Delayed cerebral ischemia remains an important cause of death and disability in patients who have suffered an SAH. The extracellular Ca\(^{++}\) influx into VSMCs plays a fundamental role in the development and chronic effects of vasospasm after subarachnoid hemorrhage (SAH). The Ca\(^{++}\)-permeable nonselective cation channels (NSCCs) are activated by several endothelium-derived constricting factors such as endothelin 1 (ET-1) and thromboxane A\(_2\). Moreover, the receptor-operated Ca\(^{++}\) channel blocker LOE 908 inhibits ET-1–induced extracellular Ca\(^{++}\) influx via NSCCs in the VSMCs of the basilar artery (BA) and the NSCC-dependent part of ET-1–induced vasoconstriction of BA rings. The purpose of the present study was to evaluate the in vivo role of LOE 908 on SAH-induced vasospasm.

Methods. Forty-two Japanese white rabbits were assigned to seven groups. Treatment groups consisted of the following: 1) control rabbits without SAH that received a cisternal injection of saline; 2) rabbits with SAH that were subjected to the intravenous administration of saline; 3 through 6) rabbits with SAH that underwent the intravenous administration of 0.01, 0.1, 1, or 10 mg/kg LOE 908, respectively; and 7) rabbits without SAH that underwent the intravenous administration of 10 mg/kg LOE 908. Autologous blood was injected into the cisterna magna. The caliber of the BA was measured on angiographic studies before and after the cisternal injection of autologous blood.

The intravenous injection of LOE 908 inhibited the magnitude of an SAH-induced vasospasm. In addition, the concentration of LOE 908 required to relax vasospasm (1 mg/kg) correlated with that required to block Ca\(^{++}\) influx into VSMCs.

Conclusions. The Ca\(^{++}\) channel blocker LOE 908 may inhibit the magnitude of an SAH-induced vasospasm by blocking the influx of Ca\(^{++}\) through NSCCs in rabbit BAs. Blocking the NSCCs may represent a new treatment for cerebral vasospasm after SAH.

**KEY WORDS** • vasospasm • Ca\(^{++}\) influx • nonselective cation channel • rabbit

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**Object.** The Ca\(^{++}\) influx into vascular smooth-muscle cells (VSMCs) plays a fundamental role in the development and chronic effects of vasospasm after subarachnoid hemorrhage (SAH). The Ca\(^{++}\)-permeable nonselective cation channels (NSCCs) are activated by several endothelium-derived constricting factors such as endothelin 1 (ET-1) and thromboxane A\(_2\). Moreover, the receptor-operated Ca\(^{++}\) channel blocker LOE 908 inhibits ET-1–induced extracellular Ca\(^{++}\) influx via NSCCs in the VSMCs of the basilar artery (BA) and the NSCC-dependent part of ET-1–induced vasoconstriction of BA rings. The purpose of the present study was to evaluate the in vivo role of LOE 908 on SAH-induced vasospasm.

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**Abbreviations used in this paper:** BA = basilar artery; [Ca\(^{++}\)]\(_i\) = intracellular free Ca\(^{++}\) concentration; ET-1 = endothelin 1; NSCC = nonselective cation channel; SAH = subarachnoid hemorrhage; SOCC = store-operated Ca\(^{++}\) channel; VA = vertebral artery; VICC = voltage-independent Ca\(^{++}\) channel; VOCC = voltage-operated Ca\(^{++}\) channel; VSMC = vascular smooth-muscle cell.

**Effects of the Ca\(^{++}\)-permeable nonselective cation channel blocker LOE 908 on subarachnoid hemorrhage–induced vasospasm in the basilar artery in rabbits**

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well with those for the ET-1–induced \([\text{Ca}^{2+}]\) response. These results suggest that LOE 908 inhibits ET-1–induced vascular contractions of BA rings by blocking extracellular \(\text{Ca}^{2+}\) influx. Moreover, LOE 908 inhibits ET-1–induced vascular contractions of BA rings by blocking extracellular \(\text{Ca}^{2+}\) influx through NSCCs, but not SOCCs.\(^7\) Given that some researchers assert that ET-1 may be one of the major contributors to vasospasm after SAH,\(^1,9,11,18,19\) extracellular \(\text{Ca}^{2+}\) influx through NSCCs may play an important role in vasospasm post-SAH. Therefore, we attempted to evaluate the in vivo efficacy of extracellular \(\text{Ca}^{2+}\) influx via NSCCs on SAH-induced vasospasm by using LOE 908. Note that LOE 908 has been developed as an inhibitor of receptor-operated \(\text{Ca}^{2+}\) channels,\(^6,7\) and the intravenous or subcutaneous administration of it at concentrations of approximately 0.5 to 30 mg/kg reduces ischemic lesion volumes by blocking extracellular \(\text{Ca}^{2+}\) influx in experimental stroke models.\(^8\) Authors of a previous report have demonstrated that LOE 908 penetrates the blood–brain barrier.\(^13\)

### Materials and Methods

#### Experimental Protocols in an SAH-Induced Vasospasm Model

Japanese white rabbits were randomly assigned to seven experimental groups of six animals each (Table 1). The control group (Group 1) consisted of animals that had received a cisternal injection of saline. Animals in five groups (Groups 2–6) were subjected to SAH. Rabbits in the first of these five groups received an intravenous injection of saline. The other four groups of animals received intravenous injections of 0.01, 0.1, 1, or 10 mg/kg LOE 908, respectively, which was kindly provided by Boehringer Ingelheim K. G. (Ingelheim, Germany). We injected these doses of LOE 908 three times per day. The intravenous administration of LOE 908 (every 8 hours) was initiated just after the induction of SAH and continued until the follow-up angiography study was completed (a total of nine intravenous administrations was performed). The final dose was administered 30 minutes before performing follow-up angiography. Six additional rabbits (Group 7) received 10 mg/kg LOE 908 without being subjected to SAH.

#### Construction of an SAH-Induced Vasospasm Model

The rabbits, each weighing 3 kg, were anesthetized with sodium pentobarbital (35 mg/kg intravenously) and allowed to breathe spontaneously with room air supplemented with O\(_2\). A catheter was introduced into the VA via the transfemoral approach. A VA angiography was performed using a bolus injection of 3 ml Iopamiron (Dai-ichi Pharmaceuticals, Tokyo, Japan).

In this study, SAH was stimulated by a single injection of autologous arterial blood into the cisterna magna, as described previously.\(^17\) After control angiography, the cisterna magna puncture was performed percutaneously by using a 21-gauge butterfly needle. Autologous arterial blood (1 ml/kg) was then injected. To avoid a marked increase in intracranial pressure, the same amount of cerebrospinal fluid was removed before injection. After the injection of blood, the animals were placed supine in animal incubators and allowed to recover. The animals were monitored postoperatively for infections, hydration, and signs of postoperative pain. Antibiotic agents and fluids were administered as appropriate. Three days after SAH, VA angiography was performed again to obtain the magnitude of the BA contraction. The caliber of the BA was measured on x-ray studies at three corresponding cross sections: just below the BA–posterior cerebral artery junction, just above the BA–VA junction, and midway between these locations. Values from these three locations were averaged.

#### Statistical Analysis

All results were expressed as the means ± standard errors of the means. Data were subjected to a two-way analysis of variance and when a significant F value was encountered, the Newman–Keuls multiple-range test was used to calculate significant differences between treatment groups. A probability value less than 0.05 was considered statistically significant.

#### Results

### Effects of LOE 908 on an SAH-Induced Vasospasm

There was no significant difference among the seven groups regarding any of the physiological parameters (pH, PCO\(_2\), PO\(_2\), or mean arterial blood pressure) measured at the time of follow-up angiography.

The mean diameter of the BA for each group is shown in Table 2. The mean diameter of the BAs in the six rabbits in Group 1 (723 ± 42 \(\mu\)m) was similar to that of the control BA in 24 rabbits (738 ± 48 \(\mu\)m; Fig. 1A and B). All animals in the SAH groups exhibited reduced BA diameters relative to those in the control group. In the six animals in Group 2, the diameters of the BAs were reduced by 46.6% (386 ± 38 \(\mu\)m). The intravenous injection of 0.01 (Group

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**TABLE 1**

<table>
<thead>
<tr>
<th>Group No.*</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>control rabbits receiving a cisternal injection of saline</td>
</tr>
<tr>
<td>2</td>
<td>rabbits w/ SAH receiving an intravenous dose of saline</td>
</tr>
<tr>
<td>3</td>
<td>rabbits w/ SAH receiving an intravenous dose of 0.01 mg/kg LOE 908*</td>
</tr>
<tr>
<td>4</td>
<td>rabbits w/ SAH receiving an intravenous dose of 0.1 mg/kg LOE 908*</td>
</tr>
<tr>
<td>5</td>
<td>rabbits w/ SAH receiving an intravenous dose of 1 mg/kg LOE 908*</td>
</tr>
<tr>
<td>6</td>
<td>rabbits w/ SAH receiving an intravenous dose of 10 mg/kg LOE 908*</td>
</tr>
<tr>
<td>7</td>
<td>rabbits w/o SAH receiving an intravenous dose of 10 mg/kg LOE 908 every 8 hours, starting after the first angiography study, for a total of nine doses</td>
</tr>
</tbody>
</table>

* Doses were begun just after induction of SAH and administered every 8 hours, for a total of nine doses.

**TABLE 2**

<table>
<thead>
<tr>
<th>Group No.*</th>
<th>Diameter ((\mu)m)**</th>
<th>Reduction (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>723 ± 42</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>386 ± 38</td>
<td>46.6</td>
</tr>
<tr>
<td>3</td>
<td>393 ± 30</td>
<td>45.6</td>
</tr>
<tr>
<td>4</td>
<td>404 ± 27</td>
<td>44.1</td>
</tr>
<tr>
<td>5</td>
<td>526 ± 388</td>
<td>27.2</td>
</tr>
<tr>
<td>6</td>
<td>556 ± 308</td>
<td>23.1</td>
</tr>
<tr>
<td>7</td>
<td>743 ± 35</td>
<td>—</td>
</tr>
<tr>
<td>control BA</td>
<td>738 ± 48</td>
<td>—</td>
</tr>
</tbody>
</table>

* See Table 1 for a definition of each group. — = not applicable. ** Data are expressed as means ± standard errors of the means. *** Data are expressed as the percentage reduction of the control values. § p < 0.05, compared with Group 2.
Effects of LOE 908 on vasospasm after subarachnoid hemorrhage

3; Fig. 1C and D) or 0.1 (Group 4) mg/kg LOE 908 hardly reduced the amount of BA contraction after SAH. In contrast, 1 (Group 5) or 10 (Group 6) mg/kg LOE 908 reduced the amount of BA contraction and this reduction reached statistical significance (Fig. 1E and F). On the other hand, 10 mg/kg LOE 908 did not affect the control BA diameter.

Discussion

Data from the present study demonstrate that the intravenous administration of the receptor-activated Ca\(^{++}\)/H\(_{11001}/\)H\(_{11001}\) channel blocker LOE 908\(^{6,7}\) relaxes SAH-induced vasospasm in the rabbit BA. The circulating blood volume in the rabbit was approximately 90 ml/kg.\(^7\) Therefore, the concentration of LOE 908 (molecular weight 808.96) in circulating blood after injecting 0.01, 0.1, 1, or 10 mg/kg LOE 908 was approximately 0.15, 1.5, 15, or 150 \(\mu\)M. The mechanism underlying the LOE 908-induced relaxation of the vasospasm is likely to result, at least partially, from the blockade of extracellular Ca\(^{++}\) influx through NSCCs for the following reasons; 1) the effects of a 1- to 10-mg/kg intravenous injection of LOE 908 on experimental focal ischemia correlate with the blockade of Ca\(^{++}\) influx;\(^8\) and 2) LOE 908 administered at concentrations higher than 3 \(\mu\)M relaxes ET-1-induced contraction in the rabbit BA by blocking extracellular Ca\(^{++}\) influx via NSCCs.\(^5\) Nonetheless, we could not produce a better dose–response curve of LOE 908 in an SAH-induced BA contraction in this experiment. The exact median inhibitory concentration of LOE 908 in the SAH-induced cerebral vasospasm remains to be determined. There is increasing evidence to support the role of ET-1 in SAH-induced cerebral vasospasm.\(^1,9,11,19,20\) Moreover, NSCCs are activated by several endothelium-derived constricting factors such as thromboxane A\(_2\).\(^14\) Therefore, the relaxation of the SAH-induced vasospasm by LOE 908 may result, at least partially, from the blockade of extracellular Ca\(^{++}\) influx through NSCCs activated by several constricting factors including ET-1. Note that LOE 908 also has inhibitory effects on L-type VOCCs and voltage-dependent Na\(^{+}\) channels.\(^13\) The exact magnitude of the contribution of the Ca\(^{++}\) influx through the NSCCs to the SAH-induced cerebral vasospasm remains to be determined.

Data from previous reports demonstrate that the extracellular Ca\(^{++}\) influx through SOCCs may be responsible for a significant component of the SAH-induced vasospasm;\(^19\) however, LOE 908 does not block SOCCs activated by ET-1 in BA VSMCs in the rabbit.\(^3\) In addition, LOE 908 fails to inhibit the SOCC-dependent part of ET-1–induced vascular contraction.\(^5\) Therefore, VICCs other than SOCCs may also be involved in SAH-induced vasospasm. Given the data indicating that LOE 908 blocks extracellular Ca\(^{++}\) influx through NSCCs activated by ET-1 in BA VSMCs in the rabbit,\(^5\) NSCCs as well as SOCCs may play important roles in SAH-induced vasospasm. These results suggest that the blockade of the NSCCs may be a new treatment for cerebral vasospasm after SAH.

Conclusions

In summary, LOE 908 may inhibit the magnitude of SAH-induced vasospasm by blocking Ca\(^{++}\) influx via NSCCs in BAs in rabbits. Blocking NSCCs may represent a new treatment for cerebral vasospasm after SAH.

References

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