Effect of heparin bonding on catheter-induced fibrin formation and platelet activation

ALLEN B. NICHOLS, M.D., JOHN OWEN, M.D., BETTY A. GROSSMAN, B.A., JOSEPH J. MARCELLA, M.D., LLOYD N. FLEISHER, PH.D., AND MARY M. L. LEE, PH.D.

ABSTRACT Pathologic and experimental evidence indicates that platelet activation and fibrin formation contribute to the pathogenesis of angina pectoris, coronary vasospasm and myocardial infarction. Detection of localized intravascular platelet activation and fibrin formation in vivo by selective blood sampling requires catheters that do not induce coagulation ex vivo. We studied the effect of heparin bonding of catheter surfaces on activation of the coagulation system by cardiovascular catheters. Woven Dacron, polyvinylchloride, and polyurethane catheters were tested and compared with identical catheters with heparin-bonded surfaces in 47 patients undergoing percutaneous cardiac catheterization. Platelet activation was measured by radioimmunoassay of plasma platelet factor 4 (PF4), β-thromboglobulin (BTG), and thromboxane B2 (TXB2) in blood samples withdrawn through catheters, and fibrin formation was assessed by determination of fibrinopeptide A (FPA) levels. In blood samples collected through conventional catheters, FPA, PF4, BTG, and TXB2 levels were markedly elevated; blood sampling through heparin-bonded catheters had no significant effect on FPA, PF4, BTG, or TXB2 levels. Scanning electron microscopy disclosed extensive platelet aggregates and fibrin strands adherent to the surface of conventional catheters but not to heparin-bonded catheter surfaces. This study demonstrates that (1) collection of blood samples through cardiovascular catheters causes artifactual elevation of FPA, PF4, BTG, and TXB2 levels, and (2) heparin-bonded catheter surfaces effectively prevent catheter-induced platelet α-granule release and fibrin formation on catheter surfaces. Heparin-bonded catheters will facilitate investigation of the role of intravascular coagulation in coronary artery disease by eliminating catheter-induced fibrin formation and platelet activation.

avoid spurious elevation of FPA, PF4, BTG, and TXB2 levels ex vivo. An important methodologic issue, which has not yet been systematically investigated, is whether collection of blood samples through cardiovascular catheters artfactually elevates these levels.

In the present study, activation of hemostasis by catheters was assessed by measuring FPA, BTG, PF4, and TXB2 levels in serial blood samples withdrawn through catheters in patients undergoing percutaneous cardiac catheterization. In addition, platelet aggregates and fibrin strands adherent to catheter surfaces were viewed by scanning microscopy. The objectives of this study were to test the hypothesis that conventional cardiovascular catheters cause activation of the hemostatic mechanism and that heparin bonding passivates catheter surfaces, preventing catheter-induced fibrin formation and platelet activation.

Methods

**Patients.** Studies were conducted in 47 patients undergoing diagnostic cardiac catheterization. Included were 28 men and 19 women, with a mean age of 51 years (range 18 to 76). Patients receiving heparin or coumadin, patients with thromboembolic disorders, and patients with implanted prosthetic devices or permanent pacemakers were excluded, as were those with mitral stenosis or previous transmural myocardial infarction. None of the patients had indwelling venous or arterial cannulas in place during the catheterization study. Informed consent was obtained from each patient according to a protocol approved by the Institutional Review Board of Columbia University.

**Catheters.** Twenty-nine heparin-bonded woven Dacron, polyurethane, or polyvinylchloride catheters were compared with 18 identical catheters that were not heparin bonded. Ten Gorlin pacing electrode catheters, eight Lehman and Goolland-Lubin right heart catheters, 16 Judkins-Schmidt Nycore coronary catheters, and 13 Swan-Ganz balloon-tipped, triple-lumen, thermodilation catheters were studied. The Gorlin, Lehman, and Goolland-Lubin catheters were constructed of woven Dacron coated with a high-density urethane finish. The coronary catheters were constructed of a nylon core coated with polyurethane and the same high-density urethane finish. The Swan-Ganz flow-directed catheters were constructed of polyvinylchloride. Catheters were coated with heparin by a noncovalent bonding method that involves impregnation by solvent evaporation and results in physical immobilization of the heparin-bonded compound (USCI Cardiology and Radiology Division, Billerica, MA).21

**Experimental protocol.** FPA, BTG, PF4, and TXB2 levels were determined in blood samples withdrawn through the catheters over a 30 min period. Levels were also determined in peripheral venous blood samples collected by separate venipunctures before and after catheter insertion and again after the 30 min catheter sample was collected. All blood samples were collected before intracardiac pressure was recorded or contrast medium was injected for angiograms.

Right heart woven Dacron and polyvinylchloride catheters were inserted through Teflon vascular sheaths (Cordis Corp., Miami) introduced percutaneously into the femoral, internal jugular, or antecubital veins. Coronary catheters were inserted through Teflon vascular sheaths placed in the femoral artery and were advanced into the aorta without guidewires. Right heart catheters were positioned in the main pulmonary artery, and coronary catheters were positioned in the aorta.

From each patient three blood samples were withdrawn from the catheter lumen at 15 min intervals beginning 5 min after catheter insertion. All catheters were flushed with saline before insertion and after withdrawal of each sample. Saline (0.9%) was infused continuously, except during blood sampling, at 1.0 ml/min through the catheter lumen.

**Technique of catheter insertion.** The effect of insertion of percutaneous catheters over guidewires by the Seldinger technique22 was tested in another subgroup of five patients. In these patients heparin-bonded coronary catheters were advanced over 145 cm heparin-coated 0.0038 inch guidewires into the femoral artery without use of vascular sheaths. After removal of the guidewires, the catheters were flushed with saline and blood samples were collected, as described above, over 30 min.

In 10 additional patients the effect of percutaneous insertion of Teflon vascular sheaths was tested by measuring FPA, PF4, BTG, and TXB2 levels in peripheral venous blood collected by separate venipunctures before and after sheath insertion.

**Blood processing and radioimmunoassay.** Peripheral venous blood samples were collected with a 21-gauge scalp-vein needle inserted into a antecubital vein. Nine milliliters of venous blood was withdrawn into a 10 ml polypyrrole syringe and immediately transferred into a tube containing 1.0 ml of the following anticoagulant solution: 0.10M NaCl, 0.05M HEPES buffer (pH 7.4), 1400 U/ml heparin, 10 mM adenosine, 20 mM theophylline, and 1000 U/ml aprotinin (Trasyloil) (FBA Pharmaceuticals, Inc., New York). Catheter blood samples were withdrawn directly from the catheter lumen and immediately transferred into identical tubes containing the same anticoagulant platelet-inhibitory mixture. Blood samples were immediately placed on melting ice and within 1 hr were centrifuged at 3000 g and 4°C for 20 min. The supernatant plasma was transferred to polypyrrole tubes with a siliconized pipette and centrifuged at 49,000 g for 15 min at 4°C. The resulting platelet-poor plasma was stored frozen at 80°C. FPA, PF4, BTG, and TXB2 levels were measured by radioimmunoassay as previously described.8, 14, 23, 24

**Scanning electron microscopy.** One catheter of each type was flushed with saline on removal from the vein or artery and incised longitudinally. A segment of the catheter was fixed overnight at 4°C in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4). After postfixation in 1% osmium tetroxide, the segment was stained in 2% uranyl acetate for 30 min and dehydrated through an ascending ethanol series. Next, the specimen was critical-point dried, mounted on aluminum stubs, coated with gold-palladium, and examined in a JEOL-25 scanning electron microscope.

**Statistical analysis.** Plasma levels of FPA, PF4, BTG, and TXB2 are skewed to the right, but are adequately described by log-Gaussian distributions. Thus, logarithmic transformation was used, and the data are reported as geometric means and standard errors of the geometric mean. Significant differences between geometric mean levels were assessed by Duncan’s multiple-range test and by analysis of variance.25

**Results**

Analysis of variance did not disclose any significant differences among the results obtained with the three different types of catheters. Therefore, the data obtained from all studies of heparin-bonded catheters were pooled and compared with combined data from the studies of control catheters without heparin bonding. Figures 1 to 4 show geometric mean plasma FPA,
PF4, BTG, and TXB₂ levels measured in peripheral venous blood and in blood samples collected through catheters for patients catheterized with control and heparin-bonded catheters.

**Effect of Teflon sheath insertion.** Ten patients had peripheral samples collected by separate venipunctures before and immediately after percutaneous insertion of the Teflon vascular sheaths into the femoral vein. The second blood sample was obtained before a catheter was advanced through the sheath. After sheath insertion, mean levels of PF4 (19.9 vs 14.7 ng/ml) and BTG (26.6 vs 25.5 ng/ml) were not significantly elevated above control values. The mean FPA level was significantly higher after sheath placement (3.5 vs 1.7 nM/ml; p < .05).

**Effect of catheter insertion.** After insertion of control catheters, peripheral FPA levels rose to 9.4 nM (figure 1), which was significantly higher (p < .05) than the 3.4 nM level observed after sheath insertion. After insertion of heparin-bonded catheters, the mean FPA level in peripheral venous blood was 3.4 nM (figure 1), which was not different from the level resulting after sheath insertion. Peripheral venous levels of PF4, BTG, and TXB₂ were not significantly different after insertion of either heparin-bonded or conventional catheters (figures 2 to 4).

The effect of indwelling catheters on peripheral venous blood levels was assessed by comparing peripheral levels immediately after catheter insertion with levels observed 30 min later. The elevated FPA levels, which were observed immediately after insertion of control catheters, persisted but did not increase further.
Serial blood sampling through catheters. To evaluate platelet activation and fibrin formation induced by collection of blood samples through catheters, levels of FPA, PF4, BTG, and TXB, measured in samples withdrawn through catheters were compared with levels in peripheral venous blood collected at the same time. The mean FPA level (26.5 nM) in blood collected through control catheters 5 min after catheter insertion was significantly higher (p < .01) than the level (8.9) in peripheral venous blood collected simultaneously (figure 1). Over the next 30 min, FPA levels rose progressively in blood samples drawn through control catheters (mean 75.6 nM) and were significantly higher (p .01) than the corresponding peripheral venous level (10.2) (figure 1). In contrast, FPA levels in serial blood samples collected through heparin-bonded catheters were not significantly elevated compared with levels in peripheral venous blood either 5 min after catheter insertion or 30 min later (figure 1).

Five minutes after catheter insertion, PF4 levels in blood collected through control catheters were elevated (11.2 ng/ml) compared with levels in peripheral venous blood (6.9 ng/ml), but this difference was not significant (figure 2). Thirty minutes later PF4 levels (46.3 ng/ml) were significantly (p < .01) elevated above the corresponding venous level (6.7 ng/ml). PF4 levels were not elevated in blood collected through heparin-bonded catheters compared with those in peripheral venous blood (figure 2).

BTG levels were significantly (p < .01) elevated (48.3 ng/ml) in catheter samples collected after insertion of control catheters compared with venous levels (27.5 ng/ml), and rose progressively over the next 30 min to 108.7 ng/ml (figure 3). In blood collected through heparin-bonded catheters, BTG levels were not elevated during the 30 min period.

TXB2 levels were elevated in blood samples drawn through control catheters 5 min after catheter insertion (mean 157.1 pg/ml) compared with the levels in peripheral venous blood (99.4 pg/ml), but this difference did not reach statistical significance. After 30 min TXB2 levels were markedly elevated (557.8 pg/ml) in catheter samples compared with those in peripheral venous blood (111.6 pg/ml; figure 4). In blood collected through heparin-bonded catheters TXB2 levels were not elevated during the 30 min period and were not significantly different from levels in simultaneously collected peripheral blood.

Scanning electron microscopy. Scanning electron micrographs of the inner surfaces of representative catheters without heparin bonding are shown in figure 5. A network of fine fibrin strands with platelet ag-
gregates and trapped red cells was observed on the inside surfaces of all of these catheters. Scanning electron micrographs of surfaces of heparin-bonded catheters showed no fibrin strands or platelet aggregates (figure 6).

Effect of catheter insertion over guidewires. Shown in figure 7 are mean FPA, PF4, BTG, and TXB2 levels for five patients in whom heparin-bonded polyurethane coronary catheters were inserted over 145 cm guidewires. Levels in this subgroup were significantly elevated, as measured in blood samples collected through the catheters 5 min after insertion. Over the ensuing 30 min, FPA, PF4, BTG, and TXB2 levels gradually declined.

Discussion

Clinical studies suggest that platelet activation and aggregation, thromboxane generation, and fibrin formation in the coronary circulation may have important roles in unstable angina, variant angina, sudden death, and coronary thrombosis. Several groups of investigators have reported that levels of TXB2, the stable metabolite of TXA2, are elevated in coronary sinus blood of patients with variant angina. Other investigators have reported elevated TXB2 levels in coronary sinus blood of patients with coronary artery disease after episodes of spontaneous angina, in association with pacing-induced angina, or within 24 hr of an episode of chest pain. Elevated BTG levels have been reported in coronary sinus blood of patients with coronary artery disease and have been interpreted as demonstrating abnormal platelet activation in the atherosclerotic coronary circulation.

In all these studies, blood samples were collected from the coronary sinus with woven Dacron catheters. As shown in the present study, mean FPA levels in peripheral venous blood increased significantly immediately after insertion of catheters of this type. Mean FPA levels in blood drawn through the woven Dacron catheters were significantly elevated above those in simultaneously drawn peripheral venous samples after catheter insertion and rose significantly to very high levels over 30 min. These data suggest that fibrin formation was occurring on the outside and inside surfaces of the woven Dacron catheters, and this was confirmed by electron micrographs that showed fibrin strands adherent to the internal catheter surfaces. Although levels in peripheral venous blood did not change, the levels of PF4, BTG, and TXB2 in samples withdrawn through the conventional catheters were also significantly elevated above levels measured simultaneously in venous blood; all three rose progressively and markedly over 30 min. These data suggest that platelet activation occurred as blood was withdrawn through the woven Dacron catheters. Scanning electron micrographs confirmed the presence of aggregates and trapped red cells was observed on the inside surfaces of all of these catheters. Scanning electron micrographs of surfaces of heparin-bonded catheters showed no fibrin strands or platelet aggregates (figure 6).

FIGURE 6. Scanning electron micrographs (original magnification x 3000) of the internal surfaces of heparin-bonded catheters. (A) Polyvinylchloride flow-directed catheter; (B) woven Dacron right heart catheter; (C) polyurethane coronary catheter. Neither platelet nor fibrin deposits are evident on any heparin-bonded catheter surface.
gated platelets adherent to the internal surfaces of the
catheters.
These findings demonstrate that artifactual fibrin
formation and platelet activation are induced as blood
samples are drawn through woven Dacron catheters.
These results indicate that blood collection through
standard cardiovascular catheters is an unreliable
method for measuring levels of FPA, PF4, BTG, and
TXB₂ in vivo, particularly if the catheters remain in
the circulation for more than a few minutes or if multiple
blood samples are withdrawn through the same cath-
ether. Because conventional woven Dacron catheters
were used for sampling of coronary sinus blood in
previous studies of spontaneous, variant, and pacing-
induced angina, the conclusions that elevated
coronary venous levels of TXB₂ or platelet proteins
reflect platelet activation in vivo must be interpreted
with caution.
In contrast, insertion of heparin-bonded catheters
was not associated with a rise in peripheral venous
FPA levels above the slight increase that was noted
during percutaneous insertion of cardiovascular
sheaths. In addition, there was no increase in FPA
levels in blood drawn through heparin-bonded cath-
eters above simultaneous venous levels over a 30 min
period. Electron micrographs obtained at completion
of the study of the heparin-bonded catheters showed no
fibrin strands on the inner catheter surfaces. Similarly,
plasma PF4, BTG, and TXB₂ levels in blood with-
drawn through heparin-bonded catheters were not sig-
nificantly higher than those in peripheral venous blood
over the 30 min. The inference that platelet activation
did not occur on the inner surfaces of heparin-bonded
catheters was supported by the absence of aggregated
platelets on catheter surfaces, as demonstrated by elec-
tron microscopy. These data indicate that heparin
bonding effectively inhibits catheter-induced platelet
activation and fibrin formation at least over a 30 min
period. Since this is a suitable time for completion of
an experimental protocol, the data suggest that hepa-
rin-bonded catheters will be suitable for clinical in-
vestigations of fibrin formation or platelet activation in
vivo in the coronary and other regional circulations.
The mechanism of the thromboresistance of hepa-
rinized surfaces is not understood, but probably
involves an interaction with one or more plasma hepa-
arin cofactors. In several previous studies, the anti-
 thrombotic effect of heparinized surfaces has been at-
tributed to desorption of ionically bonded heparin.
However, it is unlikely that heparin desorption caused
the antithrombotic effect observed in the present study.
Doherty and Lanteigne observed that heparin bonded
to surfaces by the present method desorbs slowly. In
experiments in anesthetized dogs they demonstrated
that the present method of heparin bonding prevents
accumulation of ¹¹¹In-labeled platelets on catheter shafts
for at least 48 hr. Furthermore, in studies of heparin-
bonded catheters remaining in patients for a week or
more, it was found by carbolfuchsin staining that
more than half of the heparin remained on the
catheter surfaces after removal. Since each catheter is
coated with approximately 1500 U of heparin, it is
unlikely that a small amount of desorption would cause
systemic heparinization. Lastly, the observation in the
present study that PF4 levels were not elevated in pe-
ripheral venous blood after insertion of heparin-bond-

*Patterson FV: Personal communication.
ed catheters is additional evidence that the antithrombotic effect of these catheters does not result from local heparin desorption, since heparin administered intravenously elevates PF4 levels markedly. 33

An advantage of the use of heparin-bonded catheters for investigative studies is that the need for systemic heparinization is eliminated. Systemic heparinization is routinely used during most cardiac catheterization and angiographic procedures to prevent catheter-induced thromboembolic complications. For investigative studies of intravascular platelet activation and fibrin formation, systemic heparinization is disadvantageous for the following reasons. First, heparin elevates PF4 levels markedly, possibly by displacing PF4 bound to vascular endothelium, 33 second, heparin inhibits thrombin action and promptly reduces FPA levels to the normal range, 34 and third, heparin may affect platelets directly, altering either aggregation or activation. 35, 36

Several techniques of percutaneous catheter insertion were also evaluated and found to affect FPA, PF4, PTG, and TXB2 levels significantly. Insertion of vascular sheaths caused slight but significant elevation of FPA in peripheral venous blood. Insertion of heparin-bonded coronary catheters over 145 cm heparin-coated guidewires into the femoral artery by the Seldinger technique resulted in immediate elevation of FPA, PF4, BTG, and TXB2 levels in blood withdrawn through the catheters. This observation suggested that the guidewires reversed the passivating effect of the heparin coating, possibly by abrading the inner surface of the catheters. Direct insertion of catheters through sheaths placed percutaneously eliminated the need for guidewires and did not elevate PF4, BTG, or TXB, either in peripheral venous blood or in blood samples collected through the catheters.

The results of the present study indicate that heparin-bonded catheters will be useful for selective intravascular blood sampling without artifactual activation of platelets or marked formation of fibrin. This approach will facilitate detection and localization of intravascular platelet release and fibrin formation in future investigations. Both venous and arterial catheters can be effectively heparin-bonded, making it possible to detect platelet release regionally in the coronary, cerebrovascular, renal, or peripheral circulations.

We gratefully acknowledge the technical assistance of Thomas Lim and Karen Terwilliger. We also thank Robert R. Sciaccia for assistance in statistical analysis and Leonard L. Norbert for preparing the manuscript.

References

Effect of heparin bonding on catheter-induced fibrin formation and platelet activation.
A B Nichols, J Owen, B A Grossman, J J Marcella, L N Fleisher and M M Lee

Circulation. 1984;70:843-850
doi: 10.1161/01.CIR.70.5.843

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/70/5/843

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 Circulation is online at:
http://circ.ahajournals.org/subscriptions/