Accumulation of Intimal Platelets in Cerebral Arteries Following Experimental Subarachnoid Hemorrhage in Cats

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From 2 hours to 23 days following experimental subarachnoid hemorrhage, the accumulation of indium-111-labeled platelets on the intimal surface of the middle cerebral artery was studied in 23 cats. Subarachnoid hemorrhage was produced by transorbital rupture of the right middle cerebral artery. Of the 23 cats, 17 exhibited right middle cerebral artery/left middle cerebral artery radioactivity ratios of >1.25. When these results were compared with those of 12 control cats, 0.001 < p < 0.005 (χ² test). Thus, the results from the control and experimental groups are significantly different and indicate early (after 2 hours) preferential accumulation of intimal platelets in the ruptured right middle cerebral artery compared with the unruptured left middle cerebral artery and new platelet deposition continuing for up to 23 days. However, the experimental group did not reveal a clear pattern for platelet accumulation following subarachnoid hemorrhage. There was no simple correlation between the magnitude of the radioactivity ratios and the time after hemorrhage when the cats were killed although the ratios for 2 hours to 7 days seemed greater than those for 8 to 23 days. Assuming the pivotal role of platelets in the angioopathy of subarachnoid hemorrhage, the administration of antiplatelet agents as soon as possible following its occurrence may be of value. (Stroke 1988;19:898-902)

The leading cause of death and disability in patients suffering from aneurysmal subarachnoid hemorrhage (SAH) is vasospasm of one or more major cerebral arteries.1 This condition remains largely unmanageable because of poor understanding of its pathogenesis.2 The conclusion of a recent review on the current management of cerebral aneurysms corroborates our own studies, which indicate that chronic and irreversible vasospasm following SAH is not due primarily to physiologic muscular constriction but results from anatomic alterations within the cerebral arterial wall.3 Further, the chronic angiographic arterial narrowing observed following SAH correlates well with pathologic events occurring within the vessel.4,5

The pathogenesis of structural changes in cerebral arteries following SAH is unknown. The interaction between extravascular and/or intravascular blood components and the arterial wall may play a significant role.6,7 The idea that platelets could be important in this process has been stressed.8-10 Whereas the precise kinetics of platelet accumulation have not been delineated, platelets are known to aggregate on the intimal surface of cerebral arteries within minutes or hours after SAH.9,11-13 This platelet accumulation is accompanied by early and definite pathologic changes, particularly endothelial damage, in the cerebral artery.13-21 Platelet aggregation is thought to occur as a physiologic response to endothelial injury. Whether platelets mediate subsequent structural change in deeper layers of the arterial wall is unclear. Platelets contain bioactive substances such as norepinephrine, virtually all of the serotonin in the blood, and platelet-derived growth factors (PDGFs), all capable of producing changes within the cerebral artery under altered biophysiologic conditions.8,22-25

Since platelets have been implicated as key agents in the production of pathologic changes in cerebral arteries following SAH, a better knowledge of their accumulation pattern (time sequence and quantitative deposition) is of paramount importance. Intimal platelet adhesion in cerebral arteries early after SAH is a well-recognized and well-documented phenomenon. However, it has not been established
whether subsequent events in the artery involve intimal platelet adhesion. By using indium-111–labeled platelets, our study examines their accumulation pattern on the intimal surface of the middle cerebral artery of cats early and late after experimental SAH.

**Materials and Methods**

Thirty-five mongrel cats (2.5–3.5 kg) were divided randomly into three groups (two controls and one experimental) for our study. Control Group IA consisted of six intact, unoperated cats, whereas control Group IB consisted of six sham-operated cats: a microhook was placed into the wall of the right middle cerebral artery (RMCA) but was never pulled. Group II consisted of 23 cats subjected to experimental SAH (microhook pulled) and later killed between 2 hours and 23 days after SAH. 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 of producing SAH has been described and is based on the transorbital rupture of the RMCA. Each cat was anesthetized with a combination of 22 mg/kg i.m. ketamine and 5.5 mg/kg i.m. xylazine, intubated, and allowed to breathe spontaneously; its head was immobilized in a stereotactic instrument. The right eye was then 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 RMCA proximal to the lateral fissure. A microhook made from a sharpened 27-gauge needle with attached suture was placed through the wall of the artery and out again; the attached suture was led out through the cranietomy. The orbit was packed with gelatin sponge so that the cranial space remained closed. Seven days were allowed for healing. Following preparation (microhook placement), each cat received 7.5 mg/kg i.m. meperidine hydrochloride every 8–12 hours for 5 days after surgery. After this 7-day healing period, the cat was lightly anesthetized with ketamine and SAH was produced by pulling the microhook from the vessel wall by means of the suture that had been left in the orbit.

To determine intimal platelet accumulation in the cerebral arteries, indium-111–labeled platelets were prepared according to the technique of Hawker et al except that commercially available indium-111 oxine (Amersham Corp., Arlington Heights, Illinois) was used as the labeling agent. Four milliliters of autologous arterial blood were supplied the 1 ml citrated platelet-poor plasma in which the labeled platelets were resuspended for return to the cat. Between 70 and 90 μCi indium-111 were used to label each batch of platelets. Labeling efficiency was usually ≥80% measured by use of 0.01 ml of resuspended labeled platelets and an aliquot of supernatant from the labeling medium. The remaining (0.99 ml) labeled platelet suspension was immediately infused into the donor cats via the femoral vein. Because of the half-life of indium oxine (2.8 days), cats were killed as follows. Group IA and IB cats were killed 24 hours after infusion of the labeled platelets. Group II cats that were killed ≤48 hours after SAH received their labeled platelets immediately after rupture of the RMCA. When a Group II cat was killed >48 hours after SAH, the labeled platelets were prepared and infused 24 hours before sacrifice.

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 origination) of both the RMCA and left middle cerebral artery (LMCA) were dissected from their vascular beds. The vessel segments were placed in 12×75 mm tubes containing 2.5% phosphate-buffered glutaraldehyde solution, and their radioactivities in counts per minute were measured by use of a Searle Analytic, Inc. Model 1185 automatic gamma counter (Des Plaines, Illinois). After correction for background, radioactivity of the RMCA segment was divided by that of the LMCA segment to produce the radioactivity ratio. This ratio is the important figure inasmuch as the LMCA is an internal control. As long as the ratio is significantly >1, preferential accumulation in the RMCA is indicated regardless
of the magnitude of radioactivity. The ratio for each cat was scored as positive (preferential aggregation of platelets in the RMCA) if it was >1.25 and as negative (no difference in platelet aggregation) if it was <1.25. The \( x^2 \) test (2\( \times \)2 table) with Yates' correction\(^2^7\) served to determine if there were significant differences among the groups. A positive score indicated that preferential platelet accumulation in the RMCA compared with the LMCA.

### Results

Radioactivity ratios for Groups IA and IB are given in Table 1. There were no significant differences between the two control groups. Thus, microhook implantation alone (without SAH) did not result in preferential platelet accumulation in the RMCA compared with the LMCA.

The results for Group II are given in Table 2. Of the 23 cats subjected to SAH, 17 exhibited radioactivity ratios of >1.25. When these results were compared with those of pooled Groups IA and IB, 0.001<\( p < 0.005 \). Even when the control results were not pooled, \( p < 0.05 \). Thus, the results from the control and experimental groups are significantly different and indicate preferential accumulation of platelets in the ruptured RMCA for as long as 23 days after SAH.

On the other hand, the data for Group II did not reveal a clear pattern for platelet accumulation following SAH. There was no simple correlation between the magnitude of the radioactivity ratio and the time after SAH when the cat was killed, although the radioactivity ratios for \( \leq 7 \) days after SAH seem to be greater than those of \( \geq 8 \) days after SAH.

### Discussion

The pathologic changes seen in cerebral arteries following SAH are similar to those seen in early atherosclerosis. Such changes include endothelial injury, intimal platelet aggregation, subintimal smooth muscle proliferation, and fibrosis. In both conditions, the interaction between platelets and the arterial wall may play a major causal role.\(^8\)-\(^1^0\)

Following aneurysmal rupture, the artery may undergo an acute contraction similar to that produced mechanically in the laboratory or by irrigation with blood or blood products. This immediate constriction may be related to direct vessel wall nerve injury and/or the sudden release of vessel wall catecholamines. Endothelial cell injury and intimal platelet accumulation sometimes follow.\(^2^8\)

The endothelial injury may result from several other potential sources, including ischemia due to the initial vessel constriction or norepinephrine and serotonin released from intimal platelets.\(^1^5\),\(^2^2\),\(^2^4\) Since both angiographic and clinical vasospasm occur in association with a ruptured intracranial aneurysm,\(^5\),\(^2^9\) mechanical trauma may play a primary role. As endothelial cells degenerate, the underlying subendothelium is exposed to circulating elements. PDGF released by the aggregating platelets promotes the migration and proliferation of medial smooth muscle cells into the subintimal layer of the vessel.\(^9\) As this proliferation continues, the vessel's lumen becomes anatomically narrowed. However, functional proliferating intimal smooth muscle cells and intimal myofibroblasts may reduce lumen diameter much earlier after SAH than can be attributed to arterial wall thickening alone.\(^4\) Platelet aggregation and release of PDGFs at sites of endothelial injury are necessary for intimal smooth muscle proliferation,\(^3^0\)-\(^3^2\) but the effect of antiplatelet drugs in preventing chronic vasospasm and related structural changes following aneurysmal SAH is still unclear. However, a recent report does establish a positive linear correlation between arterial platelet deposition in vivo and localized vasospasm and demonstrates the beneficial effect of platelet inhibitor therapy.\(^3^3\)

After endothelial injury, such as that which follows aneurysmal rupture, platelet-induced smooth muscle proliferation develops rapidly, and once initiated, may remain active for 3 months.\(^3^4\) This laboratory finding correlates well with the clinical observation of a significant delay in the development of cerebral infarction produced by vasospasm following SAH.\(^5\),\(^3^3\) Since our results show an early and prolonged accumulation of intimal blood platelets after SAH, the implementation of early and continuous antiplatelet therapy at that time could prove worthwhile.
Various aspects of platelet kinetics (such as aggregation, adherence to damaged endothelium, thrombus formation, and platelet survival) have been studied primarily in large, extracranial, peripheral arteries. Although numerous studies have reported the early accumulation of intimal platelets in cerebral arteries following SAH, there are no reports of sequential platelet accumulation over the extended period we used in our study. Our results indicate an early preferential accumulation of platelets in the ruptured RMCA (compared with the unruptured LMCA) and new deposition continuing for at least 23 days after SAH. The accumulation pattern was highly variable between cats, however. The lack of a clear correlation between the amount of radiolabel deposited in the cerebral artery at a given time after SAH in different cats may well be due to our method of producing SAH. That is, the severity of the vessel tear was not necessarily the same in each cat. Thus, the magnitude of SAH may vary between cats.

The late and continuing deposition of platelets seen after SAH raises an obvious question. Since platelet aggregation is positively correlated with endothelial damage, our study suggests that endothelial cell loss may be an ongoing process, lasting for weeks after SAH. The agent(s) responsible have not been delineated, but the time course over which aggregation takes place is not out of phase with resolution of the subarachnoid blood clot, the clinical course, or angiographic patterns after SAH. In many cases, angiographic constriction is seen well into the second month after SAH. Of the pathologic changes in cerebral arteries following SAH, severe subintimal smooth muscle proliferation could well account for late, irreversible arterial narrowing. The initiation and propagation of this proliferative muscular response depends on an interaction of platelets and a damaged arterial wall.

References

KEY WORDS • cerebral arteries • platelet aggregation • subarachnoid hemorrhage • cats
Accumulation of intimal platelets in cerebral arteries following experimental subarachnoid hemorrhage in cats.
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doi: 10.1161/01.STR.19.7.898

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/19/7/898

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