Thromboxane Synthesis and Platelet Protein Release During Simulated Extracorporeal Circulation


Platelet secretion induced by certain soluble aggregating agents is associated with thromboxane formation. Activation of platelets by artificial surfaces may involve similar biochemical pathways. Therefore, we monitored platelet release and formation of thromboxane B₂ during simulated extracorporeal circulation where contact between blood and synthetic surfaces is extensive. Fresh heparinized human blood was recirculated for 2 hr at 37°C at 1 liter/min in circuits (0.95 sqm) constructed of standard silicone rubber components (0.1 sqm) and a spiral coil membrane oxygenator (0.85 sqm). Within 2 min of recirculation, plasma levels of the platelet-specific protein, low-affinity platelet factor 4 (LA-PF₄) rose from <1.5% to 11% ± 3% SEM of that which was released by triton X-100, indicating significant release of platelet α-granule contents. This occurred despite the absence of detectable thromboxane B₂ in plasma (<0.5 pmole/ml). Between 2 and 15 min, plasma levels of LA-PF₄ increased from 11% to 42% ± 3%, and plasma thromboxane B₂ concentrations rose to 3.2 pmole/ml. Subsequently, thromboxane B₂ levels continued to rise to 9.8 pmole/ml by 2 hr. Thromboxane B₂ was not detected in blood from donors who had ingested aspirin, and the extent of platelet secretion was reduced by about 50% (25% at 2 hr). Thus, although a component of platelet granule release during extracorporeal recirculation appears to be associated with thromboxane synthesis, considerable release of LA-PF₄ may occur by thromboxane-independent pathways.

CONTACT between blood and artificial surfaces is associated with extensive release of platelet granule contents during simulated extracorporeal circulation¹,² during total cardiopulmonary bypass in rhesus monkeys³,⁴ and during open cardiac surgery in humans.⁵ The release of granule contents induced by aggregating agents is thought to proceed by at least two mechanisms of stimulus–response coupling. The release reaction may be independent of aggregation and insensitive to indomethacin (e.g., thrombin-induced), or mediated through aggregation and abolished by indomethacin (e.g., adenosinediphosphate [ADP]-induced). Since indomethacin is a cyclooxygenase inhibitor, the products of arachidonate metabolism, such as the thromboxanes, are necessary for release mediated by aggregation.⁶,⁷ Stimulation of platelets by physical contact with synthetic surfaces might
involves functional pathways that are similar to those activated by aggregating agents, thus, we measured both platelet secretion and thromboxane synthesis during simulated extracorporeal circulation.

MATERIALS AND METHODS

Perfusion circuits containing a spiral coil membrane oxygenator (Sci-Med Life Systems, Inc., Minneapolis, Minn. —0.85 sqm) were assembled from standard silicone rubber components (0.1 sqm) and polycarbonate connectors. Circuits, priming procedures, and the condition of recirculation were identical to those described previously. Briefly, blood and gas compartments of the oxygenator were flushed with 100% carbon dioxide for 15 min prior to filling the circuit (priming). Oxygenators were primed by applying a vacuum to the lower gas port, and blood was permitted to enter the device by gravity. Blood was recirculated by a precisely shimmed, barely occlusive, calibrated double-roller pump at 1000 ml/min for 2 hr. Oxygenators were ventilated with 5% carbon dioxide in oxygen at 1 liter/min.

Blood (500 ml) from random normal donors, who abstained from all aspirin-containing medicines for 2 wk, was drawn directly into circuit venous reservoirs (N = 5). Heparin (beef lung, 5 U/ml; Upjohn Co., Kalamazoo, Mich.) and glucose (3.3 mg/ml) were added to the collection chamber prior to venipuncture. Blood was maintained at 37° C during recirculation by immersing the venous reservoir in a constant-temperature water bath. For 5 additional trials, donors were instructed to take 2 aspirin tablets 1 hr prior to sleep the evening prior to donating blood and again at 8 a.m. on the morning of the experiment. Finally, in 3 additional experiments, enough prostaglandin (PG)E1, alone (kindly provided by Dr. John Pike Upjohn Company, Kalamazoo, Mich.), PG12, alone, or a combination of PGE1 and lidocaine (Elkins-Sinn Inc., Cherry Hill, N. J.) were added to the venous reservoir to give final whole blood concentrations of 3.6 ug/ml PGE1, 39 ng/ml PG12, and 108 ug/ml lidocaine, respectively.

Aliquots of blood (30 ml) were withdrawn from the circuit at 30 sec and 2, 15, 30, 60, and 120 min for plasma and platelet studies. These samples were compared to control aliquots of blood drawn directly from the donor into syringes containing heparin and glucose in sufficient concentrations to mimic the circuit and incubated at 37° C for the duration of the experiment. Additional control samples from all donors were allowed to clot and the serum assayed for thromboxanes.

Platelet counts were obtained with a Coulter ZF cell counter (Coulter Electronics, Hialeah, Fla.) or by phase microscopy.

Since the appearance of LA-PF4 in plasma closely parallels the release of ADP and 5-HT induced by aggregating agents, and since interference from ADP released by erythrocytes and platelet uptake of 5-HT by platelets is avoided, LA-PF4 was measured in plasma by modified radial immunodiffusion with a monospecific antibody against purified LA-PF4, as described by Niewiarowski. Samples (5 ml) for low-affinity platelet factor 4 (LA-PF4) determinations were drawn into syringes containing 0.75 ml acid citrate dextrose and immediately processed. Platelet-poor plasma (PPP—<1000 platelets/µl) was prepared as described previously. PPP was then quickly frozen and stored at −60° C until assayed. Release of LA-PF4 into plasma is expressed as a percent of total LA-PF4, released in 1 ml of platelet-rich plasma (PRP) by triton X-100 (0.8%), as determined in 15 consecutive normal donors (32 µg).

Samples (5 ml) for thromboxane determinations were drawn into syringes containing sufficient EDTA to give a final concentration of 5 mM and immediately processed. Platelet-rich plasma was prepared from these samples as described previously. Prior to high-speed centrifugation to prepare PPP, enough indomethacin was added to the PRP to give a final concentration of 10 µM. EDTA was prepared as a stock solution (100 mM in saline), and indomethacin was dissolved in 100% ETOH for use on the day of the experiment. Thromboxane B2 was measured in plasma by radioimmunoassay specific for thromboxane B2 as described by Lewy et al. The mean amount of thromboxane B2 found in the sera of the 6 untreated donors used in these experiments was 614 pmole/ml.

RESULTS

Platelet counts in extracorporeal circuits decreased to 32% ± 6% of initial levels at 30 sec and to 14% ± 5% by 15 min. Subsequently, they rose slowly to 65% ± 2%
Platelet counts. Platelet counts are plotted as a percent of the platelet count of whole blood drawn directly from the donor. The symbol i represents 2 min of recirculation. The points represent means (in Figs. 1 and 2) and the error bar represents one standard error of the mean. (o—o) Blood recirculated after addition of PGE, and lidocaine to the venous reservoir; N = 1. (o-----o) Recirculated blood obtained from donors who had ingested aspirin; N = 5. (o--o) Recirculated blood obtained from donors who had abstained from all aspirin-containing medicines; N = 5.

SEM by 2 hr; these results agree closely with those previously reported (Fig. 1). Plasma LA-PF₄ levels rose from <1.5% to 11% ± 3% of total released LA-PF₄ (see Materials and Methods) within 30 sec of extracorporeal circulation. By 15 min, plasma LA-PF₄ concentrations had risen to 42% ± 3%, indicating extensive release of platelet granule contents (Fig. 2). For comparison, thrombin, collagen, and ADP release was 100%, 70%, and 40% of total LA-PF₄, respectively. No release of LA-PF₄ (not shown) was detected in blood incubated for a corresponding length of time. Thromboxane B₂ was undetectable in plasma after 2 min of recirculation, although only 32% of the platelets remained in circulation. By 15 min of recirculation, however, plasma thromboxane B₂ levels had risen to 3.1 ± 1.8 pmole/ml and continued to rise with time to 9.6 ± 1.9 pmole/ml at 2 hr (Fig. 2). The appearance of detectable levels of thromboxane B₂ appeared to be associated with a further increase in the plasma levels of LA-PF₄ (Fig. 2).

When blood was obtained from donors who had ingested aspirin and recirculated, platelet counts declined 47% at 30 sec and to 26% ± 9% by 15 min. Subsequently, they rose slowly to 62% ± 5%. These results were not significantly different (p > 0.05 by unpaired Student’s t test) from those obtained with blood from untreated donors. Plasma levels of LA-PF₄ rose to 7.5% within 30 sec of recirculation and continued to rise slowly to 25.3% ± 3.8% by 2 hr (Fig. 2). Thus, aspirin appeared to slow the increase in plasma LA-PF₄ levels and reduced the...
ultimate extent of platelet secretion by approximately 50%. Aspirin, however, completely abolished thromboxane \( B_2 \) synthesis. In serum of donors ingesting aspirin, thromboxane \( B_2 \) levels were \(<0.5\) pmole/ml compared to the mean of 614 pmole/ml in serum of our 6 untreated donors.

When blood containing PGE\(_1\) (not shown), PGI\(_2\) (not shown), or a mixture of PGE\(_1\) and lidocaine was recirculated in the extracorporeal circuit, platelet loss was prevented (Fig. 2), and neither platelet secretion (plasma LA-PF\(_4\) \(<1.5\%\) after 2 hr of recirculation; not shown) nor thromboxane \( B_2 \) release was detected.

**DISCUSSION**

The formation of prostaglandin endoperoxides and thromboxanes is closely associated with the platelet release reaction.\(^{14,16}\) Indeed, there is strong evidence that thromboxane \( A_2 \) is a necessary mediator of one pathway leading to platelet secretion.\(^{17,18}\) Our studies indicate that when platelet release occurs in an extracorporeal circuit it is also associated with thromboxane synthesis. Furthermore, inhibition of thromboxane synthesis with aspirin (an inhibitor of cyclooxygenase) appeared to alter the temporal pattern of release and reduced platelet secretion by approximately 50%. Clearly, the products of cyclooxygenase activity are a factor in the release of platelet constituents occurring during recirculation.

Substantial platelet secretion persists during recirculation despite effective inhibition of platelet thromboxane synthesis (donor serum \(<0.5\) pmole/ml), indicating that the extracorporeal circuit can induce release via pathways that are independent of cyclooxygenase activity. Inducing platelet secretion by aspirin sensitive and insensitive pathways is a characteristic shared by several activating agents including collagen.\(^{7}\) Platelets respond similarly to both collagen and biopolymers in that adhesion is a preliminary step in platelet activation. For collagen, however, platelet secretion and synthesis of thromboxanes have not been temporarily dissociated. In contrast, during recirculation, release of LA-PF\(_4\) clearly precedes the onset of detectable thromboxane synthesis. Furthermore LA-PF\(_4\) release coincides with the decline in the circulating platelet count, an indirect measure of platelet adhesion. Detectable thromboxane synthesis, however, occurs several minutes after maximum platelet loss and is less clearly associated with initial platelet adherence. Thus, it appears that the extracorporeal circuit provides a system for dissociating adhesion-mediated from thromboxane-mediated platelet secretion.

Extensive contact between blood and synthetic surfaces results in pronounced quantitative and qualitative alterations in platelet function. Inhibition of platelets with aspirin clearly does not prevent platelet loss and only attenuates the release of LA-PF\(_4\) during simulated extracorporeal circulation. The inadequacy of aspirin contrasts with the demonstrated efficacy of prostaglandins \( I_2 \) and \( E_1 \)\(^{1,2}\) alone or prostaglandin \( E_i \) and lidocaine. By stimulating adenylcyclase and stabilizing the platelet membrane, these agents may be providing a more effective degree of platelet inhibition. Furthermore, the ability of prostaglandin \( E_i \), which acts through the cAMP system, and surface-adsorbed albumin to prevent release of LA-PF\(_4\) suggests that release of LA-PF\(_4\) following aspirin ingestion is not the result of mechanical lysis but a consequence of the secretion process. Release of approximately 10% of LA-PF\(_4\) induced by ADP, however, has been detected despite
negligible release of 5HT, implying that early release of LA-PF4 during recirculation might reflect secretory pathways unique to protein-containing granules.12

Thromboxane synthesis has been detected during clinical cardiopulmonary bypass.19,20 Our studies demonstrate that induction of thromboxane synthesis occurs to a limited extent in the extracorporeal circuit where platelet adhesion is pronounced.11 Platelet adherence to a biopolymer may be influenced, in part, by release of a soluble platelet-recruiting agent.21 The time course of thromboxane formation and the inability of aspirin to limit platelet loss during recirculation make it unlikely that thromboxanes are functioning in this fashion. The detection of plasma thromboxane B2 formation in the extracorporeal circuit, however, indicates prior release of thromboxane A2, which is a potent vasoconstrictor and platelet activator. Released thromboxane A2 may thus compromise capillary perfusion and contribute to platelet microaggregate formation22 during cardiopulmonary bypass.

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