Adventitial Red Blood Cells Produce Intimal Platelet Accumulation in Cerebral Arteries of Cats Following Subarachnoid Hemorrhage

Yoshihiro Yamamoto, MD; Ben R. Clower, PhD; Joseph L. Haining, PhD; Shoji Asari, MD; and Robert R. Smith, MD

After dividing 21 cats into three equal groups, we exposed their right middle cerebral arteries transorbitally and adventitiously irrigated them with 2 ml washed red blood cells, blood plasma, or saline. To determine arterial intimal platelet accumulation in each cat, we injected [111In]oxine-labeled platelets intravenously immediately before injecting the various experimental solutions. Animals were sacrificed 2 or 4 hours following the injection of labeled platelets. Irrigation with washed red blood cells produced a significantly greater intraluminal accumulation of platelets than irrigation with saline (p<0.05). Plasma tended to have less of an effect on platelet accumulation than washed red blood cells, but this difference was not significant. These data suggest that the adventitial blood fraction responsible for intimal platelet accumulation in cerebral arteries following subarachnoid hemorrhage may be derived mainly from the red blood cell fraction. (Stroke 1991;22:373-377)

Cerebral vasospasm remains a major cause of morbidity and mortality in patients suffering subarachnoid hemorrhage (SAH) from a ruptured intracranial aneurysm. This constrictive vascular disorder occurs a few days after the initial bleeding and may persist for days or weeks. Whether this phenomenon is a result of physiologic or anatomic alterations is not clear. The fact that constricted vessels have been resistant to pharmacologic vasodilation strongly suggests a structurally based condition.

There is general agreement, however, that the disorder results only after cerebral arteries are exposed to blood in the subarachnoid space. The precise blood fraction responsible for the constriction has not been identified.

The results of our clinical and experimental studies have clearly and consistently demonstrated a variety of pathologic changes in major cerebral arteries following SAH. Since the occurrence of many of these alterations are not out of phase with the time sequence of clinical events, we believe these changes to be relevant to cerebral vasospasm. Our studies also indicate a correlation between intimal platelet accumulation and pathologic changes in vessels following experimental SAH.

Platelets accumulate on the intraluminal side of the vessel wall within minutes after SAH. The degree of platelet accumulation appears to be related to both the amount of subarachnoid blood and, subsequently, the severity of vessel alterations. Platelets contain various spasmogenic agents and growth factors capable of altering both the physiologic and anatomic natures of a vessel. Whether platelet accumulation can be considered a cause of morphologic alterations or whether it occurs as a secondary response to these changes is unclear. In either case, intimal platelet accumulation appears to be a reliable marker indicating endothelial damage due to yet unidentified toxic substance in blood.

Employing radioactive techniques, we have previously demonstrated that whole blood placed on the adventitia of cerebral arteries in vivo results in intimal platelet accumulation. This study examines the effect of various blood fractions in producing a similar response.

Materials and Methods

Twenty-one mongrel cats weighing 2.5-3.5 kg were divided randomly into three groups of seven cats each. Groups I and II were subjected to the subarachnoid injection of washed red blood cells (RBCs)
and blood plasma, respectively, while group III was subjected to the subarachnoid injection of saline. Each cat received 2 ml of the respective solution. These studies are in compliance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" and were approved by the local Institutional Animal Care and Use Committee.

The method for introducing solutions into the subarachnoid space was based on the transorbital injection of 2 ml of each blood fraction or saline into the cisternal space in the region of the proximal main trunk of the right middle cerebral artery (MCA). Each cat was anesthetized with a combination of 22 mg/kg i.m. ketamine hydrochloride and 5.5 mg/kg i.m. xylazine. The head was immobilized in a stereotactic instrument, and the right eye was removed. After orbital exenteration, a small (4 mm) craniectomy was performed just superior to the right optic canal, exposing a short segment of the main trunk of the right MCA proximal to the lateral fissure. Following vessel exposure, 2 ml of the respective solution was injected onto the adventitial surface of the artery.

Washed RBCs or plasma were prepared from autologous blood collected from the femoral artery. Four milliliters of arterial blood was collected in a 17×100 mm sterile polystyrene tube containing 0.7 ml citric acid and centrifuged at 200g for 10 minutes. The supernatant plasma was removed and used for platelet preparation and injection in group II. For group I, the packed cells were resuspended in sterile physiologic saline to the original blood volume (4 ml) and centrifuged at 200g for 10 minutes. The supernatant was discarded, and the washing process was repeated once. After the second washing, the packed cells were suspended in sterile saline again to a volume of 4 ml; 2 ml of the suspension was injected into the subarachnoid space.

To determine platelet accumulation in the cerebral arteries, indium-111–labeled platelets were prepared according to the technique of Hawker et al except that commercially available [111In]oxine was used as the labeling agent. The labeled platelets were injected intravenously immediately before the transorbital injection of the above-mentioned solutions. In each group, the cats were sacrificed 2 or 4 hours following the injection of labeled platelets.

At sacrifice, each cat was given 100 mg i.m. ketamine, and the femoral vein was exposed and infused with 150 mg sodium pentobarbital. After excision of the brain, measured segments of equal length (approximately 15 mm from the origin) of both the right and left MCAs were dissected from their vascular beds. The vessel segments were placed in 12×75 mm tubes containing 2.5% phosphate-buffered glutaraldehyde solution. The radioactivity of each segment as counts per minute was measured by using an automatic gamma counter (Model 1185, Searle Analytic, Inc., Des Plaines, Ill.). After correction for background, radioactivity of the right MCA segment was divided by that of the left MCA segment to produce the radioactivity ratio. This ratio is an important figure inasmuch as the left MCA is an internal control. As long as the ratio is significantly greater than 1, preferential accumulation of platelets in the right MCA is indicated regardless of the amount of radioactivity. Since the samples were small, a randomization test as well as a Kruskal-Wallis test were performed to determine significant differences in the radioactivity ratio among the groups.

### Results

The net radioactivity of the MCA segments and the radioactivity ratio in all 21 cats are listed in Table 1; the mean and standard deviation of the radioactivity ratio for each group are plotted in Figure 1. There was a significant difference between groups I and III (p<0.05, Kruskal-Wallis test). Cats in group II tended to show smaller radioactivity ratios than those in group I, but the group means failed to differ significantly.

The results of the randomization test are summarized in Table 2. There were no significant differences in the radioactivity ratio between groups II and III. There were significant differences, however, between groups I and III (p<0.05). This study showed

### Table 1. Net Radioactivity of Middle Cerebral Artery Segments and Radioactivity Ratio in 21 Cats

<table>
<thead>
<tr>
<th>Cat</th>
<th>Time (hr)</th>
<th>Right (cpm)</th>
<th>Left (cpm)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A98</td>
<td>2</td>
<td>868</td>
<td>332</td>
<td>2.61</td>
</tr>
<tr>
<td>B13</td>
<td>2</td>
<td>410</td>
<td>209</td>
<td>1.96</td>
</tr>
<tr>
<td>B18</td>
<td>2</td>
<td>357</td>
<td>150</td>
<td>2.38</td>
</tr>
<tr>
<td>A87</td>
<td>4</td>
<td>759</td>
<td>415</td>
<td>1.83</td>
</tr>
<tr>
<td>A67</td>
<td>4</td>
<td>574</td>
<td>339</td>
<td>1.69</td>
</tr>
<tr>
<td>A59</td>
<td>4</td>
<td>368</td>
<td>213</td>
<td>1.73</td>
</tr>
<tr>
<td>A74</td>
<td>4</td>
<td>515</td>
<td>402</td>
<td>1.28</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A88</td>
<td>2</td>
<td>278</td>
<td>765</td>
<td>0.36</td>
</tr>
<tr>
<td>B01</td>
<td>2</td>
<td>1,989</td>
<td>1,756</td>
<td>1.08</td>
</tr>
<tr>
<td>A95</td>
<td>2</td>
<td>241</td>
<td>97</td>
<td>2.48</td>
</tr>
<tr>
<td>A99</td>
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<td>1.07</td>
</tr>
<tr>
<td>A98</td>
<td>4</td>
<td>270</td>
<td>306</td>
<td>0.88</td>
</tr>
<tr>
<td>B17</td>
<td>4</td>
<td>218</td>
<td>119</td>
<td>1.83</td>
</tr>
<tr>
<td>B20</td>
<td>4</td>
<td>902</td>
<td>552</td>
<td>1.63</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F10</td>
<td>2</td>
<td>430</td>
<td>479</td>
<td>0.90</td>
</tr>
<tr>
<td>P02</td>
<td>2</td>
<td>42</td>
<td>39</td>
<td>1.08</td>
</tr>
<tr>
<td>B20A</td>
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<td>477</td>
<td>527</td>
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<tr>
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<td>252</td>
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</tr>
<tr>
<td>Q47</td>
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<td>8</td>
<td>6</td>
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<tr>
<td>B31</td>
<td>4</td>
<td>272</td>
<td>464</td>
<td>0.59</td>
</tr>
<tr>
<td>B28</td>
<td>4</td>
<td>71</td>
<td>148</td>
<td>0.48</td>
</tr>
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</table>
TABLE 2. Statistical Comparison of Radioactivity Ratio for Three Groups of Cats Using Randomization Test

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Statistic compared</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups I and II</td>
<td>Mean</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.058</td>
</tr>
<tr>
<td>Groups I and III</td>
<td>Mean</td>
<td>0.00087</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.0117</td>
</tr>
<tr>
<td>Groups II and III</td>
<td>Mean</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.441</td>
</tr>
</tbody>
</table>

Group I, injection of washed red blood cells; group II, injection of blood plasma; group III, injection of saline.

FIGURE 1. Mean±standard deviation of radioactivity ratio (counts per minute in right middle cerebral artery segment divided by that in left segment [RMCA/LMCA]) of each group of cats. RBC, red blood cells. *p<0.05 different from group III by Kruskal-Wallis test.

Discussion

No single mechanism has been identified that proves the cause or pathogenesis of chronic vasospasm after SAH.1-7,9,12-27 Whether the observable luminal narrowing is attributed to the contraction of vascular smooth muscle cells or results from some organic change in the arterial wall remains controversial. In human and animal models, there appears to be little doubt that pathologic changes occur in cerebral arteries following SAH due to aneurysmal rupture, induced vessel trauma, or cisternal blood injection.1-7,12-19

Many authors have stressed that luminal platelets could be important in the pathologic process occurring after SAH.2-3,7,9,19,20,28 Although the precise kinetics of platelet accumulation have not been established, platelets are known to aggregate on the intimal surface of cerebral arteries within hours following SAH.7,9 Platelets contain norepinephrine, serotonin, and various growth factors, all capable of producing vascular alteration under altered biophysical conditions.23,38,28 Platelet-derived growth factor in particular promotes both the proliferation and migration of cellular elements into the subintimal zones.28,29 In addition, aggregated platelets may also create emboli, resulting in cerebral infarction distally in the arterial tree. Thus, the extent of platelet aggregation within an arterial segment may well serve as a positive linear marker for ongoing endothelial injury. Our previous studies indicate a correlation between intimal platelet accumulation and vessel wall changes following experimental SAH.2-3,7

The cat model described here produces consistent intimal platelet accumulation, as well as structural alterations, in the cerebral arteries following SAH.2-3,7,9 Transorbital rupture of the right MCA, resulting in SAH, induced an early and prolonged (up to 23 days) accumulation of platelets.7 A more recent study revealed that whole blood placed on the adventitial surface of intact cerebral arteries resulted in platelet aggregation to a degree comparable to that after vessel rupture in the early stages following SAH.5 This suggests that the noxious agents responsible for endothelial injury and, perhaps, the subsequent pathologic changes arise primarily from the abluminal surface of the cerebral arteries.9

Our data show that the adventitial blood fraction responsible for platelet accumulation may be derived mainly from the RBC compartment. This notion is further strengthened by our observation that washed RBCs induced intimal platelet accumulation to a degree comparable to that produced by whole blood in a previous study.9 Adventitial platelets could also induce intraluminal platelet accumulation, as shown by group II. However, the radioactivity ratios of cats in group II tended to be smaller than those of cats in group I. If the \( p<0.1 \) level of significance had been accepted to increase the power of the tests, group II would have differed significantly from group I while there would have been no difference between groups II and III. Although there is insufficient evidence to reject the null hypothesis at the usual \( p<0.05 \) level, there is enough evidence to suggest that accepting the null hypothesis of no difference between groups I and II would be a mistake.11 Whatever is in RBCs that causes preferential accumulation of platelets in the right MCA may very well also be present in plasma, but at lesser concentrations, and may vary from cat to cat. Further studies to elucidate this possibility will address the accuracy of the statistical analysis.

To our knowledge, there have been no reports indicating that RBCs induce intimal platelet accumulation. It is well known, however, that RBCs and their hemolysate in the blood clot covering the cerebral arteries contain vasoconstrictive substances that have the potential to contract smooth muscle cells.20-23 Fujii and Fujitsu34 demonstrated that oxyhemoglobin preferentially contracts cultured smooth muscle cells. It has also been demonstrated that hemoglobin (Hb)
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Our understanding of mechanisms involving platelets by 7-9 days.30-31 Further studies employing Hb deriv-
ate over the next several days, then gradually diminishes by 7-9 days.30,31 Further studies employing Hb deri-
atives or RBC membrane components may enhance our understanding of mechanisms involving platelets as an initiator of chronic vasospasm and the related pathologic changes in the vessels.

Acknowledgments

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