The effects of HA compound calcium antagonists on delayed cerebral vasospasm in dogs

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The authors have examined the effects of the HA compounds HA1004 (N-(2-guanidinoethyl)-5-isoquinolinesulfonamide) and HA1077 (1-(5-isoquinolinesulfonyl)homopiperazine), which are intracellular calcium antagonists, on delayed cerebral vasospasm from subarachnoid hemorrhage (SAH). The modes of action of these compounds were compared with those of the more commonly used calcium entry blockers. Calcium ionophore A23187 (4.8 x 10^-6 M)-induced contraction of a canine basilar artery strip was completely antagonized by the HA compounds (10^-5 M) but not by the entry-blocking calcium antagonists nicardipine, diltiazem, and verapamil (10^-5 M), suggesting that the HA compounds act differently. Delayed cerebral vasospasm was induced by a "two-hemorrhage" canine model. The magnitude of the vasospasm and the effects of the HA compounds were determined angiographically. On SAH Day 7, a significant vasospasm was observed in every dog. The diameter of the basilar artery had diminished to 59% ± 2% (mean ± standard error) of the control value obtained before SAH (on Day 1). The intravenous administration of HA1004 caused a mild dilation of the basilar artery of 10% and 11% at doses of 3 and 10 mg/kg, respectively; however, HA1077 produced a more marked dilation of 19% and 27%, respectively, at the same doses. Both of these drugs lowered mean arterial blood pressure to about 80% and 50% at doses of 3 and 10 mg/kg, respectively. Intracisternal administration of the HA compounds (6 mg) completely reversed cerebral vasospasm without much effect on the blood pressure. The intracellular calcium antagonists of the HA compound group appear to be promising agents for the treatment of intractable cerebral vasospasm.

KEY WORDS • cerebral vasospasm • HA compound • calcium antagonist • subarachnoid hemorrhage • dog

Cerebral vasospasm is frequently associated with delayed ischemic neurological deficits from brain infarction, and is considered to be the principal cause of morbidity and mortality in patients with subarachnoid hemorrhage (SAH) due to aneurysm rupture. In spite of extensive research, the pathogenesis of cerebral vasospasm remains obscure, and no specific therapeutic method for the treatment of cerebral vasospasm has been established.

Recently, much attention has been paid to calcium antagonists since it was found that they could alter the fundamental mechanism of smooth-muscle contraction (that is, the calcium influx) irrespective of the cause of vasospasm. However, the effects of calcium antagonists on cerebral vasospasm are still controversial. Most of the commonly used calcium antagonists are calcium entry blockers which act primarily by inhibiting the influx of extracellular Ca++ into the cells through calcium channels in the cell membrane and do not directly affect the intracellular Ca++. During a search of the various sulfonamide derivatives, the HA compounds HA1004 (N-(2-guanidinoethyl)-5-isoquinolinesulfonamide) and HA1077 (1-(5-isoquinolinesulfonyl)homopiperazine) (Fig. 1), both intracellular calcium antagonists, were prepared by modifying the structure of a calmodulin antagonist (W-7). HA1004 was demonstrated to act on intracellular calcium but not on calmodulin. The present paper describes the differences in the mechanism of...
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FIG. 1. Molecular structures of the HA compounds.

action between the HA compounds and the entry-blocking calcium antagonists nicardipine, diltiazem, and verapamil,* as studied with an in vitro basilar artery model and an in vivo experimental delayed cerebral vasospasm model in dogs.

Materials and Methods

In Vitro Study

The canine basilar artery was removed rapidly, and spiral strips, approximately 2.0 cm in length, were prepared for isometric recording under 1.5 gm of tension in 20-ml jacketed tissue baths. The strips were submerged in an aerated Krebs-Henseleit solution (5% CO₂ in O₂) which was maintained at 37°C and was composed of the following (in mM concentrations): NaCl 115; KCl 4.7; CaCl₂ 2.5; MgCl₂ 1.2; NaHCO₃ 25; KH₂PO₄ 1.2; and dextrose 10.0. The tissue was allowed to equilibrate for 30 minutes to 1 hour before the start of each experiment. Force generation was monitored using an isometric transducer coupled to a polygraph recorder.†

Tissue contraction was induced with addition of the calcium ionophore A23187 (4.8 x 10⁻⁵ M). The intracellular calcium antagonists (HA1004 and HA1077) and entry-blocking calcium antagonists (nicardipine, diltiazem, and verapamil) were each added to a tissue bath, and their effects on the artery strips were examined.

In Vivo Study

A “two-hemorrhage” canine model of SAH was used in this study because it reliably produces a chronic and intractable cerebral vasospasm similar to that seen in humans.₅,₁₀,₁₈,₂₀,₂¹,₂₄ The method of inducing a cerebral vasospasm was essentially the same as reported by Varsos, et al.²₄ Two successive 4-ml administrations of fresh autogenous blood were made into the cisterna magna, one on Day 1 and one on Day 3. A control angiogram was obtained by direct puncture of the vertebral artery on Day 1 before the blood injection. A second angiogram to confirm the presence of vasospasm was obtained on Day 7 instead of Day 5 as reported by Varsos, et al.

Twenty-one mongrel dogs of either sex, each weighing 7 to 11 kg, were used for this study. All procedures were performed under anesthesia with intravenous sodium pentobarbital (20 to 30 mg/kg) after intramuscular premedication with ketamine hydrochloride (12 mg/kg) and with spontaneous respiration via endotracheal intubation.

On Day 7 of the study, one femoral artery was cannulated for continuous blood pressure monitoring with a strain gauge transducer and for arterial blood sampling. After the occurrence of chronic vasospasm was confirmed angiographically, each dog received drug treatment(s) according to the protocol (Table 1). The second or third drug administration was started after the diameter of the basilar artery had returned to its pretreatment size and the systemic blood pressure to its control level. For intravenous administration, 3 or 10 mg/kg of the HA compounds dissolved in 30 ml of saline was infused with a Harvard pump over a period of 30 minutes. Angiography was performed at 15, 30, 45, 60, 90, and 120 minutes after the initiation of the infusion, and at 60-minute intervals after while the drug was still effective. For intracisternal administration, 6 mg of the HA compounds in a 3-ml saline solution was injected into the cisterna magna over a period of 3 minutes. Angiograms were taken at 15, 30, 45, 60, 90, and 120 minutes after the intracisternal injection. At the end of the study, the dogs were sacrificed and autopsies were performed to examine the spread of the subarachnoid blood clot.

The diameter of the middle third of the basilar artery at its narrowest point was measured on the angiograms with the use of a microdensitometer and was expressed as a percentage of the diameter of that arterial segment before SAH (%diameter). The two-tailed Student's t-test was applied for statistical analysis.

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* The agents used were HA1004 and HA1077 (obtained from Asahi Chemical Industry, Tokyo, Japan), nicardipine (obtained from Yamanouchi Pharmaceutical, Tokyo, Japan), diltiazem (obtained from Tanabe, Tokyo, Japan), and verapamil (obtained from Eisai, Tokyo, Japan).

† Polygraph recorder, Model RM6000, manufactured by Nihon Kohden (America) Inc., Irvine, California.

Table 1

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>1st Treatment</th>
<th>2nd Treatment</th>
<th>3rd Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3</td>
<td>04 (3 mg/kg iv)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4, 5</td>
<td>04 (10 mg/kg iv)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>06 (mg ic)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>77 (3 mg/kg iv)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8, 9</td>
<td>77 (10 mg/kg iv)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10, 11</td>
<td>04 (3 mg/kg iv)</td>
<td>77 (3 mg/kg iv)</td>
<td>—</td>
</tr>
<tr>
<td>12, 13, 14</td>
<td>04 (10 mg/kg iv)</td>
<td>04 (6 mg ic)</td>
<td>—</td>
</tr>
<tr>
<td>15, 16, 17</td>
<td>77 (3 mg/kg iv)</td>
<td>77 (6 mg ic)</td>
<td>—</td>
</tr>
<tr>
<td>18, 19</td>
<td>77 (10 mg/kg iv)</td>
<td>77 (6 mg ic)</td>
<td>—</td>
</tr>
<tr>
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</tr>
<tr>
<td>21</td>
<td>04 (3 mg/kg iv)</td>
<td>77 (10 mg/kg iv)</td>
<td>04 (6 mg ic)</td>
</tr>
</tbody>
</table>

* Abbreviations: 04 = HA1004; 77 = HA1077; iv = intravenous administration; ic = intracisternal administration; — = no treatment.
Results

In Vitro Study

The calcium ionophore A23187 elicited a concentration-dependent contraction of the canine basilar artery strips when administered in concentrations of $10^{-7}$ to $10^{-5}$ M. The basilar artery that had been contracted with A23187 ($4.8 \times 10^{-6}$ M) responded differently to the HA compounds and the entry-blocking calcium antagonists (Fig. 2). The A23187-induced contraction of the canine basilar artery was not antagonized by the entry-blocking calcium antagonists nicardipine, diltiazem, or verapamil at concentrations of $10^{-7}$ to $10^{-5}$ M. However, the HA compounds HA1004 and HA1077 ($10^{-5}$ M) completely antagonized the A23187-induced contraction of the canine basilar artery. The effects of HA1004 and HA1077 on well-matched arterial samples were similar.

In Vivo Study

The diameter of the basilar artery of 21 dogs on angiograms obtained before SAH averaged 1.43 ± 0.05 mm (mean ± standard error). On SAH Day 7, significant vasospasm, with reduction in the arterial diameter of 25% or more, was noted in every case; the diameter and the %diameter were 0.84 ± 0.04 mm and 59% ± 2.1%, respectively. Autopsies at the end of the study demonstrated a dense blood clot encasing the basilar artery and the anterior brain stem in every case.
Table 2 summarizes the results of the study. On intravenous administration, HA1004 produced only mild dilation of the basilar artery of 10% and 11% at doses of 3 and 10 mg/kg, respectively; however, HA1077 produced dilation of 19% and 27% at the same doses, respectively. With intracisternal administration, both HA1004 and HA1077 not only reversed the cerebral vasospasm completely but also produced vasodilation of 12% and 15%, respectively, above the control level.

Time courses of the effects of the HA compounds on basilar artery vasospasm are shown in Figs. 3 and 5. Changes in the mean arterial blood pressure (MABP) on intravenous administration of the compounds are also illustrated (Fig. 3). There was some variation among the dogs as to the peak dilation time, ranging from 15 to 90 minutes after the start of the infusion; however, in all the dogs maximum hypotension occurred at the end of the infusion. Intravenous HA1004 lowered the MABP by 17% (3 mg/kg) and 52% (10 mg/kg) with only a mild dilating effect on the basilar artery (Fig. 3 left). In contrast, intravenous HA1077 produced a greater dilation of the basilar artery in a shorter period of time accompanied by a similar degree of hypotension (Fig. 3 right). The angiograms of representative animals are shown in Fig. 4. Dog 11 (Fig. 4 upper) received 3 mg/kg of HA1077 and showed vasodilation from 15 minutes to 2 hours after administration. The peak dilation occurred at 90 minutes after the drug. Dog 9 (Fig. 4 lower) received 10 mg/kg of HA1077 and showed sustained vasodilation from 15 minutes to 3 hours after administration. Intracisternal administration of the HA compounds completely reversed the cerebral vasospasm without any effect on the blood pressure (Fig. 5). Representative angiograms showed that HA1077 (Fig. 6 upper) was quicker in its vasodilating effect than was HA1004 (Fig. 6 lower). This effect lasted at least 4 hours (Figs. 5 and 6).

Discussion

A variety of pharmacological, mechanical, and electrical stimuli are capable of causing a vascular smooth-muscle cell to contract. Regardless of the type of stimuli, the concentration of cytosolic intracellular Ca++ rises to a critical level, either by the influx of extracellular Ca++ or by its release from intracellular Ca++ storage. Intracellular free Ca++ activates calmodulin by binding to it. Then this activated Ca++-calmodulin complex interacts with the myosin light-chain kinase, triggering the development of cross-linkages between myosin and actin which are associated with increased phosphorylation and mechanical shortening. Vasodilation can be produced by the variety of mechanisms which lower the intracellular Ca++ levels.

The etiology of cerebral vasospasm has not been elucidated. Although there are some investigators who believe that cerebral vasospasm is an acute proliferative vasculopathy or vasculitis, it is generally believed to be caused by vascular smooth-muscle contraction. Because calcium antagonists can affect the fundamental mechanism of a smooth-muscle contraction (that is, calcium influx) regardless of the cause of the vasospasm, they have been used not only in experimental but also in clinical vasospasm.

FIG. 4. Angiograms showing the effects of the intravenous administration of HA1077 on the basilar arteries of two dogs: Dog 11 receiving 3 mg/kg, upper row, and Dog 9 receiving 10 mg/kg, lower row. Compared with angiograms on Day 1 before subarachnoid hemorrhage (A,D), chronic vasospasm is obvious on Day 7, prior to administration of the drug (B,E). Reversal of vasospasm is demonstrated on the angiograms obtained 90 minutes after intravenous infusion of HA1077, 3 mg/kg (C), and 30 minutes after intravenous administration of HA1077, 10 mg/kg (F).

FIG. 5. Time courses of the effects produced by the intracisternal administration of HA1004 and HA1077 (6 mg) on the diameter of the basilar artery. The vasodilation was significant (p < 0.05) after 15 minutes.
TABLE 2
Summary of the effects of the HA compounds on dog basilar artery

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>No. of Dogs</th>
<th>% Arterial Diameter*</th>
<th>Before Drug</th>
<th>After Drug†</th>
</tr>
</thead>
<tbody>
<tr>
<td>intravenous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA1004</td>
<td>3 mg/kg</td>
<td>7</td>
<td>52 ± 3.7</td>
<td>62 ± 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mg/kg</td>
<td>5</td>
<td>63 ± 4.2</td>
<td>74 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>HA1077</td>
<td>3 mg/kg</td>
<td>7</td>
<td>61 ± 2.3</td>
<td>80 ± 3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mg/kg</td>
<td>5</td>
<td>60 ± 3.4</td>
<td>87 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>intracisternal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA1004</td>
<td>6 mg</td>
<td>5</td>
<td>71 ± 2.0</td>
<td>112 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>HA1077</td>
<td>6 mg</td>
<td>5</td>
<td>71 ± 2.4</td>
<td>115 ± 6.8</td>
<td></td>
</tr>
</tbody>
</table>

* Values represent the mean ± standard error. Percentage of the basilar artery diameter on Day 7 before and after treatment compared to that on Day 1 (before subarachnoid hemorrhage).
† Maximum dilated diameter after drug administration.

The calcium antagonists (also known as calcium channel blockers, calcium entry blockers, and slow channel blockers) were first grouped by Fleckenstein, et al., in the late 1960's. Later, Rahwan, et al., proposed that calcium antagonists could be classified into two groups: 1) calcium entry blockers, which act primarily by inhibiting the influx of the extracellular Ca++ into cells through the slow channel in the cell membrane; and 2) intracellular calcium antagonists, which interfere with the physiological functions of the Ca++ by inhibiting the action or mobilization of the intracellular Ca++, by enhancing the sequestration of this cation by intracellular organelles, or by enhancing the efflux from the cells. Most of the calcium antagonists, such as nifedipine, nimodipine, nicardipine, diltiazem, and verapamil, are classified as calcium entry blockers.

In our in vitro study, both intracellular calcium antagonists, HA compounds HA1004 and HA1077, completely antagonized the contraction of the canine basilar artery induced by the calcium ionophore A23187, which transports the Ca++ as a lipid-soluble complex through a number of natural and artificial membranes. However, the entry blocker calcium antagonists did not inhibit the A23187 (4.8 μM)-induced contraction of the canine basilar artery. Furthermore, the HA compounds have antagonized the contraction produced by phenylephrine in a Ca++-free solution, which is considered to induce mobilization of Ca++ from an intracellular pool. Thus, both HA compounds appear to be intracellular calcium antagonists.

The effect of these intracellular calcium antagonists on experimental chronic cerebral vasospasm was studied. In the present study, a "two-hemorrhage" canine model was used which reliably caused severe diffuse vasospasm in the intradural vertebral and basilar arteries 6 days after the first injection of blood. Two different groups of investigators have studied the effects of calcium antagonists on cerebral vasospasm by using this model. Varsos, et al., failed to relieve cerebral vasospasm with high doses of intravenous nifedipine, which lowered the blood pressure by 40%. Gioia, et al., were also unable to resolve vasospasm with high doses of sublingual nimodipine which again lowered blood pressure; however, they did show that intracisternal administration of 10-3 M nimodipine completely reversed cerebral vasospasm. Using another excellent model for chronic cerebral vasospasm in monkeys, Espinosa, et al., and Nosko, et al., also failed to improve the incidence or severity of cerebral vasospasm by the oral administration of nimodipine for 7 days after the injection of blood.

Intracisternally administered HA compounds, like nimodipine, completely reversed cerebral vasospasm in every case. However, in contrast with nimodipine or nifedipine, HA1077, administered in intravenous doses which lowered the blood pressure only slightly, significantly reversed cerebral vasospasm. As far as is known, this is the first drug that can reverse chronic cerebral vasospasm by its systemic use. The results also suggest...
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that cerebral vasospasm is not an irreversible process, at least on the 7th day of SAH, and furthermore that it could be reversed by lowering the intracellular Ca++. HA1004 was less effective than HA1077 and was slower to act on basilar artery vasospasm in dogs, possibly because of its lower affinity to the artery.

Since both HA1004 and HA1077 are light-stable and water-soluble, they are easier to prepare than dihydropyridine derivatives such as nifedipine and nimodipine, which are inactivated by ordinary light and are water-insoluble, thus needing special solvents. There were no serious side effects in the dogs treated under anesthesia in this study. Further investigation of the side effects of these drugs is now being performed. The HA compounds, which are presumed to act on intracellular Ca++, appear to be promising agents for the treatment of cerebral vasospasm.

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References


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