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Effects of Penicillin G and Cephalothin on Platelet Function *in Vivo* (39458)

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Penicillin G and related antibiotics have been shown by a number of investigators to inhibit platelet reactions *in vitro* (1-5). These antibiotics have also been reported to cause excessive bleeding in patients, and to increase the bleeding times in patients and volunteers receiving high doses of these drugs (1, 3-7).

In previous studies ((2), and Cazenave *et al.* Brit. J. Haematol., in press) we showed that penicillin G, carbenicillin, and cephalothin inhibit the adherence of platelets to a collagen-coated surface and to the damaged aortic surface of rabbits. We also found that penicillin G and cephalothin became associated with the platelets and that their inhibitory effects *in vitro* persisted after the platelets were washed and resuspended in fresh media.

Although the antibiotics have been shown to affect platelet function *in vitro*, it has not been established whether these antibiotics prolong the bleeding time *in vivo* by affecting the vessel wall, the plasma proteins, or the platelets. To investigate this question, we have treated platelets with penicillin G or cephalothin *in vitro*, washed the platelets, resuspended them in fresh media, reinfused them into thrombocytopenic rabbits, and examined their ability to shorten the prolonged bleeding times of these animals. We have also studied the effect of pretreatment of rabbit platelets with penicillin G, on platelet survival time upon infusion into rabbits.

Materials and methods. Reagents. Penicillin G (USP sodium, 1650 units/mg) was obtained from General Biochemicals, Chagrin Falls, Ohio). Cephalothin (Keflin) was a gift of Dr. R. S. Dolman, Eli Lilly and Co. (Canada), Toronto, Ontario. The antibiotics were dissolved in water at a concentration of 150 m*M*. Dilutions to the required concentrations were made with modified Tyrode solution (no calcium or magnesium); the pH was adjusted to 7.35 and the osmolarity to 290 mOsm. Fresh solutions were prepared immediately before each experiment.

Preparation of suspensions of washed platelets labeled with ^{51}Cr. Suspensions of twice-washed platelets from rabbits were prepared in Tyrode solution containing 0.35% bovine albumin (8). They were labeled with ^{51}Cr in the first washing fluid (2). For the bleeding time studies, the platelets were incubated for 30 min at 22° with 1.5 m*M* (880 units/ml) penicillin G or 1.5 m*M* (0.606 mg/ml) cephalothin before being centrifuged and resuspended in fresh medium at a platelet count of 2,000,000/mm^3 for infusion. For the platelet survival studies, the washed platelets were incubated for 20 min at 37° with 15 m*M* (8800 units/ml) penicillin G and then resuspended in fresh medium at a platelet count of 3,000,000/mm^3 before injection into the rabbits.

Platelet survival. The *in vivo* survival of washed rabbit platelets treated with penicillin G was measured by a modification of the method of Reimers *et al.* (9) using platelet suspensions labeled with ^{51}Cr. Control experiments were done with platelets that had not been treated with the antibiotic, but had been subjected to the same washing and resuspending procedures. The radioactivity of samples of whole blood was determined by the method of Aster and Jandl (10).

Preparation of antiserum to rabbit platelets. The antiserum was raised in sheep and was kindly provided by Dr. J. Gauldie, Department of Pathology, McMaster University, Hamilton, Ontario, Canada. Approximately 200 ml of rabbit blood, pooled from six animals, was collected into 2% EDTA-0.33% saline (9 parts of blood to 1 part anticoagulant). Platelet-rich plasma was centrifuged at 2300*g* for 15 min and the

platelet pellet was resuspended in 5 ml of 0.85% saline at a platelet count of 5,000,000/mm^3. The platelets were disrupted by sonication and the sonicate was stored at −20° in 0.1-ml aliquots. For injection, an aliquot was thawed, mixed with 0.1 ml of complete Freund's adjuvant, and injected subcutaneously into a sheep; this was repeated once a week for 6 weeks. At 7 weeks, the sheep was bled and the serum was prepared, heated for 30 min at 56° to destroy complement, and stored at −20°. The presence of antiplatelet antibody was confirmed by the formation of a precipitin line against a sonicated rabbit platelet suspension on an Ouchterlony plate.

Production of thrombocytopenia. Thrombocytopenia in rabbits was produced by whole body irradiation with a cesium source (930 rad, 465 rad on each side for 14.5 min). During irradiation, both sides of the necks of the rabbits were shielded with a lead ribbon to prevent radiation damage to the blood vessels. The rabbits were given injections of 1 ml penicillin-streptomycin (Derapen-C, with dihydrostreptomycin, Ayerst Laboratories, Montreal, Canada) 24 and 48 hr after irradiation. Seven days later the rabbits received an intravenous injection of 0.3 ml of sheep antiserum to rabbit platelets, 12 to 16 hr before the bleeding time studies were to be done.

Platelet counts and bleeding time determinations. One hour before the bleeding time studies, samples of blood were taken from the ear veins of the thrombocytopenic rabbits and the platelets were counted by the method of Brecher and Cronkite (11). Platelets that had been pretreated with penicillin G or cephalothin (or control platelets) were then injected intravenously into the thrombocytopenic rabbits. Each rabbit received 10^{10} platelets in a volume of 5 ml (the weights of the rabbits were similar (mean ± SEM, 2527 ± 47 g)). Thirty minutes later the rabbits were anesthetized with sodium pentobarbital, the jugular veins were exposed and each was arranged above a small trough. Blood samples were taken by cardiac puncture for platelet counts and for measurement of the percentage recovery of ^{51}Cr-labeled platelets injected. Then a puncture wound was made in a jugular vein with a 23 gauge needle. The wound site was observed until bleeding ceased and the time was recorded. To prevent accumulation of blood at the wound site, a flow of 0.85% saline was directed into the trough below the exposed jugular vein. The bleeding time was then measured again in the same way by puncturing the other jugular vein.

Statistical considerations. In experiments with thrombocytopenic rabbits the relation between the platelet count and the bleeding time has been found to resemble an exponential function (Hirsh, J., and Senyi, A. F., unpublished observation). Therefore, the bleeding time values were transformed to logarithms before determining the mean for each experiment. The significance between any two of the three means was determined by a two-way analysis of variance. This analysis also provides the best common estimate of the standard deviation. This was then used to develop the 99% confidence intervals for each of the two treatment means and the control mean. The calculated means and confidence intervals from the transformed data were then transformed back to the original scale.

Results. Platelet survival. Pretreatment of platelets with penicillin G had no apparent effect on their survival *in vivo* (Fig. 1).

Bleeding times. The range of bleeding

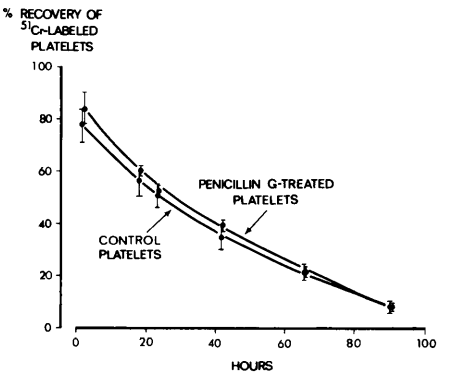

FIG. 1. The effect of penicillin G on survival of control washed rabbit platelets and platelets that had been incubated in vitro with penicillin G (15 m*M* for 20 min at 37°) and resuspended in fresh medium before infusion into the rabbits. Platelets were labeled *in vitro* with ^{51}Cr before penicillin G treatment. Means and standard errors of the means are shown. Four rabbits in each group.

times of 41 untreated rabbits (mean platelet count ± SEM, 414,000 ± 12,900/mm³) was 60 to 180 sec [geometric mean and (99% confidence intervals), 100 (73–136)sec]. Bleeding time from puncture wounds in the jugular veins of thrombocytopenic rabbits with platelet counts of 4000 to 16,000/mm³ (mean ± SEM, 8000 ± 130/mm³) ranged from 764 to 1220 sec [geometric mean and (99% confidence intervals), 1012 (934–1098) sec]; these values were determined in a separate group of 12 rabbits that did not receive injections of platelets. Platelets which had not been treated with an antibiotic shortened the bleeding time of thrombocytopenic rabbits to within the range of bleeding times of untreated rabbits with normal platelet counts (Table I). Although the platelets that had been pretreated with penicillin G or cephalothin did shorten the bleeding time from the puncture wounds in the jugular veins, they were much less effective than the control platelets which had not been treated with an antibiotic (Table I). Injection of platelets pretreated with penicillin G shortened the bleeding time more than injection of cephalothin pretreated platelets. The observed difference between any two of the three means was statistically highly significant ($p < 0.001$).

The mean platelet counts of the 21 thrombocytopenic rabbits used for studies with the antibiotic-treated platelets are shown in Table I. The mean platelet counts and the percentage recovery of ^{51}Cr-labeled platelets before the bleeding time determinations are also shown. In no instance was there a statistically significant difference ($p < 0.5$) between the three groups of animals; thus, the effect of the antibiotics on the bleeding time cannot be attributed to differences in platelet counts or platelet recovery.

Discussion. Pretreatment of rabbit platelets with a high concentration of penicillin G *in vitro* did not affect their recovery or survival *in vivo* following reinjection.

The concentration of the antibiotics to which the platelets were initially exposed was much greater than the concentrations usually achieved in man (0.03 to 12 units of penicillin/ml). However, maximal serum concentrations of 1000 to 2000 units/ml have been reported in man following intravenous administration of 20 to 100 million units of penicillin G (12).

The diminished ability of platelets preincubated with penicillin G or cephalothin to shorten the bleeding time of thrombocytopenic rabbits is in keeping with our observations from earlier studies of the effects of these antibiotics on platelet function (Cazenave *et al.*, Brit. J. Haematol., in press). We found that platelets incubated with penicillin G or cephalothin, washed and resuspended in fresh medium responded less strongly to aggregating and release-inducing agents (ADP, collagen, or thrombin) than control platelets. It seems likely that the antibiotics became bound to the platelets. Thus, it is not surprising that platelet function *in vivo* is inhibited by preincubation of the platelets with the antibitoics *in vitro*. The impairment of the ability of platelets to adhere to subendothelial structures and to

TABLE I. BLEEDING TIMES[a] OF THROMBOCYTOPENIC RABBITS GIVEN PLATELETS PREINCUBATED IN VITRO WITH PENICILLIN G, CEPHALOTHIN, OR NEITHER ANTIBIOTIC.

Antibiotic used for pretreatment of platelets	Number of determinations	Mean platelet count[b] (number/mm³)		Percent of recovery of ^{51}Cr-labeled platelets[b]	Bleeding time[c] (sec) mean (confidence interval)
		Before infusion	Before bleeding time determinations		
None (control)	14	4600 ± 600	62,700 ± 4700	68 ± 9	119 (98–144)
Penicillin G 1.5 m*M*	14	4000 ± 500	56,600 ± 6900	69 ± 8	375 (309–455)
Cephalothin 1.5 m*M*	14	4600 ± 300	55,100 ± 6200	65 ± 4	616 (507–748)

[a] Bleeding times of rabbits that had been made thrombocytopenic in this way and were not given platelet infusions ranged from 764 to 1220 sec (see text).

[b] Mean ± SEM.

[c] Geometric mean and 99% confidence intervals. Difference between any two of the three treatment means ($p < 0.001$, two-way analysis of variance).

react to aggregating agents would account for the observation that the antibiotic-treated platelets were much less effective than control platelets in shortening the bleeding times of thrombocytopenic rabbits.

Thus, the effects of penicillin G and related antibiotics on the bleeding times of patients (1, 5–7) and volunteers (3, 4) given high doses of these drugs is, at least in part, attributable to the inhibitory effects of the antibiotics on the platelets. Our results, however, do not exclude the possibilities that the antibiotics also affect the vessel wall and/or the plasma proteins.

Our results support the suggestion of Brown *et al.* (4) that "patients with underlying hemostatic defects, especially patients with thrombocytopenia, may be at an increased risk of hemorrhage" when penicillin G and related antibiotics are administered in high dosages.

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