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-SAH 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.

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

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 fact that endothelial injury is always associated with the occurrence of vasospasm. From observations of the changes in plasma and cerebrospinal fluid (CSF) endothelin in patients with SAH, we hypothesized that endothelial injury triggers an imbalance in endotheli-um-derived relaxing factors (EDRF; nitric oxide and prostacyclin) and endothelium-derived constrictive factor (EDCF; endothelin-1) and that vasocostriction occurs because of the action of EDCF. According to this theory, the factor responsible for vasocostriction 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 vasocostrictive effect or its release of superoxide anion as it is autoxidized to methemoglo-bin. Many experimental studies have proven that erythrocytes are essential for causing vasospasm and

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more specifically that oxyhemoglobin itself produces the vasospasm. In contrast, recent observations have revealed that inflammatory or immunologic reactions can produce vasospasm without participation by erythrocytes. From these two different phenomena, it was learned that the common pathway to producing endothelial injury may be the superoxide anion. Superoxide anions can cause many free radical reactions in the arterial wall and may cause endothelial injury. We can speculate, therefore, that the superoxide anion plays a pivotal role in causing vasospasm. Based on this theory, many free radical scavengers have been used in clinical or experimental SAH, although the efficacy of these drugs has not been clearly demonstrated.

In the present study we studied the ability of human recombinant copper-zinc superoxide dismutase (h-r SOD), a newly developed free radical scavenger, 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 pentobarbit-al 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
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></td>
<td>3</td>
<td>Saline 3 mL</td>
<td>TEM</td>
</tr>
<tr>
<td>SAH</td>
<td>20</td>
<td>Blood 3 mL</td>
<td>Angiography</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Blood 3 mL</td>
<td>TEM</td>
</tr>
<tr>
<td>SOD</td>
<td>5</td>
<td>Blood 3 mL followed by 30,000 U SOD b.i.d. for 72 h</td>
<td>Angiography</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Blood 3 mL followed by 30,000 U SOD b.i.d. for 72 h</td>
<td>TEM</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.

punctured with a 23-gauge butterfly needle, and 1.5 mL of CSF was withdrawn. For the control study, 3 mL saline was injected in the major cistern in 6 rabbits (control group). 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 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 (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

![Fig 1. Bar graph showing sequential changes in the diameter of the basilar artery compared with baseline. 1Hr indicates 1 hour after blood injection; D1, 1 day after blood injection; D2, 2 days after blood injection; D4, 4 days after blood injection; D7, 7 days after blood injection; and D11, 11 days after blood injection. The diameter of the basilar arteries was significantly reduced on days 1 and 2 in the subarachnoid hemorrhage (SAH) group. The peak of the constriction was seen on day 2.](http://stroke.ahajournals.org/)

![Fig 2. Changes in the diameter of the basilar artery from baseline.](http://stroke.ahajournals.org/)
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>Vacuolation 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>
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<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
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<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>
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</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.26 On the other hand, inflammation associated with SAH alone may induce persistent and severe cerebroarterial constriction.10 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.5,7 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.20 Superoxide anion and hydroxyl radical are generated as follows21:

\[
\begin{align*}
2O_2^- & \rightarrow H_2O_2 + O_2^- \\
O_2^- + H_2O_2 & \rightarrow O_2 + \cdot OH + OH^- \\
Fe^{2+} + O_2^- & \rightarrow Fe^{3+} + O_2 \\
Fe^{2+} + H_2O_2 & \rightarrow Fe^{3+} + \cdot OH + OH^- 
\end{align*}
\]

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 initiated22,23 and finally causes endothelial damage. During inflammation, free radicals such as superoxide or hydroxyl radicals generated in the arterial wall may induce endothelial injury10 and breakdown of the physical blood-brain barrier.1,2 Once endothelial injury occurs, endothelin production by the endothelium is stimulated by oxyhemoglobin,24 and prostacyclin25 and EDHF synthesis are reduced. Then the imbalance between EDCF and EDHF induces vasoconstriction3 and is manifested as angiographic cerebral vasospasm. That may be followed by platelet aggregation on the injured surface of the arterial wall. Then serotonin and thromboxane released from platelets enhance vasoconstriction and produce microemboli that accelerate brain ischemia, leading to symptomatic vasospasm.26

Superoxide Dismutase

According to the above hypothesis, SOD is thought to inhibit the progress of cerebral vasospasm as follows. First, SOD prevents initiation of the chain reaction of lipid peroxidation by eliminating superoxide anions that lead to endothelial injury. Second, SOD inhibits inactivation of EDHF27 released mainly by endothelial cells by trapping superoxide anions in the arterial wall or in CSF.

Many antioxidants have been studied in clinical or experimental SAH models.13,28,29 Kamiyama et al50 found that SOD and catalase are effective inhibitors of oxyhemoglobin-induced contraction of cat basilar arteries exposed in situ. However, many other reports failed to confirm that SOD can prevent the occurrence of vasospasm.13,28,29 Recently h-r SOD, which was reported to be stable similar to the native enzyme SOD,31 has been used effectively to prevent transient ischemic injury of CA1 neurons in gerbils.32 We examined the effects of h-r SOD in our rabbit SAH model. Multiple intracisternal injections of h-r SOD prevented the occurrence of cerebral vasospasm in oxyhemoglobin-induced SAH in a monkey model. Our results may differ from those of Macdonald et al because of differences between the experimental models, particularly temporal changes in the concentration of oxyhemoglobin and SOD activity in CSF.

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

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References

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 these authors 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 has to 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.

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