Induction of Transient Neurological Dysfunction in Baboons by Platelet Microemboli

Christof Kessler, MD; Andrew B. Kelly, DVM; William D. Suggs, MD; Joseph D. Weissman, MD; Charles M. Epstein, MD; Stephen R. Hanson, PhD; and Laurence A. Harker, MD

Background and Purpose: To investigate experimental mechanisms of reversible cerebral dysfunction, we produced transient focal cerebral ischemia in five baboons by unilateral perfusion of internal carotid territories with platelet microemboli generated endogenously.

Methods: Platelet microemboli were formed by incorporating segments of Dacron vascular graft for 1 hour as unilateral carotid arterio-arterial shunts. Platelet embolization was assessed by ultrasonography and isotopic imaging; cerebral function was evaluated by measurements of somatosensory evoked potentials and clinical motor performance.

Results: Platelet microemboli, detected by transcranial Doppler ultrasonography, accumulated rapidly in the shunted carotid hemispheric territory. Indium-lll-labeled platelets reached a maximum value of \(3.2\pm0.8\times10^8\) platelets in the dependent hemisphere of five animals after 20 minutes of carotid blood flow through the grafts when measured in real time by continuous scintillation camera imaging. The retained \(^{111}\text{In}-\text{platelet microemboli cleared from the cerebral vasculature within 1 hour after removing the grafts. Corresponding blood markers of in vivo thrombus formation, }\beta\text{-thromboglobulin, platelet factor 4, and fibrinopeptide A, increased eightfold to 20-fold after incorporating graft segments and normalized within 1 hour after removing the grafts. Coincidentally, focal neurological function was temporarily impaired, as shown by the ipsilateral loss of somatosensory evoked potentials 5 minutes after initiating platelet microembolization, with restoration 1 hour after removing the grafts in five baboons, and contralateral hemiparesis in two recovered baboons, with complete resolution by 24 hours.}

Conclusions: Endogenously generated platelet microemboli accumulate transiently in the dependent cerebral circulation and produce corresponding focal neurological dysfunction that resolves within hours after microembolization. (Stroke 1992;23:697-702)

KEY WORDS • blood platelets • embolism • baboons

Whereas cardiogenic strokes typically present as unheralded thromboembolic events,1-3 other ischemic strokes are commonly preceded by transient ischemic attacks (TIAs).4-5 These transient episodes of cerebral ischemia have generally been attributed to platelet microemboli arising from atherothrombotic lesions in carotid arteries or aorta,6-8 although the putative relation between platelet microembolization and reversible neurological dysfunction has not been directly established experimentally. To test this postulated mechanism of transient cerebral ischemia in vivo, we studied the effects of endogenous platelet microemboli on cerebral function in baboons. The nonhuman primate was used in modeling reversible cerebral ischemic episodes to simulate relevant human vascular responses and blood thrombotic processes in producing experimental ischemic events.7-10

The experiments were designed to assess neurological function during and after transient unilateral perfusion of carotid artery territories with endogenously generated platelet microemboli, using the nonembolized contralateral hemispheres as concurrent controls. Neurological function was measured as changes in somatosensory evoked potential (SEP) during embolization and by clinical scoring of motor function in subsequently awakened animals. Platelet microemboli were generated by segments of Dacron vascular graft incorporated as unilateral carotid arterio-arterial (A-A) shunts and perfused at physiological carotid blood flow rates. Embolization was documented by Doppler flow analysis and measured as the hemispheric accumulation of autologous indium-111-labeled platelets using scintillation camera imaging of the affected territories.

Materials and Methods

We used five juvenile male baboons (Papio cynocephalus) weighing 12-18 kg as experimental animals. They were observed to be free of disease for at least 3
months. All experiments were previously approved by the Institutional Care and Use Committee and complied fully with the procedures and methods outlined in the National Institutes of Health publication 85-23, "Guide for the Care and Use of Laboratory Animals," as well as Public Health Service policy, the Animal Welfare Act, and applicable University policies.

Anesthesia was carried out using ketamine hydrochloride for induction (20 mg/kg) and halothane (1-2%) in oxygen with endotracheal intubation. Under aseptic conditions one carotid artery was isolated in each baboon, and a temporary nonthrombogenic A-A shunt composed of sterile, saline-prefilled silicone rubber tubing, 3 mm i.d. (Dow Corning Corp., Midland, Mich.) and approximately 75 cm long, was placed in the common carotid artery (Figure 1). This length of vascular graft was selected because it generated abundant amounts of platelet microemboli.1 Blood flow was measured using a Doppler blood flowmeter (model T206, Transonic Systems Inc., Ithaca, N.Y.).

After 1 hour the segment of Dacron graft was removed; blood flow through the temporary shunt of silicone rubber tubing was maintained for a second hour during which no microembolization was detected by Doppler assessment and gamma camera imaging, SEPs normalized, and the results of blood thrombosis tests fell to basal levels. Immediately thereafter, the carotid A-A silicone rubber shunt was removed, the artery repaired, and blood flow restored (confirmed by Doppler study). Subsequently, the complete cerebral vasculature and entire brain of each of three baboons was pressure-perfusion fixed for histological studies (see below). The two remaining baboons were clinically recovered from anesthesia and serially evaluated for clinical neurological function (see below); 24 hours later the cerebral vasculature and brain of each baboon underwent pressure-perfusion fixation for histology.

Autologous baboon platelets were labeled with $^{111}$In-oxine using a technique described previously; 1.5 mCi $^{111}$In-platelets were injected 1-2 hours before placing the shunt. Images of the brain were obtained in a flexed posteroanterior position with a General Electric 400 T MaxiCamera (General Electric Co., Milwaukee, Wis.) interfaced with a Medical Data System and A2 image processing computer (Medtronic, Ann Arbor, Mich.). A medium energy collimator was used to capture both $^{111}$In energy peaks (172 keV and 247 keV) with 15% energy windows. The images were recorded at intervals of 5 minutes. Scintigraphic images were also acquired of 4-ml samples of whole blood (blood standard). $^{111}$In-platelet deposition onto the Dacron vascular grafts was also measured after removing the graft from the shunt and clearing it of luminal blood.

The $^{111}$In-platelet activity in each hemisphere was determined by measuring $^{111}$In-platelet activity (counts per minute) in standardized regions of interest (ROI) encompassing the entire hemisphere. From each hemispheric ROI an equal-sized contralateral background ROI was subtracted, and the total number of deposited platelets (labeled plus unlabeled) was calculated by multiplying the plateau count per milliliter of whole blood. Subtraction of the number of deposited platelets in the unaffected hemisphere from the number of platelets in the affected hemisphere resulted in the estimation of the net number of embolized platelets at each time point.

Median nerve SEPs were continuously measured with a Nicolet 1170 signal averager (Nicolet Biomedical, Madison, Wis.) and PS11 amplifier (Grass Instruments, Quincy, Mass.). The scalp was shaved and carefully cleaned with alcohol. Needles were inserted into the scalp 1 cm posterior to the coronal suture and 1.5-2 cm lateral to the midline with the exact position adjusted.
for maximum SEP amplitude. A reference needle electrode was inserted into the scalp 1–2 cm superior to the nasion. A needle ground electrode was placed proximal in the stimulated limb. The stimulating bar electrode was placed at the volar surface of the wrist in the contralateral arm. Typically, 256 stimuli were delivered at a rate of 5.9 per second. The high and low bandpass filter settings were 10 and 1,500 Hz, respectively. Amplitudes were measured from the baseline to the peak of the large negative wave.

Transcranial Doppler sonography was performed using a Transpect TCD (Medasonics, Fremont, Calif.) with a 2-mHz probe. Continuous measurements of the blood flow velocities in the internal carotid artery were obtained in the transorbital window in the embolized side in 4.0–5.0-cm depth throughout the entire period of $^{111}$In-platelet imaging. Because of the thick temporal bone in baboons, measurements in the middle cerebral artery were of variable quality.

Before and 60 and 120 minutes after inserting the segments of Dacron vascular graft, platelet counts, white cell counts, plasma fibrinogen levels, and activated partial thromboplastin time were measured. Fibrinogen was estimated as total thrombin clottable protein. Activated partial thromboplastin time was assayed using Thrombosil I (Ortho Diagnostic Systems, Raritan, N.J.).

As markers of thrombus formation in vivo, the plasma levels of $\beta$-thromboglobulin, platelet factor 4, and fibrinopeptide A were measured from blood collected in an inhibitor/anticoagulant mixture. $\beta$-Thromboglobulin and platelet factor 4 were measured by commercially available radioimmunoassays (Amersham, Arlington Heights, Ill., and Abbott Laboratories, Chicago, Ill., respectively). The fibrinopeptide A levels were measured by radioimmunoassay as described elsewhere.

Two baboons were recovered after surgically closing their carotid artery shunt sites, and neurological function was objectively assessed at 60 minutes and 12 and 24 hours after they regained consciousness. The examination used the quantitative neurological scale as developed by Spezler et al. Whole brain in situ pressure-perfusion fixation was performed in three animals using a method described previously. Fixed brain tissue was obtained from all areas of the brain and prepared as serial coronal sections, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The slides were examined by light microscopy, giving particular attention to both the small and large vessels in the territory supplied by the ipsilateral middle cerebral artery.

Results

After establishing blood flow through the carotid A–A shunts and before incorporating the segments of Dacron vascular grafts, the hemispheres were compared with respect to determinations of platelet imaging, transcranial Doppler, and SEP. Hemispheric $^{111}$In-platelet activities were equivalent on the shunted and contralateral sides (0.36±0.11×10⁹ and 0.38±0.10×10⁹ platelets, respectively; Figure 2, top left panel). Similarly, no microemboli were detected by Doppler assessment, and no abnormalities were apparent in comparing hemispheric SEP responses (data not shown).

Immediately after incorporating segments of thrombogenic Dacron vascular graft into A–A carotid shunts, microparticles were detected in the effluent blood by transcranial Doppler analysis. Coincidently, $^{111}$In-platelet activity accumulated rapidly in the ipsilateral cerebral hemispheres (Figures 2 and 3), reaching the maximum value equivalent to 3.2±0.8×10⁹ platelets within 20 minutes (Figure 2, top right panel; p<0.001 compared with baseline values). Thereafter, the $^{111}$In-platelet microemboli progressively disappeared from the affected carotid territories, falling to 1.30±0.4×10⁹ platelets (p<0.01 for both basal and peak values) 40 minutes later, despite the continued presence of the thrombogenic vascular graft segments in the carotid shunts (Figure 3). After removing the grafts, $^{111}$In-platelet microemboli continued to clear from the cerebral circulation, approaching basal levels after 60 minutes (0.48±0.13×10⁹ platelets, p>0.05 compared with basal values) (Figures 2 [bottom left panel] and 3). No change in $^{111}$In-platelet activities from baseline was apparent in the contralateral hemispheres. The quantitative accumulation and subsequent clearance of platelet microemboli in the ipsilateral internal carotid territories, calculated by subtracting the platelets in the unaffected hemispheres from the platelets in the affected hemispheres, are presented in Figure 3.

The initial platelet counts averaged 331,000±57,900 platelets/µl (range 165,000–463,000 platelets/µl). The mean white cell count was 5,330±1,580 cells/µl, and the hematocrits were 33.5±2.1%. During the period when platelet microemboli were being generated, the mean peripheral platelet count fell to 247,000±45,600 (p<0.01) but remained constant after the grafts were removed (p>0.1). Plasma $\beta$-thromboglobulin, platelet factor 4, and fibrinopeptide A increased in parallel with the hemispheric accumulation of platelet microemboli and subsequently fell to near basal values within an hour after removal of the graft segments (Table 1). There was no change in hematocrit, white cell counts, or fibrinogen levels during the experiment.

Somatosensory evoked potentials from the affected hemispheres were abolished in all baboons within 20 minutes after initiating the microembolic shower (Figure 4). After the microemboli began to clear from the affected hemispheres, SEPs of reduced amplitude reappeared (Figure 4). One hour after removing the grafts from the carotid shunt, while maintaining the animals under anesthesia, the mean amplitude of the SEPs was 74% of the initial amplitude.

The basal pulsatile transcranial Doppler spectra of the internal carotid arteries were modified to sawtooth patterns when the roller pump was used to maintain flows. Immediately after initiating the embolic process, transcranial Doppler analysis detected large numbers of abnormal signals, resembling closely the broad, high signals produced by air bubbles. During the subsequent 20–30 minutes, low-amplitude signals were prominent, persisting at a lower frequency during the hour when the graft was in place but disappearing after its removal.

After recovery from anesthesia the two awake baboons exhibited contralateral hemiparesis, including paresis of facial muscles (clinical scores of 54 and 35 compared with basal scores of 100). Twelve hours later the animals were normal with respect to motor and
FIGURE 2. Gamma camera anteroposterior images of brain during platelet thromboembolization. Top left panel: Before incorporating Dacron segment but after placing arterio-arterial shunt composed of silicone rubber tubing, baseline hemispheric images are symmetrical with minimal $^{111}$In-platelet activity. Top right panel: The same baseline cerebral scintogram is shown with regions of interest depicted. Bottom left panel: Twenty minutes after incorporating graft segments, maximum $^{111}$In-platelet activity accumulates in shunted carotid cerebral territory. Bottom right panel: One hundred twenty minutes after inserting vascular grafts, $^{111}$In-platelet microemboli are cleared from affected hemisphere.

cranial nerve function as well as in behavior (clinical score of 100).

The neuropathological examination performed on sections prepared after pressure-perfusion fixation demonstrated patent vessels without detectable thrombotic occlusion of either large or small intracerebral arteries, including the embolized cerebral territories, except for a single thrombus found in a superficial meningeal vessel.

Discussion

This study demonstrates that platelet microemboli generated endogenously in carotid arteries and perfused into the dependent cerebral vasculatures are temporarily retained in the affected carotid territories and produce transient neurological dysfunction that resolves many hours after the microemboli clear from the circulation. These findings of experimental hemispheric platelet embolization simulate clinical TIAs and support the previous postulate that platelet emboli arising from proximal carotid thrombogenic atherosclerotic lesions constitute one pathogenetic mechanism for the development of TIAs and stroke. Since this primate model of cerebral platelet microembolization is quantitative, it may be useful in further defining pathophysiologically important variables and in the objective evaluation of different therapeutic strategies.

Several stroke models have been developed in nonhuman primates. Generally, they have used either acute reversible or irreversible mechanical occlusion of the middle cerebral artery or embolization with microspheres. The present model was developed to study the interactions among formed platelet microemboli, retention by cerebral vasculature, and reversible alterations in neurological function, processes not readily investigated by existing model systems. Using the present model, the accumulation of $^{111}$In-labeled platelet emboli within the brain, their subsequent clearance, and the effects on neurological function have been evaluable noninvasively. The generation of platelet thromboemboli by vascular graft segments incorporated into A-A in baboons has been previously demonstrated and has been used in the present experimental design. The exposure of the graft to flowing native blood for 1 hour actively consumes platelets from the circulation, as shown by the accumulation of platelets on the graft segment and the reciprocal reduction of the platelet count by one fourth (a reduction by 84,000 plate-
TABLE 1. Blood Markers of Thrombosis

<table>
<thead>
<tr>
<th></th>
<th>Pregraft</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Thromboglobulin (ng/ml)</td>
<td>6.9±1.7</td>
<td>127.0±51.6</td>
<td>33.3±12.1</td>
</tr>
<tr>
<td>Platelet factor 4 (ng/ml)</td>
<td>7.3±0.6</td>
<td>54.0±3.9</td>
<td>10.8±4.0</td>
</tr>
<tr>
<td>Fibrinopeptide A (nmol/ml)</td>
<td>3.4±1.6</td>
<td>23.1±2.8</td>
<td>2.3±1.2</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>53.93±21.6</td>
<td>86.23±12.4</td>
<td>70.5±2.3</td>
</tr>
</tbody>
</table>

Values are mean±1 SD of serial blood samples obtained from five study animals. APTT, activated partial thromboplastin time.

FIGURE 3. Line graph showing accumulation and subsequent clearance of platelet microemboli in brain as measured by $^{111}$In-platelet scintillation camera imaging.

lets/μl from the baseline count of 331,000 platelets/μl. The thromboembolic mechanism for platelet consumption is confirmed by the striking transient appearance of microparticles detected by transcranial Doppler analysis in blood effluent from the graft segments and by the increased plasma levels of β-thromboglobulin, platelet factor 4, and fibrinopeptide A, blood markers of ongoing thrombosis in vivo (Table 1). The placement and presence of the silicone rubber tubing comprising the A-A carotid shunts do not contribute per se to the formation of platelet microemboli, as demonstrated by the absence of detectable microemboli by either imaging or Doppler evaluation during the 30-minute control period preceding the incorporation of the segments of vascular graft.

Gamma camera imaging documents both the rapid accumulation and subsequent disappearance of hemispheric embolized platelets (Figure 3). However, in a previous study using segments of Dacron vascular graft as generators of platelet microemboli for femoral artery vascular territory in baboons, Schneider et al14 demonstrated that $^{111}$In-platelet emboli are detectably retained in the vasculature of legs for many hours, i.e., only 25% of platelet emboli in the leg are cleared during a 4-hour period. These differences in retention of thromboembolic material by different vascular beds may reflect protective mechanisms characteristically operating in the cerebral circulation. For instance, clearance of cerebral microemboli might be enhanced either by increasing embolus-initiated vasodilatation or through more rapid embolic disruption. In this regard, testable possibilities include differences in the production of vasoregulatory factors that also inhibit platelet function, i.e., prostacyclin or nitric oxide.19-20 Alternatively, differences in endothelial-derived fibrinolytic factors may play a role, such as increased production/release of tissue plasminogen activator or decreased production/release of its inhibitor. Clearance of microemboli from the cerebral circulation is confirmed by neuropathology, demonstrating an absence of embolized material in the affected vasculature at 3 hours.

Somatosensory evoked potentials have been useful objective measures of neurological function in experimental stroke.21-22 For instance, Matsumiya et al23 reported a reversible decrease of amplitudes of ipsilateral SEPs after 60 minutes of middle cerebral artery occlu-

FIGURE 4. Left panel: Somatosensory evoked potentials (SEPs) in baboons recorded ipsilateral to embolization. A, baseline SEPs before graft insertion. A small negative wave (P) precedes the major negative wave (N). Five minutes after starting the embolization the amplitude of N markedly decreases (B) and recovers within another hour (C). Right panel: Line graph showing mean (±1 SEM) SEP amplitudes of embolized hemispheres from five animals. During the experiment the SEPs disappear within 20 minutes and partially recover thereafter.
sion and subsequent recovery of SEPs after reperfusion of the previously occluded vessels. In our experiments, SEPs were sensitively suppressed by the initiation of the embolic process and were restored following the clearance of platelet microemboli from the affected hemispheres, but their full amplitudes were not attained at 3 hours. Although both ketamine and halothane have been reported to impair SEP responses, neither of these agents appeared to compromise the interpretation of our findings because all animals exhibited full SEP production in unshunted control cerebral hemispheres. We did not collect late time-course SEP data because the two recovered animals used to assess clinical motor function could not be restudied. The present results indicate that full recovery of neurological function occurs despite complete transient loss of SEPs.

Several investigators have also used transcranial Doppler for the detection of cerebral emboli produced by thrombogenic endarterectomy sites. Both Padayachee et al and Spencer et al detected high-amplitude Doppler signals after the incorporation of shunts or following cross-clamping of the common carotid artery. Although these signals have been attributed to the presence of "air bubbles," Spencer et al have recorded transient signals of smaller amplitude coincident with transient clinical symptoms, which they interpreted to be formed-element emboli. Our findings support this interpretation because the predominant signals during the period of embolization were also small-amplitude signals that were different from those produced by air influx. Nevertheless, transcranial Doppler may not discriminate larger formed-element emboli from air bubble signals.

In summary, endogenously formed platelet microemboli in baboons produce experimental transient ischemia and corresponding reversible neurological dysfunction that simulate clinical TIAs. This quantitative model of hemispheric embolization may be useful for experimental studies examining the pathophysiology and therapeutic intervention of TIAs.

Acknowledgment

We gratefully acknowledge the technical expertise of Deborah L. White and Ulla M. Marzec.

References

Induction of transient neurological dysfunction in baboons by platelet microemboli.
C Kessler, A B Kelly, W D Suggs, J D Weissman, C M Epstein, S R Hanson and L A Harker

Stroke. 1992;23:697-702
doi: 10.1161/01.STR.23.5.697

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 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/23/5/697

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/