Medical College of Virginia
Richmond, Va

Reference
The role of superoxide anions in the pathogenesis of cerebral vasospasm.

T Shishido, R Suzuki, L Qian and K Hirakawa

Stroke. 1994;25:864-868
doi: 10.1161/01.STR.25.4.864

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/25/4/864

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/