Impairment of Anti-Platelet-Aggregating Activity of Endothelial Cells After Experimental Subarachnoid Hemorrhage

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Background and Purpose: Serial changes of anti-platelet-aggregating activity in the endothelial cells after experimental subarachnoid hemorrhage were studied in 30 feline two-hemorrhage models.

Methods: One hour or 2, 4, 7, or 14 days after mimic subarachnoid hemorrhage, ADP (40 mg/kg) was infused into the basilar artery via the right vertebral artery to activate circulating platelets. Immediately after ADP infusion, the basilar artery was fixed by intra-arterial perfusion with 1.5% glutaraldehyde in 0.1 mol/L phosphate buffer and was removed. The luminal surface was examined under a scanning electron microscope.

Results: One hour after subarachnoid hemorrhage, no platelets adhered or aggregated on the luminal surface. However, 4 to 7 days after subarachnoid hemorrhage, many platelets were observed adhering or aggregating on the luminal surface.

Conclusions: These findings suggest the impairment of anti-platelet-aggregating activity of endothelial cells after subarachnoid hemorrhage. This impairment may be involved in inducing cerebral ischemia during cerebral vasospasm by causing platelet adhesion and aggregation. (Stroke. 1993;24:1541-1546.)

Key Words: cerebral vasospasm • endothelium • platelet aggregation • subarachnoid hemorrhage • cats

Cerebral vasospasm is an important cause of mortality and morbidity in patients with aneurysmal subarachnoid hemorrhage (SAH), but its pathogenesis has not yet been fully clarified.1 Some reports suggest a possible role of the platelet system in this pathogenesis. Time-sequential changes of platelet functions were examined with affirmative results in patients with symptomatic cerebral vasospasm,2,3 and multiple cerebral microthrombi were observed in patients who died of fatal cerebral vasospasm.4 Thromboxane A2, which is synthesized mainly in circulating platelets and released from aggregating platelets, has been suggested to play some role in this pathological condition.6 However, the factors that induce platelet adhesion and aggregation during cerebral vasospasm have not been explained.

Some current investigations indicate the possible role of endothelial damage in cerebral vasospasm. Ultrastructural observation revealed various morphological changes of endothelial cells after SAH.7,8 Disruption of the blood–arterial wall barrier after SAH has also been suggested to be involved in the pathogenesis of cerebral vasospasm.9 Endothelial synthesis of potent vasodilators, such as prostacyclin (prostaglandin [PG] I2) and endothelium-derived relaxing factor (EDRF), has been reported to decrease during vasospasm.10-16

On the other hand, endothelial cells per se are known to have antithrombotic properties, one of which is an inhibitory activity against platelet adhesion and aggregation on the luminal surface (anti-platelet-aggregating activity) by synthesizing EDRF17 and PGI2,18,19 The decrease of EDRF and PGI2 synthesis in endothelial cells, therefore, may bring on the state of platelet adhesion and aggregation during vasospasm. However, a correlation between the decrease of EDRF and PGI2 synthesis in endothelial cells and the impairment of their anti-platelet-aggregating activity in vivo after SAH, is still obscure. In the present study, the serial changes in this activity of endothelial cells after SAH were investigated. ADP was infused intra-arterially to activate circulating platelets in vivo after experimental SAH, and immediately after the ADP infusion the degree of platelet adhesion and aggregation on the arterial luminal surface was observed under a scanning electron microscope (SEM).

Materials and Methods

Thirty adult cats, weighing 2.5 to 4.3 kg, were anesthetized with intraperitoneal injection of sodium pentobarbital (30 to 40 mg/kg). The cats were placed in the lateral position, and the cisterna magna was punctured with a No. 22 needle. After removal of 3 mL of cerebrospinal fluid, fresh autologous nonheparinized
Summary of Experimental Groups and Results

<table>
<thead>
<tr>
<th>Time After SAH</th>
<th>No. of Cats</th>
<th>Intimal Changes, Mean±SD</th>
<th>ADP Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 h</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 d</td>
<td>2</td>
<td>3.2±1.6</td>
<td>0</td>
</tr>
<tr>
<td>4 d</td>
<td>2</td>
<td>12.2±4.8</td>
<td>9.2±4.8</td>
</tr>
<tr>
<td>7 d</td>
<td>2</td>
<td>9.6±4.3</td>
<td>7.9±4.2</td>
</tr>
<tr>
<td>14 d</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

SAH indicates subarachnoid hemorrhage.

arterial blood (3 mL) obtained from the femoral artery was slowly injected over 1 minute, and the cats were kept in the head-down position for 30 minutes to facilitate the blood accumulation in the basal cisterns. Then the cats were permitted to awaken and were returned to the kennel. Forty-eight hours after the first cisternal injection, the second injection was carried out in the same manner.

One hour or 2, 4, 7, or 14 days after the first cisternal blood injection, the cats were similarly anesthetized and were intubated endotracheally to control their respiration with an animal respirator (model R-60, Aika Inc, Tokyo, Japan). The subjects were divided into two groups: the ADP-infused group and the ADP-uninfused group (Table). In the ADP-infused group, a polyethylene catheter was inserted via the right axillar artery, and its tip was advanced to the right subclavian artery at the origin of the right vertebral artery. The right costocervical trunk and the right internal thoracic artery were ligated at their origin, and the subclavian artery was ligated together with the catheter tip so that the catheter became wedge-shaped (Fig 1). Through the catheter, 40 mg/kg of ADP (Sigma Chemical Co, St Louis, Mo) dissolved in 3 mL saline solution was infused into the right vertebral artery over 3 minutes with an auto-

FIG 1. Schematic diagram of feline arterial anatomy and method for infusing ADP.

FIG 2. Photomicrograph of the endothelial surface of the basilar artery taken from a normal control cat. Endothelial cells are arranged regularly and smoothly, and marginal folds (arrowheads) and nuclear protrusions (arrow) are seen. Bar, 10 μm.
matic injector (model STC-521, Terumo Inc, Tokyo, Japan) to activate circulating platelets. Immediately after the end of ADP infusion, the cats were killed by perfusion-fixation, and their brains were taken out. In the other three cats without cisternal blood injection, ADP was infused in the same manner after the basilar artery was exposed transclivally, and, using a surgical microscope, dynamic changes were observed in the caliber of the artery during ADP infusion. Immediately after the completion of ADP infusion, those cats were also killed by perfusion-fixation.

For evaluation of serial morphological changes on the luminal surface of the basilar artery after SAH per se, in the ADP-uninfused group 12 cats were killed by perfusion-fixation without ADP infusion at the same intervals after the first cisternal blood injection.

Scanning electron microscopic observation was conducted as follows. A brief perfusion with saline solution was started through a catheter placed in the ascending aorta, followed by perfusion with 1.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) under a pressure of 130 mm Hg. Immediately after the perfusion-fixation, the brain was taken out, and the basilar artery was dissected. The artery was immersed in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for 24 hours at 4°C and incised longitudinally under a surgical microscope. The specimens were postfixed with 1% osmium tetroxide in 0.1 mol/L phosphate buffer (pH 7.4) for 90 minutes at 4°C, dehydrated in a graded ethanol series, dried at the critical point, and sputter-coated with gold (Eiko Engineering Co, Tokyo, Japan). The luminal surface was observed with an SEM (Hitachi S-405, Hitachi, Tokyo, Japan). In the ADP-uninfused group, the serial changes of the intima after SAH, such as intercellular gap, ballooning, and crater, were examined, counting these findings within a $35 \times 42$-$\mu m^2$ field at a magnification of $\times 2000$ in 10 nonoverlapping areas in each specimen. In the ADP-infused group, the degree of platelet adhesion and aggregation on the luminal surface was mainly examined, counting the adhering platelets within a $35 \times 42$-$\mu m^2$ field at a magnification of $\times 2000$ in 10 nonoverlapping areas in each specimen.
Margins, and intercellular gaps began to appear. Four days after SAH, intercellular gaps became more marked (Fig 3B), and cellular balloonings and crater formations began to be observed (Fig 3C). These changes persisted until 7 days after SAH. Fourteen days after SAH, such findings were minimal with vestigial longitudinal folds, and cellular marginal folds reappeared clearly (Fig 3D).

In the surgical microscopic observation of the transclivally exposed basilar artery in cats without SAH, intra-arterial ADP infusion produced immediate constrictions of the basilar artery and the right vertebral artery where ADP was perfused (Fig 4A and 4B). The luminal surface of the constricted basilar artery showed longitudinal folds under an SEM, which might be a result of this vasoconstriction. However, neither platelet adhesion nor aggregation could be noted (Fig 4C).

When the intra-arterial ADP infusion was conducted on cats with mimic SAH, no platelet adhesion on the luminal surface was observed 1 hour after the first cisternal blood injection. Two days after SAH, however, a few platelets could be observed adhering to the luminal surface (Fig 5A). When ADP was infused 4 days after SAH, a large number of platelets and a few erythrocytes adhered all over the intima (Fig 5B and 5C), and these findings persisted until 7 days after SAH. Fourteen days after SAH, adhesion or aggregation of blood cells became minimal. Time-sequential changes of platelet adhesion or aggregation were well expressed quantitatively (Table).

Discussion

In the present study, an intra-arterial ADP infusion method for activating circulating platelets was introduced to evaluate the serial changes of anti-platelet-aggregating activity in endothelial cells in vivo after experimental SAH, and the degree of platelet adhesion and aggregation on the arterial luminal surface was evaluated with an SEM. The ADP infusion into cerebral vessels has formerly been used in studies for transient ischemic attacks or vascular changes induced by platelet aggregation.20-22 Fujimoto et al.21 studied the vascular changes of the middle cerebral artery by injecting ADP into the carotid artery in rabbits and reported that no vascular injury was recognizable immediately after the ADP injection but at 60 minutes endothelial degeneration with platelet adhesion was seen, and that such changes were not seen when blood in the cerebral circulation was replaced by physiological saline before the ADP injection. Therefore, the possibility of endothelial cell injury by activated platelets or by ADP per se can be excluded by evaluating the vessel wall immediately after ADP infusion.

In the ADP-uninfused group, platelets adhered neither to endothelial cells nor to intercellular gaps after SAH. Therefore, in our experimental SAH model, the intercellular gaps are not suggested to involve exposed basal lamina.

In cats without mimic SAH, constriction of the basilar artery was observed during ADP infusion, and its luminal surface showed longitudinal folds. This vasoconstriction may be caused by mediators released from activated platelets such as thromboxane A₂, serotonin, and PGF₂α, or by ADP direct effect. But no platelet adhesion on the luminal surface could be seen. In the ADP-infused group, platelet adhesion on the luminal
surface was scarcely observable until 2 days after SAH, while marked platelet adhesions or aggregations were seen 4 to 7 days after SAH. These findings suggest that the anti–platelet-aggregating activity of endothelial cells was impaired 4 to 7 days after SAH. In the ADP-uninfused group, on the other hand, cellular balloonings, crater formations, and intercellular gaps were notable 4 to 7 days after SAH. Such morphological changes are considered to be associated with the functional impairments of endothelial cells and to represent their regressive degeneration. However, this functional impairment may not be irreversible, because platelet adhesions on the luminal surface were reduced 14 days after SAH. Sasaki et al\textsuperscript{16} reported that the synthetic activity of PGI\textsubscript{2} in the canine basilar artery exposed to subarachnoid blood injection had diminished remarkably on days 3 and 8. Nakamura et al\textsuperscript{14} indicated that the 6-keto-PGF\textsubscript{1}\textalpha levels in the feline basilar and pial arteries had decreased after the production of SAH. Nakagomi et al\textsuperscript{15} reported that the vasodilatory response to ATP of the basilar artery was suppressed in the rabbits killed 2 days after SAH and suggested that the production of EDRF by endothelial cells was inhibited after SAH. Results of our study correlated well with those in vitro studies.

We reported that the patients with delayed ischemic symptoms due to cerebral vasospasm after aneurysmal SAH showed platelet hyperactivity.\textsuperscript{3} In addition, multiple cerebral microthrombi were observed in patients who died of cerebral vasospasm.\textsuperscript{4,5} Furthermore, some degree of efficacy of antiplatelet drugs in the prevention of symptomatic cerebral vasospasm was reported.\textsuperscript{6,22} Therefore, the platelet system may play an important role in cerebral ischemia during cerebral vasospasm. However, the factor that induces platelet adhesion and aggregation during cerebral vasospasm has not been explained. Our results suggest that the impairment of anti–platelet-aggregating activity of endothelial cells can be a factor causing platelet adhesion and aggregation during cerebral vasospasm.

References


Editorial Comment

Ohkuma et al show enhanced platelet adhesion and aggregation in the basilar artery after experimental subarachnoid hemorrhage (SAH). They suggest that loss of antiplatelet properties of endothelium causes this enhanced adhesion/aggregation. They also suggest that the adhesion/aggregation may be clinically important. One of the antiplatelet substances produced by endothelium and lost as a result of SAH is so-called
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