

Defective Platelet Function Following the Administration of Penicillin Compounds

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Platelet function and blood coagulation were studied in five human volunteers receiving penicillin-G in incremental doses of 1.2–48 million U/day, in six volunteers receiving ampicillin in incremental doses of 60–300 mg/kg/day (4–20 g/day), and in six volunteers receiving methicillin in incremental doses of 60–300 mg/kg/day. Coagulation tests remained normal in all 17 volunteers. However, ADP-induced platelet aggregation became abnormal in every subject except one receiving ampicillin and one receiving methicillin. Defective aggregation occurred with predictability with the following doses: penicillin-G, 24 million U/day; ampicillin, 300 mg/kg/day; methicillin, 300 mg/kg/day. All volunteers given penicillin-G and

all given ampicillin experienced dose-related prolongation of bleeding time which did not occur with methicillin. Striking prolongation of bleeding time occurred only with penicillin-G in doses of 48 million U/day. Other tests of platelet function including clot retraction, platelet factor 3 availability, and collagen-induced or epinephrine-induced aggregation remained normal during the administration of these drugs. Measurement of intracellular adenine nucleotides revealed that the ADP and ATP content of platelets was unaffected. It appears that at least one mechanism by which the penicillin compounds alter platelet behavior is by interfering with activation of these cells by ADP.

RECENT ADDITIONS to the list of pharmacologic agents capable of inhibiting platelet function¹ include the closely related semisynthetic penicillins, carbenicillin,^{2,4} and ticarcillin.⁵ Indeed, bleeding ascribed to defective platelet function has been reported in several patients receiving carbenicillin.^{2,4}

The most predictable abnormality of platelet function in these subjects is a reduced aggregation response to adenosine diphosphate (ADP).^{2,4,5} Since the degree of derangement is dose dependent, it is not surprising that carbenicillin, which is usually prescribed in very large doses of 300–600 mg/kg/day (20–40 g/day), was the first penicillin compound to be recognized as having an effect on platelets.² Now it is clear that even penicillin-G in extremely large doses alters platelet function.^{3,6}

Since all penicillins share the same basic structure, 6-amino-penicillanic acid (differing only in the radical attached to the free amino group of this acid moiety⁷), it may be that all penicillin compounds have the capability of altering platelet function but that very high doses must be administered before the defect becomes apparent. The present study was carried out to examine this possi-

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bility. The effects of three commonly used penicillin compounds on platelet function and blood coagulation were investigated using human volunteer subjects. The penicillins employed were: penicillin-G, since it is the penicillin most commonly prescribed and is not infrequently given in very large doses; ampicillin, a widely used broad-spectrum penicillin; and, methicillin, a penicillinase-resistant semisynthetic penicillin.

Another objective of the study was to investigate further the mechanism whereby these drugs alter platelet function.

MATERIALS AND METHODS

Subjects

For this study, human volunteers obtained through the Texas Department of Corrections were admitted to the General Clinical Research Center of The Methodist Hospital. The purpose, methods, and hazards of the study were explained in detail and presented in writing to each volunteer. The option to withdraw from the study at any time was emphasized. All subjects signed written, informed consent forms before receiving any study drug. A detailed investigational protocol was submitted and approved by both committees on research involving human beings from the two institutions involved in the study. All volunteers were in good health as judged by physical examination, complete blood counts, blood chemical studies, urinalysis, stool examination for occult blood, electrocardiography, and radiographic examination of the upper gastrointestinal tract. Each gave a negative history for bleeding tendency, peptic ulcer disease, renal disease, or previous allergic reaction to penicillin. Drugs other than those under investigation were not administered to any volunteer during the study and no aspirin-containing medications for the month prior to the study.

Drug Administration

Following the establishment of baseline values for tests of blood coagulation and platelet function, five volunteers received incremental doses of penicillin-G intravenously beginning with 1.2 million U/day* for 3 days. This period was followed with 6 million U/day for 4 days, 12 million U/day for 3 days, 24 million U/day for 4 days, and finally 48 million U/day for 3 days. Two groups with six volunteers in each received incremental doses of either ampicillin or methicillin intravenously. These two drugs were given sequentially as follows: 60 mg/kg/day for 3 days, 100 mg/kg/day for 4 days, 200 mg/kg/day for 3 days, and finally 300 mg/kg/day for 4 days. Daily doses of each of the drugs were given in divided doses on a 4-6-hr basis. Otherwise, there were no interruptions in drug administration for any volunteer in the study. The duration of drug administration for different doses was chosen for convenience.

Blood Coagulation and Platelet Function Studies

Before, during, and after drug administration numerous tests of blood coagulation and platelet function were performed. The methods employed, previously described,⁴ included platelet count, template bleeding time, prothrombin time, partial thromboplastin time, thrombin time, plasma fibrinogen, prothrombin consumption, thrombin-induced platelet-rich plasma clot retraction, kaolin-induced platelet factor 3 availability, and platelet aggregation. Platelet aggregation was studied in a Bryston Aggregometer (Bryston Manufacturing, Ltd., Scarborough, Ont., Canada) using collagen (bovine tendon), epinephrine, or ADP as aggregating agents. All platelet aggregation studies were carried out with a standard platelet concentration of 300×10^9 /liter. Aggregation to ADP and epinephrine was assessed by determining the presence or absence of a secondary wave and by quantification of primary aggregation. ADP-induced primary aggregation was quantified by measuring the light transmission of platelet-rich plasma (LT_{PRP}) and platelet-poor plasma

*In keeping with international convention, doses of penicillin-G in this report are given in terms of units and doses of the other penicillin compounds are given in terms of weight. To facilitate a comparison of the doses given, see Fig. 2.

(LT_{PPP}) and after completion of the primary wave of aggregation (LT_{1^*}) and then applying the following equation: $1^* \text{ aggregation} = (LT_{1^*} - LT_{PRP}) \div (LT_{PPP} - LT_{PRP})$.^{4,5} In all aggregation studies, unless indicated otherwise, the final concentrations of ADP and epinephrine were $2 \mu M$ and $20 \mu M$, respectively. The final concentration of collagen used in aggregation studies was the greatest dilution that gave a maximum aggregation response with control platelets. Control platelets were obtained, on each day of testing, from a small pool of normal donors with known aggregation responses. Collagen was kept frozen at $-20^\circ C$ in small quantities until thawed for use, at which time serial twofold dilutions were made. All collagen that was thawed and/or diluted was discarded after a single day's use.

Platelet Adenine Nucleotides

For the purpose of assessing the effects of penicillin compounds on the adenine nucleotide content of platelets, the concentrations of ADP and adenosine triphosphate (ATP) in platelets before and after drug administration were measured in three volunteers receiving penicillin-G, two receiving ampicillin, and two receiving methicillin. For these determinations, ethanol extracts of platelets in platelet-rich plasma were prepared according to the method of Holmsen and Weiss.⁸ ATP and ADP (after its conversion in the presence of phosphoenolpyruvate and pyruvate kinase to ATP⁸) in extracts were measured by a bioluminescence technique⁹ utilizing luciferase enzyme (firefly lantern extract, Sigma FLE-50, Sigma Chemical Co., St. Louis, Mo.) and a Packard 574 liquid scintillation spectrometer (Packard Instrument Co., Downer's Grove, Ill.). Results were expressed in terms of $\mu\text{moles}/10^{11}$ platelets. Results were analyzed by the Student's *t* test.

RESULTS

Coagulation and Platelet Function Studies

Tests of plasma coagulation including prothrombin time, partial thromboplastin time, thrombin time, and plasma fibrinogen levels were unaffected by the administration of penicillin-G in doses as high as 48 million U/day or by ampicillin or methicillin in doses as high as 300 mg/kg/day (approximately 20 g/day).

No significant changes in platelet count, clot retraction, kaolin-induced platelet factor 3 availability, or platelet-dependent prothrombin consumption were observed during this study. However, significant alterations in bleeding time and platelet aggregation occurred.

Progressive lengthening of bleeding time occurred in all subjects receiving penicillin-G or ampicillin (Fig. 1). No significant change in bleeding time occurred with methicillin administration. Following 4 days of penicillin-G at a dose of 24 million U/day, the mean ($\pm$ SEM) bleeding time was 6.13 ± 0.82 min (range = 3.5–7.5 min), a value significantly longer than the baseline value of 3.20 ± 0.46 min (range = 2.0–5.0 min). Following 3 days of 48 million U/day, the bleeding time was 12.25 ± 1.97 min (range = 5.5–15 min). Ampicillin doses of 100 mg/kg/day for 4 days resulted in a significant prolongation of bleeding time (4.97 ± 0.64 min versus 2.96 ± 0.27 min prior to drug administration). After 200 mg/kg/day, the difference between the treatment mean and control mean dropped to a level not significantly different from the baseline value ($p = 0.05$). This finding was the result of one volunteer experiencing a shortening of bleeding time from 7.5 to 4.0 min in spite of an increase in the dosage of ampicillin. When the dose was raised to 300 mg/kg/day, the same volunteer once again demonstrated a bleeding time of 7.5 min and the mean value for the ampicillin group (6.0 ± 0.46 min; range 5.0–7.5 min) was again significantly prolonged. Three days after discontinuation of penicillin-G and

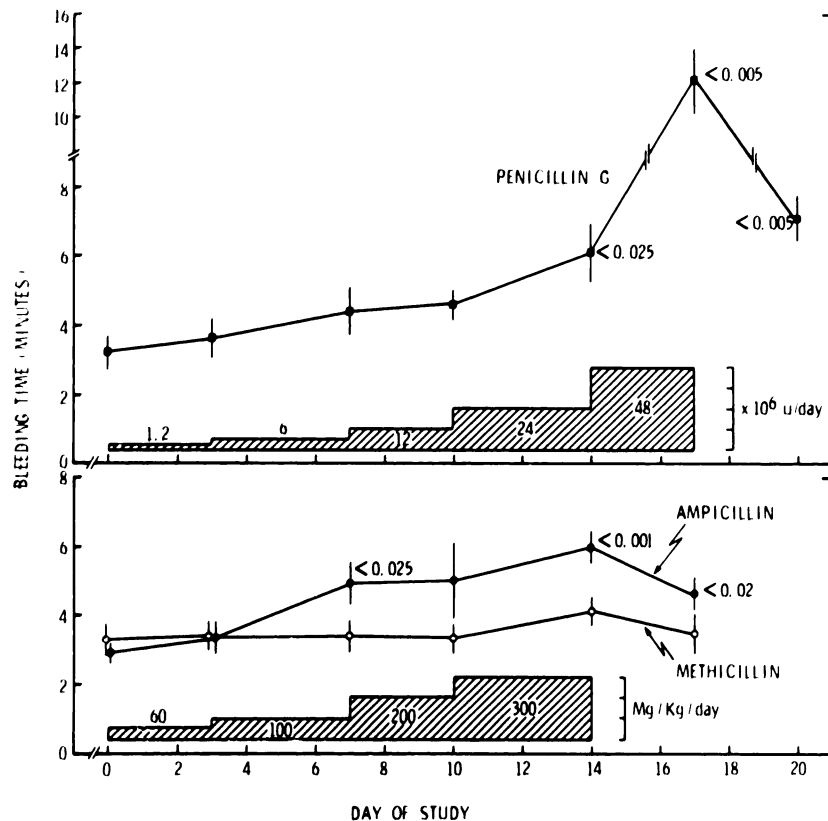


Fig. 1. Bleeding times (mean $\pm$ SEM) in volunteers receiving incremental doses of penicillin-G (■), ampicillin (●), and methicillin (○). The levels of significance (p values) for data that differ significantly from baseline are shown.

ampicillin administration, bleeding times were beginning to shorten but were still significantly prolonged (Fig. 1). Further follow-up studies were not performed.

Platelet aggregation studies revealed defects in the capacity of platelets to aggregate when ADP was employed as the aggregating agent but not when collagen or epinephrine was used. This effect was noted with all three drugs. A summary of the results of ADP-induced aggregation studies is shown in Table 1. A small but significant decrease in the degree of primary aggregation accompanied the administration of penicillin-G in doses of 24 million U/day or more. This change was also seen with ampicillin and methicillin at a dose level of 300 mg/kg/day. All subjects given penicillin-G eventually demonstrated a loss of secondary aggregation. One of the five volunteers given this drug exhibited a loss of secondary aggregation while receiving as little as 1.2 million U/day, whereas another volunteer in the penicillin-G group received 48 million U/day before exhibiting this manifestation of altered platelet function. One volunteer given 300 mg/kg/day of ampicillin and one given the same dose of methicillin showed persistent but reduced secondary aggregation.

Table 1. Summary of ADP-induced Platelet Aggregation Studies

Drug and Dose	Degree of Primary Aggregation (Relative Light Transmission) (mean $\pm$ SEM)	No. of Volunteers Demonstrating Loss of Secondary Aggregation/No. at Risk*
Penicillin-G (million U/day)		
0	0.56 $\pm$ 0.02	0/5
1.2	0.50 $\pm$ 0.04	1/5
6	0.50 $\pm$ 0.05	1/4
12	0.57 $\pm$ 0.02	0/3
24	0.46 $\pm$ 0.03†	2/3
48	0.45 $\pm$ 0.03†	1/1
Ampicillin (mg/kg/day)		
0	0.58 $\pm$ 0.02	0/6
60	0.58 $\pm$ 0.01	2/6
100	0.56 $\pm$ 0.02	0/4
200	0.50 $\pm$ 0.03	1/4
300	0.48 $\pm$ 0.02†	2/3†
Methicillin (mg/kg/day)		
0	0.57 $\pm$ 0.02	0/6
60	0.59 $\pm$ 0.01	0/6
100	0.51 $\pm$ 0.04	2/6
200	0.54 $\pm$ 0.01	3/4
300	0.52 $\pm$ 0.01†	0/1†

*Denominator represents number of volunteers who retained secondary aggregation after drug was administered at the previous dose level, and therefore were at risk to lose secondary aggregation during administration of the dosage shown.

†Significantly less than baseline value ($p < 0.05$).

‡The single volunteer who demonstrated a persistence of secondary aggregation at this dose level did exhibit a reduction in the magnitude of the secondary wave.

Platelet Adenine Nucleotides

The concentrations of ATP and ADP in platelets obtained from two subjects receiving a daily dose of 48 million units of penicillin-G, from one receiving a daily dose of 24 million units of penicillin-G, from two receiving ampicillin at a dose level of 200 mg/kg/day, and from two receiving methicillin at 300 mg/kg/day did not differ significantly from values determined for these volunteers during the baseline period (Fig. 2). Untoward bleeding was not noted in any of the 17 volunteers included in this study.

DISCUSSION

The penicillin compounds are among the most widely used drugs in medicine. It is not uncommon for patients being treated for severe infection to receive very large doses of these agents. For example, carbenicillin must be given in doses of 20–40 g/day to be maximally effective in the treatment of gram-negative septicemia.¹⁰ While such therapy is usually tolerated without significant toxicity or untoward effects, some patients receiving carbenicillin have experienced bleeding.^{2,4,11–13} Admittedly, most of these patients have had some degree of renal insufficiency which by itself can cause a hemorrhagic disorder or they have been receiving other drugs that might have contributed to a bleeding dis-

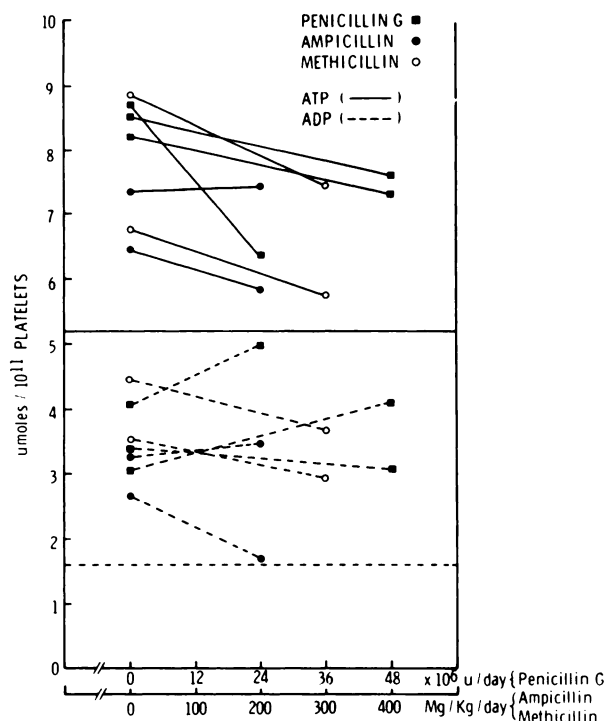


Fig. 2. Concentrations of platelet ATP and ADP before and after drug administration in three volunteers given penicillin-G (■), two given ampicillin (●), and two given methicillin (○). Doses of penicillin-G in millions of units per day and of ampicillin and methicillin in mg/kg per day are shown on the abscissa. Horizontal lines represent the lower limits of normal (mean - 2 SD) for ATP (—) and ADP (----) as determined in 18 normal control subjects.

order. When the carbenicillin is stopped, however, so does the bleeding.¹³ Further evidence that carbenicillin can create a bleeding tendency is that untoward bleeding has been noted in several patients (and human volunteers) with normal renal function who have received only carbenicillin in clinically applicable doses.⁴ Ticarcillin, an investigational semisynthetic penicillin similar in structure and antibacterial spectrum to carbenicillin but different in its greater bactericidal activity against *Pseudomonas*,¹⁴ has not produced any evidence of bleeding when given in a controlled study to human volunteers.⁵ Bleeding does not occur in spite of clear evidence that, like carbenicillin, ticarcillin causes a dose-related inhibition of platelet function. The absence of any evidence of bleeding in the ticarcillin study is explained by the fact that the largest dose of ticarcillin employed was 300 mg/kg/day, which is below the dose level of carbenicillin that is associated with bleeding in patients or volunteers with normal renal function.⁴

As a result of our earlier studies^{4,5} and those of Cazenave and co-workers³ who showed that large in vitro concentrations of penicillin-G affected platelet function and of Lacombe and associates⁶ who noted the effect in vivo, we felt that the present controlled studies would help to delineate: (1) the effects of other penicillin compounds on platelets as well as other components of the hemostatic system; (2) the dose level at which such effects occur; and (3) the mechanism of effect.

The results indicated that as with carbenicillin⁴ and ticarcillin,⁵ platelet function but not coagulation was altered by the penicillin compounds under study

and the character of the platelet defect was essentially the same as that produced by carbenicillin and ticarcillin. That is, the effect appeared to last for the life span of the exposed platelets (as evidenced by persistent prolongation of bleeding time for several days after drug was stopped) and ADP-induced platelet aggregation was the single most sensitive test for measuring the effect of these agents on platelets.

While doses of penicillin-G as small as 1.2 million U/day affected the platelets of some volunteers, significant prolongation of bleeding time required doses of 24 million U/day or more. Ampicillin in doses of 100 mg/kg/day or more caused prolongation of bleeding time but methicillin even at 300 mg/kg/day did not. Thus, it would appear that methicillin exerted less of an effect on platelets than did the other two agents. Aggregation studies, however, revealed similar degrees of effect by methicillin, ampicillin, and penicillin-G in equivalent doses. It may be that other components of platelet function such as adhesiveness were less affected by methicillin. Since we did not examine all aspects of platelet function, this remains an unresolved point.

Differences between the results of this study and those in which carbenicillin and ticarcillin were used^{4,5} were that platelet-dependent prothrombin consumption and epinephrine- and collagen-induced aggregation tests remained normal in volunteers given penicillin-G, ampicillin, or methicillin, whereas these tests became abnormal in most volunteers receiving comparable doses of carbenicillin or ticarcillin. We have no explanation for these disparate results, except for the obvious one related to the structural differences among these compounds.

It has been suggested that the penicillin compounds alter platelet behavior by coating the cell membrane, thereby interfering with sites for activation of the cell by compounds such as ADP.^{3,4} One alternative explanation for the loss of secondary aggregation is that the intracellular content of adenine nucleotides is in some manner reduced to an extent that the amount of ADP released from the platelet during the release reaction is insufficient for sustaining and augmenting aggregation.¹⁵ This alternative explanation seems untenable in view of the results of the present study that showed that the intracellular content of platelet ATP and ADP was not significantly changed by administration of these drugs.

While the degree of abnormality of platelet function observed in the subjects receiving penicillin-G, ampicillin, and methicillin did not result in an apparent bleeding tendency, there was sufficient inhibition in platelet function in several volunteers receiving high doses of penicillin-G to lengthen the bleeding time to 15 min or greater. Therefore, like aspirin and other antiaggregating agents, penicillin compounds should be considered potentially hazardous from the standpoint of bleeding when given in extremely high doses to patients with underlying disorders of hemostasis, especially those with thrombocytopenia. Furthermore, when the penicillins are given to patients with compromised renal function (who are likely to possess platelet function derangements related to uremia), careful attention should be given to the dosage regimen, since in such patients the blood levels of these drugs can become exceedingly high, which could potentiate the hazard of bleeding due to faulty platelet function.

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