Mechanisms of Cerebrovascular Events as Assessed by Procoagulant Activity, Cerebral Microemboli, and Platelet Microparticles in Patients With Prosthetic Heart Valves

Thomas Geiser, MD; Matthias Sturzenegger, MD; Urs Genewein, MD; André Haebelri, MD; Jürg H. Beer, MD

Background and Purpose—Cerebrovascular events (CVE) in patients with prosthetic heart valves (PHV) have remained a severe and frequent complication despite oral anticoagulation with or without aspirin. We studied the possible pathophysiological involvement of platelet-derived microparticles (PMP) as a contributing factor for the increased incidence of CVE in patients with PHV.

Methods—We compared in a retrospective, case-control study the clinical outcome after the implantation of the PHV with several different independent morphological and functional methods, including simultaneous transcranial Doppler monitoring of both middle cerebral arteries, PMP detection by flow cytometry with use of platelet-specific antibodies, coagulation markers, and determination of the procoagulant activity by Russell’s viper venom time, a phospholipid-dependent coagulation assay.

Results—Eight of 26 patients with PHV had 9 CVE during 136 person-years of observation. Transcranial Doppler monitoring revealed an increased frequency of microembolic signals recorded over a 30-minute period in patients with CVE (75±25; median, 55; range, 27 to 248) compared with those without CVE (23±12; median, 7; range, 0 to 153; P<0.05) or with control subjects (0; P<0.001). Flow cytometry analysis showed increased levels of PMP in patients with compared to those without CVE (4.1±0.6% versus 2.4±0.4% of all fluorescence-positive events gated; P<0.05). Increased procoagulant activity was documented by the shortened Russell’s viper venom time expressed as an increased level of platelet equivalents per microliter of plasma in patients compared with control subjects (+24.7±14.9%; P<0.01). Subgroup analysis revealed that patients with CVE had a higher excess of platelet equivalents per microliter of plasma than patients without CVE in relation to the controls (+68.7±36.7%; P<0.05). Mildly elevated thrombin–antithrombin III complexes (2.9±0.7; median, 2.3; normal, <2.0 μg/L) suggested incompletely suppressed thrombin formation, and fibrin generation (fibrinopeptide A) was in the upper normal range (2.1±0.2; median, 1.8; normal, <2.0 ng/mL), despite adequate anticoagulation (INR=3.6±0.1).

Conclusions—Our data show increased microembolic signals, platelet microparticles, and procoagulant activity in symptomatic patients with PHV and provide a potential pathophysiological explanation of CVE. (Stroke. 1998;29:1770-1777.)

Key Words: cerebral embolism • coagulation • heart valve prosthesis • platelets • procoagulant
debate over whether these HITs are formed elements (eg, platelet aggregates) or gaseous bubbles resulting from cavi-
tation is still not settled,12,13 and their clinical significance is
disputed.

Increased shear rates at the PHV can activate platelets15 and
generate platelet-derived microparticles (PMP). Such generated
platelet fragments provide negatively charged surfaces
and have a potent procoagulant activity.16 The phospholipids
facilitate the assembly of the prothrombinase complex and
may accelerate the coagulation cascade and thrombin forma-
tion several thousandfold,17 while multiple receptors (platelet
glycoprotein IIb/IIIa and GP Ib) on PMP function as
cross-links for mixed thrombi.18

We therefore hypothesized that the CVE might result from
an increased procoagulant activity mediated by PMP gener-
at at the PHV and analyzed the clinical course after
implantation of the PHV in relation to several independent
morphological and functional parameters. Microembolic sig-
nals in both middle cerebral arteries (MCAs) were measured
by transcranial Doppler ultrasound, and PMP were detected
and quantified by flow cytometry using 2 well-characterized
platelet-specific monoclonal antibodies directed against 2
different platelet glycoproteins. Procoagulant activity was
determined by the Russell’s viper venom (RVV) time
(RVVT), and thrombin and fibrin generation were assessed
by the currently available methods.

Subjects and Methods

Patients and Controls

Twenty-six patients (aged 61.5±1.8 years; 14 women and 12 men)
with PHV gave their informed consent to participate in this retro-
spective case-control study. The study was approved by the Ethics
Committee of the Medical Faculty of the University of Bern (Switzerland).

The 26 patients examined were referred to us at our request from
cardiologists in the catchment area of our hospital. (None of our
group ordinarily take care of patients with PHV.) There were no
special selection criteria other than the following: patients having a
PHV with an interval between valve implantation and study enroll-
ment of at least 3 months and patients not having a major cerebro-
vascular event before PHV implantation. Patients with major CVE
prior to the PHV implantation were excluded.

Patients were classified into the following 3 groups: A, those with
proven cerebrovascular events; B, those with nonspecific transient
neurological symptoms, such as vertigo, blurred vision, or recurrent
amnesias; and C, those without cerebrovascular events. Classifica-
tion was based on the patient’s history and the clinical and neuro-
psychological examination by the same neurologist (M.S.) and
performed before further examination.

Cardiovascular risk factors (hypertension, smoking, diabetes,
hyperlipidemia, and atrial fibrillation) were distributed similarly in
the patients with and without CVE. Patient characteristics and data
concerning the PHV implantation are summarized in Table 1. Patients who suffered from cerebral infarction or transient ischemic
attacks (TIAs) were extensively evaluated clinically, radiologically,
and with blood analysis, including cerebrovascular ultrasound and
transesophageal echocardiography studies, to exclude causes of
platelet activation other than the PHV.

Five patients were analyzed in part in a previous preliminary
study, in which markers of plasmatic coagulation and Doppler
microemboli counts were measured.19 The patients agreed to be
reevaluated according to this more elaborate protocol.

All patients were on a regimen of oral anticoagulant therapy with
either acenocoumarol (n=4) or phenprocoumon (n=22), with a
target INR of between 3.0 and 4.0.1 Actual mean INR was 3.6±0.1
throughout the observation period. Further medication consisted of
ACE inhibitors (11 patients), diuretics (10 patients), digoxin (7
patients), calcium antagonists (5 patients), β-blockers (7 patients),
nitrates (3 patients), amiodarone (2 patients), antibiotics (2 patients),
oral antidiabetics (1 patient) or insulin (1 patient), low-dose estrogen
replacement therapy (2 patients), benzodiazepines (2 patients), and
thyroxine substitution (2 patients). The distribution of the medication

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of Patients With PHV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>(n=8)</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>Indication</td>
</tr>
<tr>
<td>Aortic</td>
</tr>
<tr>
<td>Mitral</td>
</tr>
<tr>
<td>Combined</td>
</tr>
<tr>
<td>Type of PHV</td>
</tr>
<tr>
<td>St Jude Medical</td>
</tr>
<tr>
<td>Björk-Shiley</td>
</tr>
<tr>
<td>Carbomedics</td>
</tr>
<tr>
<td>Observation time, y</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Mean, per patient</td>
</tr>
<tr>
<td>CVE (No. of occurrences)</td>
</tr>
<tr>
<td>TIA (1)</td>
</tr>
<tr>
<td>Recurrent TIA (4)</td>
</tr>
<tr>
<td>PRIND (1)</td>
</tr>
<tr>
<td>CVI (3)</td>
</tr>
</tbody>
</table>

Values are mean±SEM or the absolute (and relative) number of patients in the category.
Group A indicates patients with CVE; group B, patients with nonspecific transient neurological symptoms; group C, patients without CVE.
was similar in the patients with and without CVE except for aspirin, which was given to 4 of 12 patients with CVE or nonspecific neurological symptoms and 1 of 14 patients with no CVE. One patient with CVE was on additional dipiridamole.

Control subjects were 26 healthy, age- and sex-matched volunteers who were examined according to the protocol on the same day as the patient. Except for 1 subject with well-controlled, mild hypertension on an ACE inhibitor, none of the control subjects took any medication.

Transcranial Doppler Ultrasonography
Studies were performed according to a protocol described elsewhere. Briefly, patients were examined under standard conditions in a supine position. The recording, performed with a 2-channel transcranial Doppler system (Multi-Dop X/TC7, Firma DWL, Elektronische Systeme GmbH) equipped with a specially developed software for automated emboli detection, was started after the subjects had rested for at least 20 minutes in a quiet room. The Doppler frequency spectra of both MCA were recorded simultaneously and continuously during 30 minutes. HITS were identified by their short (<0.1 second), transient, unidirectional, high-amplitude signal with a narrow spectrum and counted by 3 different methods: (1) visually (on the monitor displaying the fast Fourier transform of the color-coded Doppler spectra), (2) acoustically (by continuous on-line observation by an examiner using headphones), and (3) computer-assisted (with a special software). The detected HITS counts in the left and right MCA were added, thus reflecting the total over the registered 30 minutes.

Laboratory Studies
Blood was drawn from patients and controls by a specially trained technician through clean venipuncture from an antecubital vein under controlled venous stasis of 60 mm Hg for maximally 45 seconds. Previous studies have shown normal values for fibrinopeptide A and thrombin–antithrombin III complexes for this technique.

The Monovette system (Sarstedt) was used. A total of 45 mL of blood was drawn. The first 5 mL was discarded, then 5 parts blood was added to 1 part acid citrate dextrose (citrated 38 mmol/L, sodium citrate 74.8 mmol/L, dextrose 124 mmol/L; pH 5.0) and prepared for flow cytometry and RVVT (see below); 9 mL blood was added to 1 mL citric acid (110 mmol/L) with the inhibitors theophylline (15 mmol/L), adenosine (3.7 mmol/L), dipyridamole added to 1 mL citric acid (110 mmol/L) with the inhibitors theophylline (15 mmol/L), adenosine (3.7 mmol/L), dipyridamole (100 mmol/L), and 500 mmol/L dextrose; 45 mL blood was drawn into 0.5 mL of 100 mmol/L trisodium citrate for the assessment of the prothrombin time, and another 4.0 mL was drawn into 6.4 mg dry EDTA for the determination of leucocyte and platelet count and hemoglobin concentration. The tubes were put on melting crushed ice immediately after blood sampling for no more than 15 minutes, then centrifuged at 4°C for 30 minutes at 2000g. Multiple aliquots were snap-frozen in liquid nitrogen and stored at −70°C until analysis.

For quality control reasons, the coagulation assays were repeated in 4 patients on a separate occasion and gave essentially the same results. In these cases the mean of the 2 examinations was used. Two patients were excluded because of grossly elevated fibrinopeptide A levels (and normal thrombin–antithrombin III complex), suggesting fibrin formation in vitro. Therefore, the above constellation makes an improper blood sampling and an in vitro artifact very likely.

Flow Cytometry
Platelet-rich plasma (PRP) was prepared immediately after venipuncture by centrifugation with 800g for 2 minutes at room temperature. Platelets were counted (Coulter Counter, Coulter Electronics Ltd, or Sysmex K-1000, Toa Medical Electronics) and adjusted to 300 000/μL with sterile-filtered (0.2-μm pore size, Schleicher and Schuell) and degased PBS. Platelets were fixed within 10 minutes after venipuncture by adding paraformaldehyde (ratio 1:1) in PBS, pH 7.4, to achieve a final concentration of 0.5%. The fixation step was early because several monoclonal antibodies have been reported to induce vesiculation. For analysis by flow cytometry, the following monoclonal antibodies (Mab) were used: Mab 6D1, directed against GP Ib, and Mab 7H2, directed against GPIIb (both kindly provided by Dr B.S. Gmelin, from Dako) was then added without washing (as to not to lose any microparticles) and incubated for another 45 minutes. All steps were performed at room temperature. After a 20-fold dilution with PBS (typically, 100 μL sample was diluted with 1.9 mL buffer), the samples were analyzed on a Becton-Dickinson FACScan equipped with 15-mW air-cooled argon laser (Becton-Dickinson), and 10 000 gated events per sample were analyzed with Cell Quest software (Becton-Dickinson). Standard beads of different sizes (2, 0.5, 0.1, and 0.01 μm) were used to calibrate the system (Polysciences Inc). PMP were defined by their size and their specific positive fluorescence for platelet glycoprotein and quantified as described by Warkentin et al. Briefly, the number of GP Ib– or GP IIIa–positive particles of <87 relative fluorescence units was expressed as percentage of the total number of GP Ib– or GP IIIa–positive particles gated. This cutoff value was derived from the analysis of PRP of 25 normal donors, in accordance with the findings of Warkentin et al. This threshold (87 fluorescence units) separates optimally the well-defined platelet population from microparticles, whereas nonspecific binding of the control mouse Mab sets a much lower fluorescence margin.

Russell’s Viper Venom Time
Procoagulant activity was determined by the dilute RVVT, a sensitive, phospholipid-dependent coagulation assay. To 100 μL of pooled, ultra centrifuged (2000g for 20 minutes, followed by 15 000g for 20 minutes) and filtered (0.2-μm pore size) plasma from 12 healthy donors, we added 100 μL RVV solution (1 μL/mL) (Pen- tapharm AG, with high purity grade) and 20 μL of a PMP-containing solution of the assay was then started by recalcification (100 μL of the 20 mmol/L CaCl2). Coagulation times were recorded with use of an ST4 instrument (Diagnostica Stago). To quantify procoagulant activity, a standard curve was obtained with frozen-thawed platelets as a source for PMP, as proposed by Warkentin et al. This standard was prepared by freezing platelets (300×10^9/L) twice in liquid nitrogen for 20 minutes, followed by thawing (37°C). Final concentrations ranged from 50 000 down to 1 single platelet equivalent of frozen-thawed platelets per microliter of plasma. The RVVTs were then expressed as platelet equivalents per microliter of plasma.

Different concentrations of platelets were measured in addition to platelet-poor plasma (PPP) and high-speed PPP (HS-PPP). PPP was prepared by centrifugation of the whole blood with 2 000 for 20 minutes and HS-PPP by an additional centrifugation with 15 000g for 20 minutes, followed by filtration with a 0.2-μm filter.
Statistical Analysis

Values were analyzed by the Student t test and Wilcoxon-Mann test for paired data, the Mann-Whitney test for unpaired data, and ANOVA, where appropriate. For multiple testing, the Bonferroni-Holm test was used. Differences were considered significant at P<0.05. Values are given as mean±SEM; in some instances the median values were analyzed as well, as indicated.

Results

Clinical Course

The total accumulated observation time of the 26 patients evaluated was 136 patient-years. Time span between PHV insertion and enrollment in the study was 5.2±4.6 years (range, 8 months to 20 years). In patient group A, the following 9 CVE had occurred in 8 patients: 3 completed strokes (cerebrovascular infarctions [CVIs]), 1 prolonged reversible ischemic neurological deficit (PRIND), 1 single TIA, and 4 recurrent TIAs (2 or more events), corresponding to an incidence of 6.6 per 100 patient-years for all CVE and of 2.9 per 100 patient-years for definite stroke (CVI and PRIND). In group B, 4 patients complained of nonspecific transient neurological symptoms such as recurrent attacks of vertigo (3 occurrences); recurrent amnesia (1 occurrence); and visual disturbances, such as blurred or flickering vision (2 occurrences). All symptoms occurred exclusively after the valve replacements and at least 3 months before laboratory workup and transcranial Doppler monitoring, despite a level of oral anticoagulation considered to be therapeutically optimal for these patients (INR, 3.0 to 4.5). Extensive clinical, laboratory, and ultrasonographic evaluation in the patients with CVE did not disclose any other sources of thromboembolic events. In some instances the number of PMP was slightly higher compared with the controls, but it did not reach significance (2.9±0.3% compared with 2.2±0.2% using anti-GP IIIa and 2.2±0.2% compared with 2.0±0.2% using anti-GP Ib [both NS]; Figure 2). The Mab directed against GP IIIa appeared more sensitive for the analysis of PMP, therefore distinguishing better between the 3 patient groups and the control group.

Figure 1. Increased emboli counts in patients with CVE (group A), nonspecific transient neurological symptoms (vertigo, amnesia, and visual disturbances) (group B), and without CVE (group C). All patients and controls were monitored for HITS with transcranial Doppler ultrasound during a 30-minute period simultaneously in both MCAs. Data are mean±SEM of the total number of HITS in both MCAs. Statistical differences are indicated (*P<0.05, **P<0.001).

The binding to P-selectin or CD62P on PMP surfaces was low and similar in patients and controls (0.3% of all events gated). Microparticles derived from red cells and leukocytes were present at very low concentrations as well (0.4±0.08 in patients compared with 0.2±0.02% in controls for glycophorin-A [NS] and 0.1% in both groups for CD44).

Figure 2. Increased PMP in patients with CVE (group A), nonspecific transient neurological symptoms (vertigo, amnesia, and visual disturbances) (group B), and without CVE (group C). PMP were quantified with flow cytometry using 2 different platelet-specific antibodies (GP IIIa or GP Ib) and are shown as percentage of the total fluorescence-positive events gated. Data are presented as mean±SEM; statistical differences are indicated (*P<0.05).

The subgroup analysis of the patients in relation to their clinical events revealed 75±25 HITS (median, 55; range, 27 to 248) in the group of patients with CVE (group A, n=8); 83±41 HITS (median, 63; range, 14 to 191) in the group of patients with nonspecific transient neurological symptoms (group B, n=4); and in sharp contrast, 23±12 HITS (median, 7; range, 0 to 153; P<0.05) in patients without CVE (group C), which is a 3- to 4-fold lower emboli count (Figure 1).

Flow Cytometry

The number of PMP, as quantified from freshly drawn, carefully standardized and fixed PRP, was dramatically increased by 70.1% in patients with documented CVE (group A, n=8) compared to those without CVE (group C, n=14): 4.1±0.6% with CVE and 2.4±0.4% without CVE, using Mab 7H2; P<0.05. The latter group of patients did not differ from the healthy control subjects (2.2±0.2%). In addition, the patients with nonspecific transient neurological symptoms (group B, n=4) did not show significant differences in the number of PMP compared with control subjects or patients without CVE.

Considering all patients together, the number of PMP was slightly higher compared with the controls, but it did not reach significance (2.9±0.3% compared with 2.2±0.2% using anti-GP IIIa and 2.2±0.2% compared with 2.0±0.2% using anti-GP Ib [both NS]; Figure 2). The Mab directed against GP IIIa appeared more sensitive for the analysis of PMP, therefore distinguishing better between the 3 patient groups and the control group.

The flow cytometry of PMP was performed with 2 different platelet-specific antibodies (GP IIIa or GP Ib) and are shown as percentage of the total fluorescence-positive events gated. Data are presented as mean±SEM; statistical differences are indicated (*P<0.05).
Coagulation Markers

To detect increased thrombin or fibrin generation or fibrinolysis, prothrombin fragment 1+2, thrombin–antithrombin III complex, fibrinopeptide A, D-dimer, and β-thromboglobulin were measured. Prothrombin fragment 1+2, D-dimer, and β-thromboglobulin were within normal ranges (see Table 2), whereas the thrombin–antithrombin III complex levels (2.9±0.7 μg/L; median, 2.3; normal, <2.0) and fibrinopeptide A levels (2.1±0.2 ng/mL; median, 1.8; normal, <2.0) were slightly elevated.β-thromboglobulin (3.62 ng/mL); tragically, he developed a CVI 1 day after testing.

There were no significant differences when the results of the different clinical subgroups (groups A, B, and C) were analyzed (Table 2).

Procoagulant Activity (RVVT)

Patients had a significantly higher amount of (frozen-thawed) platelet equivalents per microliter of plasma compared with control subjects (P<0.01; Figure 3), which is also expressed in a significant shortening of the RVVT. These differences appeared most prominent in diluted samples with low platelet concentrations. When the platelets, but not the PMP, were removed by differential centrifugation (PP), the effect could still be observed, whereas it disappeared after the elimination of PMP by high speed centrifugation and filtration of the plasma (HS-PPP) (Figure 3). The excess of platelet equivalents (comparison of platelet equivalents between patients and control subjects) was most prominent in the group of patients with CVE (group A) (+68.7±36.7%; P<0.05) compared to the patients with nonspecific transient neurological symptoms (group B) or those without CVE (group C), where no significant differences compared to the controls could be found (Figure 3, insert).

Discussion

Ischemic CVE in patients with PHV may have 2 causes: (1) the level of anticoagulation is unintentionally too low or (2) thrombogenic mechanisms are not sufficiently suppressed by the reduction of coagulation factors during adequate coumadin prophylaxis. A number of well-designed and well-conducted studies have shown that it is possible to reduce (but not completely suppress) the incidence of CVE by actually achieving the targeted INR in a high percentage of the prothrombin times measured. However, the precise pathophysiology of thromboembolism in patients with PHV is still not established. A thrombogenic role has been attributed to activation of platelets or plasma coagulation on foreign surfaces, incomplete “wash out” of blood from valves, and increased shear rates with secondary platelet activation.

Therefore, we have taken a stepwise approach to analyze the thrombogenic mechanisms in 26 patients. To potentially identify subclinical events and therefore possibly a subgroup of patients particularly at risk for strokes, we have evaluated all patients and a control group for cerebrovascular microembolic signals (HITS) with transcranial Doppler monitoring. We have tried to analyze the procoagulant activity by simultaneously quantifying the circulating platelet membrane microparticles as well as their functional procoagulant impact on the RVVT, a clotting test particularly sensitive to the presence of phospholipids.

Our clinically observed CVE rate of 6.6 per 100 patient-years and the rate of definitive stroke of 2.9 per 100
patient-years is in the range found in the literature. If other transient and less specific symptoms are included as well, the rate is in the upper range (11.0 per 100 patient-years). Possible reasons include a relatively high number of patients with mitral valve replacements (n=4) and double valve replacements (aortic and mitral, n=8) known to be at higher risk for thromboembolic complications and a scrutinized neurological evaluation (MS), which may have detected discrete but relevant symptoms other studies might have missed.

The patients with CVE had significantly higher HITS counts during Doppler monitoring compared with the asymptomatic patients. It appears possible that these patients constantly embolize into the cerebral circulation platelet aggregates generated at the site of the PHV by the previously mentioned mechanisms. Other compositions of the embolic particles appear less likely, considering the results of the coagulation studies, which showed no thrombin or fibrin generation. Mixed thrombotic materials at this high embolization rate would be expected to result in positive tests for fibrin formation and/or fibrinolysis (fibrinopeptide A, D-dimer). Recent studies suggest that at least part of HITS represent air bubbles generated by the cavitation effect just behind the valve leaflets. However, these air bubbles are of a size unable to cause strokes. Because air-fluid interfaces activate platelets, this might be another mechanism generating platelet aggregates. Microbubbles may also become stabilized, probably by being coated with membrane fragments (from platelets and other cells) generated at the PHV. Otherwise, it is difficult to understand how bubbles caused by the cavitation affect of PHV should persist until their arrival in the MCA.

Almost all of these HITS, despite occurring at high frequency, are asymptomatic. Thus, postulated aggregates likely disaggregate in the cerebral microcirculation, embo-lize into clinically silent areas, or are too small to generate ischemic injury. The stabilized bubbles may also disaggregate in the periphery, as may be the case with HITS composed of particulate materials. All control subjects had HITS counts of 0, whereas even the asymptomatic PHV patients had a mean count of approximately 20 HITS. It remains to be determined whether their increased rate identifies these patients to be at risk for future strokes. Additional important determinants may include size of the emboli (which cannot be estimated by Doppler analysis), their detailed composition, and most importantly, the ability to dissolve the emboli rapidly.

PMP counts were significantly elevated in the subgroup of patients with CVE but not in asymptomatic patients. In sharp contrast to the HITS quantified by Doppler ultrasound, the PMP can be observed in the healthy control population as well; namely, at a level of approximately 2%, which is similar to that in the asymptomatic patients. The low PMP level in controls may represent background noise or an insignificant systemic level, or it may result from sample collection. This observation may reflect a threshold level for thromboembolic complications; however, the almost invariably observed shortened RVVT suggests an increased prothrombinase activity in the plasma of all (symptomatic and asymptomatic) PHV patients. Therefore, very small membrane fragments may have been missed by the higher threshold of flow cytometry. One might hypothesize that the larger microparticles are more thrombogenic. In addition, other qualitative differences of membrane microparticles may also contribute to their procoagulant role in these patients.

Preliminary results of our group suggest that the orientation of the platelet receptor, and perhaps of the phospholipid bilayer of microparticles as well, is different depending on the mechanism of their formation. Therefore, “inside-out” vesicles may provide negatively charged phospholipids to a much greater extent than those that retain the nonactivated orientation. Moreover, in contrast to the platelets, the loss of the aminophospholipid-translocase activity in the PMP may lead to permanent loss of the lipid asymmetry. The fact that we found no significant levels of P-selectin- (or CD62P-) positive PMP or platelets might suggest a predominantly mechanical generation rather than the activation-induced vesiculation. Increased levels of PMP have also been reported in patients with a vascular pathology, favoring turbulent flow and thus high shear rates as the mechanism for their generation. The levels of β-thromboglobulin were not increased in our patients and do not support significant platelet release in vivo. On the other hand, reduced levels of PMP (ie, the inability to vesiculate) are associated with a bleeding disorder, Scott syndrome. The latter biological model provides the evidence that a physiological level of PMP appears to be required for normal hemostasis. This interpretation fits well with our findings that the control subjects had no HITS at all but had a constant level of approximately 2% of PMP in their circulation.

It is well established that negatively charged phospholipids serve as a template for the tenase/prothrombinase complex of plasmatic coagulation. This can be demonstrated most sensitively by the RVVT, which is shortened by the presence of phospholipids. In our hands, as little as the equivalent of 3 frozen and thawed platelets per microliter was sufficient to accelerate the RVV clotting time detectably. RVVT were substantially shortened in patients compared with control subjects, even though the activity of prothrombin and Factors VII, IX, and X was reduced to less than one fifth the normal values because of coumadin therapy.

The fact that the relative shortening of the RVVT in patients compared with controls increases continuously when the platelet count is lowered from 100×10^9/L to 5×10^9/L indicates that it is indeed the PMP rather than activated platelets that are responsible for the effect. The argument is further strengthened by the observation that the difference disappears after high-speed centrifugation and ultrafiltration (HS-PPP) to eliminate PMP. The latter method interestingly leads to an even slightly longer RVVT in patients, which might be explained with their lower content of vitamin K–dependent coagulation factors (Figure 3).

Interestingly, we found no evidence that the CVE itself induced a persistent procoagulant state: there was no inverse correlation between the parameters measured and the time elapsed between the CVE and the individual analysis. Taken together, our data provide a potential pathophysiological model for the thromboembolic events that occur in patients with PHV and may help to explain the incomplete
efficiency of therapeutic oral anticoagulation: Elevated concentrations of PMP may be a risk factor and may decrease the threshold for thromboembolic complications; under careful oral anticoagulation only minimal, if any, \( \beta \)-thromboglobulin release, thrombin and fibrin generation, or fibrinolysis can be observed. Symptomatic patients, however, may have particularly elevated procoagulant activity provided by their PMP or may have additional congenital or acquired risk factors, which then lead to macroscopic emboli formation and cerebrovascular events. A prospective study with a larger and well-defined sample of patients is therefore needed to further elucidate this hypothesis.

It will be interesting to further evaluate platelet inhibitors as adjunct to anticoagulant therapy, particularly those that inhibit activation-induced vesiculation.\(^3\)\(^5\) Even if they will not inhibit mechanically generated PMP, they may inhibit the shear-induced activation\(^3\)\(^\text{and}\) influence the further buildup of PMP or enhance their dissolution in the cerebral circulation, thus reducing their deleterious effects.

Acknowledgments

This study was supported by the Swiss National Science Foundation (grant 31–40 822.94 to Dr Beer) and the Swiss Heart Foundation (grant to Dr Beer). We wish to thank Christa Beer, Daniela Spina, Anita Vogt, Karin Wooldt, and Cornelia Schmid for expert technical assistance; Steven Merlin for his support in the flow-cytometry analysis; and Dr Christoph Minder for his help with statistical analysis. We also thank the patients, their general practitioners, and their cardiologists who agreed to participate in this study.

References


Mechanisms of Cerebrovascular Events as Assessed by Procoagulant Activity, Cerebral Microemboli, and Platelet Microparticles in Patients With Prosthetic Heart Valves

Thomas Geiser, Matthias Sturzenegger, Urs Genewein, André Haeberli and Jürg H. Beer

Stroke. 1998;29:1770-1777
doi: 10.1161/01.STR.29.9.1770

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 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/29/9/1770

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/