"Aspirinated" Platelets are Hemostatic in Thrombocytopenic Rats With "Nonaspirinated" Vessel Walls—Evidence From an Exchange Transfusion Model

By Elisabetta Dejana, Brunella Barbieri, and Giovanni de Gaetano

The contrasting effects of aspirin on bleeding time (BT) might be related to the drug's inhibitory activity on platelets and vascular prostaglandin 12 (PGI2). To test this, we developed an exchange transfusion model in the rat and studied the BT in animals whose platelets but not vessels had been exposed to aspirin. Rats with severe experimental thrombocytopenia were exchange-transfused with blood from normocythemic rats pretreated with aspirin 6 hr before. The platelet count was raised from 2% to about 70% of basal level and the BT returned to control values even though the platelets neither responded to arachidonic acid nor produced detectable amounts of malondialdehyde and vascular PGI2 was not inhibited. These results indicate that "aspirinated" platelets may be hemostatically active and that the BT is not necessarily affected by unbalanced prostaglandin production in platelets and the vessel wall.

It has long been known that aspirin prolongs the bleeding time in man. This effect, however, may vary from one individual to another, is seldom of clinical relevance, and may be greatly influenced by the experimental conditions used.1-5

In laboratory animals the effect of aspirin on bleeding time is controversial and, in fact, both prolongation and shortening of the bleeding time have been reported in transected mesenteric vessels of rabbits. Contrasting results have also been obtained by different investigators in rats. Using a standardized "template" technique,6 we did not observe any modification of the bleeding time in normal rats given different doses of aspirin.6 However, other investigators,7,8 using different experimental conditions, found this parameter prolonged.

Besides methodological problems, the different effects of aspirin on bleeding time could be related to the drug's inhibitory activity on platelet and vascular prostaglandins.12,14 Indeed, inhibition of aggregating platelet endoperoxides and thromboxane A2 could be counterbalanced in some way by simultaneous blockade of the antiaggregating vascular prostaglandin I2, thus resulting in a modest and variable effect of aspirin on bleeding time.15 This interpretation is difficult to substantiate since the drug's effect on platelets cannot be easily dissociated from that on the vessel wall.14

We have therefore developed an exchange transfusion model in the rat and studied bleeding times in animals whose platelets but not vessels had been exposed to aspirin. In this model, selective inhibition of platelets by aspirin did not result in any prolongation of the bleeding time. This indicates that "aspirinated" platelets may be hemostatically active and that bleeding time is not necessarily affected by unbalanced prostaglandin production in platelets and vessel walls.

MATERIALS AND METHODS

Male Sprague Dawley CD-COBS rats (Charles River) weighing 250-350 g were used. Acute thrombocytopenia was induced in a group of animals (platelet recipients) by i.v. injection of specific antiserum against rat platelets obtained in rabbits as described.16 Using 0.05 ml/100 g body weight the platelet count was reduced to about 2% of basal values for at least 12 hr after injection. Neither red cell nor leukocyte counts were significantly affected.

The bleeding time was longer than 10 min and reverted to control values within 48 hr. In preliminary experiments washed platelets17 from normal rats were infused into thrombocytopenic animals (9 x 107 platelets/animal/2.5 ml/10 min) 6 hr after the induction of thrombocytopenia. One hour after the end of the infusion platelet count was about 400 x 103/liter (with a recovery of about 70%) and the bleeding time was shortened to about 4 min.

This procedure was cumbersome, required the sacrifice of five donor animals for each recipient and the infusion of an amount of platelet suspension of about 20% of the blood volume of the recipient animal without completely normalizing either platelet count or bleeding time.

For these reasons the exchange transfusion model described below was developed.

Six hours after induction of thrombocytopenia, each animal was anesthetized with sodium pentobarbital (35 mg/kg) and given heparin (Liquemin) 100 U/kg i.v. (a dose that did not affect the bleeding time measurements).18 The jugular vein and carotid artery were cannulated with a piece of PE50 tubing.

The carotid artery and jugular vein of a thrombocytopenic recipient rat were connected to the jugular vein and carotid artery of a donor rat. Three groups of donor rats were used; the first one was made thrombocytopenic as described. The other two groups consisted of rats with a normal platelet count given either isotonic saline or 0.05 ml/100 g platelet suspension of about 20% of the blood volume of the recipient animal.
saline 1 ml/kg body weight, or aspirin (50 mg/kg) (as its soluble lysine salt, Flectadol Maggioni) 6 hr before connection with the recipients. After the connection was established, the blood was allowed to flow from one animal to the other for 10 min. After that period the two animals were disconnected and the tubings were clamped. Arterial blood samples were collected from the cannulated carotid arteries for platelet count before and after the exchange transfusion in both recipient and platelet donor.

Within 10 min the platelet count reached essentially the same value in both animals (about 700-800 x 10^9/liter). After the exchange transfusion, the thrombocytopenic rat was immediately value in both animals (about 700-800 x 10^9/liter). After the exchange transfusion, the thrombocytopenic rat was immediately

Table 1 reports control values for platelet count, platelet MDA, vascular PGI2, and bleeding time of normocythemic and thrombocytopenic rats after two platelet exchange transfusions. When the donor rats were thrombocytopenic, the recipient rats' platelet count after transfusion remained about 30 x 10^9/liter and bleeding time was longer than 10 min. In contrast, following exchange transfusions with either saline or aspirin treated normocytic donors the platelet count was restored to about 900 x 10^9/liter and bleeding time became approximately 100 sec for both groups. Platelets from thrombocytopenic rats exchanged with saline treated rats, were aggregated by arachidonic acid (data not shown) and produced the same amounts of MDA as normocytic rats (Table 1). Aspirin treated donors supplied recipients with platelets that neither responded to arachidonic acid nor produced detectable amounts of MDA. Vascular PGI2 activity was within the control range in all groups of recipient animals (Table 1).

**DISCUSSION**

The prolonged bleeding time of rats with severe experimental thrombocytopenia was normalized by transfusion of platelets from either saline or aspirin pretreated rats. Since the exchange transfusion was performed 6 hr after administration of aspirin to donor animals, it is unlikely that the recipient animals' vessels were exposed to the drug. This was confirmed by the observation that PGI2 activity of the recipients' arteries and veins was normal.

In contrast, cyclo-oxygenase of transfused platelets was completely blocked, as shown by MDA assay and by the absence of aggregation induced by AA. In a few additional animals TxB2 was measured by radioimmunoassay according to Lewy et al. and it was found below detectable values (5 pmol x 10^9 platelets).

In previous studies in normal rats we did not find
the bleeding time prolonged by aspirin. Although a large range of doses of aspirin was studied at various intervals after treatment, inhibition of vascular (capillary) PG12 activity by the drug could not be excluded. The possibility, therefore, remained that the lack of effect of aspirin might be the consequence of a simultaneous blockade of the thromboxane–prostacyclin balance.

The results of the present study rule out such a possibility. They indicate that the biochemical defect induced by aspirin does not affect the hemostatic function of platelets in the bleeding time test used. This is in agreement with previous studies in humans showing that platelets from donors ingesting aspirin are still hemostatically effective.

O’Brien suggested that the hemostatic function of the platelet pool is maintained when approximately 20% of the circulating platelets have not been exposed to aspirin. A similar explanation for the hemostatic effectiveness of aspirinated platelets reported here is unlikely. The platelet count of recipient thrombocytopenic animals before exchange transfusion was about 30 x 10^9/liter, corresponding to about 3% of the total platelet count reached after completion of this procedure. Moreover, AA induced in vitro aggregation of platelets obtained from recipients (at the end of exchange) was completely inhibited.

The question remains, therefore, why aspirin did not prolong the bleeding time despite pronounced biochemical and functional changes in platelets (even in a condition where vascular PG12 activity was normal). It has been shown that aspirin does not prevent either platelet adhesion to subendothelium or subsequent release reaction of the adherent platelets. On the other hand, in vitro data have shown that release inhibition by aspirin may be overcome by high doses of collagen and thrombin-induced release is only inhibited by aspirin to a very limited extent.

Serial morphological studies of the early hemostasis in human skin wounds following aspirin ingestion showed less stable and larger plugs but normal platelet aggregation. All these data indicate that impairment of the arachidonate pathway by aspirin is not by itself sufficient to reduce the hemostatic efficacy of platelets. It may, however, become a precipitating factor when associated with other abnormalities of platelet function.

This concept is supported by the observation that aspirin dramatically prolongs the bleeding time in rats treated with dipirydamole, an inhibitor of platelet adhesion. On the other hand, it is known that aspirin may cause excessive prolongation of the bleeding time in patients with abnormal hemostasis such as in von Willebrand’s disease.

In conclusion, failure of aspirin to prolong bleeding time in rats is not related to inhibition of vascular prostacyclin activity that could mask the drug’s inhibitory effect on platelet endoperoxides and thromboxane A2 formation. More likely, the functional defect associated with the aspirin-induced modifications of the arachidonate metabolism leaves intact the platelets’ ability to take part in primary hemostasis. This suggests that thromboxane-prostacyclin balance is not the only or the most important factor regulating bleeding time.

ACKNOWLEDGMENT

Dr. Silvia Villa provided us with the antiplatelet antiserum. Judith Baggott, Anna Mancini, and Paola Bonifacio helped prepare the manuscript.

REFERENCES

5. de Gaetano G, Donati MB, Garattini S: Drugs affecting platelet function tests. Their effects on haemostasis and surgical bleeding. Thromb Diath Haemorrh 34:283, 1975
23. Baumgartner HR, Muggli R: Effect of acetylsalicylic acid on platelet adhesion to subendothelium and on the formation of mural platelet thrombi. Thromb Diath Haemorrh 60:345, 1974 (Suppl)
"Aspirinated" platelets are hemostatic in thrombocytopenic rats with "nonaspirinated" vessel walls--evidence from an exchange transfusion model

E Dejana, B Barbieri and G de Gaetano