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# Effects of Apalcillin on Platelet Function in Normal Volunteers

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**We investigated the effects of apalcillin on blood coagulation and platelet function. The drug was administered to 21 human volunteers in daily intravenous doses of 75, 150, and 225 mg/kg. These doses evoked abnormalities in platelet aggregation similar to those found with piperacillin and mezlocillin and less striking than those produced by carbenicillin and ticarcillin. Plasma coagulation as measured by prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen concentration was not affected. There were consistent and major reductions in plasma antithrombin III activity, particularly at the two higher dose levels. Of 21 patients, 7 (33%) also manifested maculopapular skin rashes which resolved after discontinuation of the drug.**

Penicillin and its semisynthetic antibiotic derivatives may affect hemostasis by causing a time- and dose-related defect in platelet aggregation (3, 9). The resultant platelet functional abnormality is most easily demonstrated in vitro by reduced aggregation to an ADP stimulus and in vivo by prolongation of the template bleeding time (7). Severity of the hemostatic defect is variable, however, and seems more predictable and profound with carbenicillin and ticarcillin, for example, than with piperacillin, mezlocillin, and ampicillin (2–4, 6, 7, 9). Apalcillin is a new semisynthetic penicillin with an extended antibacterial spectrum. It is structurally related to ampicillin. We administered apalcillin to normal human volunteers to assess its effects on hemostasis.

## MATERIALS AND METHODS

**Subjects.** A total of 21 normal, healthy human volunteers (15 males, 6 females), ranging in age from 22 to 34 years (mean age, 24 years), entered Medical Center Del Oro Hospital in Houston, Texas, where the study was done. Initial evaluation included a history and physical examination to exclude anyone with evidence of peptic ulcer disease, gastrointestinal bleeding, renal disease, or penicillin allergy. Also performed were complete blood count, blood chemistries, urinalysis, electrocardiogram, and stool examination for occult blood. Each volunteer provided informed consent.

**Drug administration.** Apalcillin (Wyeth Laboratories, Philadelphia, Pa.) was reconstituted to the desired concentration and then diluted in 100 ml of 5% dextrose in water and infused intravenously every 8 h. The volunteers were divided into three groups of seven. The volunteers received apalcillin daily at a dose of 75 (group A), 150 (group B), or 225 (group C) mg/kg for a 7-day period.

**Coagulation studies.** Blood coagulation and platelet function were evaluated upon initiation of the study, and the evaluation was repeated on days 4, 7, and 14 after the start of antibiotic infusion. Platelet count, prothrombin time, activated partial thromboplastin time, thrombin time, and template bleeding time (Simplate; General Diagnostics, Div. Warner-Lambert Co., Morris Plains, N.J.) followed standard methodology (7). Platelet aggregation was measured in a PAP-3 aggregometer (Bio/Data Corp., Horsham, Pa.). Aggregation was induced in platelet-rich plasma by epinephrine (final concentration, 45 μM; Parke, Davis & Co., Detroit, Mich.), ADP (final concentration, 2.5 μM; Sigma Chemical Co., St. Louis, Mo.), and arachidonic acid (final concentration, 0.5 mM; NuChek-Prep, Elysian, Minn.). Total platelet aggregation was measured as a function of the final changes in light transmission after aggregation in platelet-rich plasma (3, 6, 7). Aggregation was considered weak if it measured less than 75% of the base-line value in subsequent studies or if deaggregation occurred.

Prothrombin consumption was measured as previously described (3). Plasma fibrinogen concentration was estimated by the thrombokinetic technique and by quantitative Biuret digestion of the fibrin clot (6). Antithrombin III activity was measured in plasma by a chromogenic substrate technique (KabiVitrum AB, Stockholm, Sweden).

## RESULTS

One subject each in groups A and C discontinued therapy for personal reasons on day 5 and 6, respectively. One patient in group B developed a skin rash on day 5 and was dropped from further study. The prothrombin time, activated partial thromboplastin time, thrombin time, and platelet count were not significantly altered by a 7-day infusion of apalcillin at any dose. The results of platelet function tests appear in Table 1. No subject developed a prolonged template bleeding time on day 4 of the apalcillin infusion. However, on day 7 one volunteer in group A had a prolongation from the base-line value of 3 to 6.5 min; one in group B reached 7 min; and the bleeding time of one volunteer in group C was extended to 15 min. All bleeding times had returned to normal by day 14 of the study.

Platelet aggregation in all but one volunteer in group A remained normal throughout the study. This individual manifested defective aggregation to ADP, arachidonic acid, and epinephrine on day 7 only. Only two of seven individuals in group B showed normal aggregation patterns on day 4. All of the volunteers in group C developed abnormal aggregation patterns to ADP.

Prothrombin consumption times in all volunteers appeared to become more complete (longer) by day 7. In addition, the patients in groups B and C were noted to have apparent rises in plasma fibrinogen concentration as measured by a thrombokinetic technique but not by actual Biuret fibrin clot digestion. These changes suggested potentiation of the thrombin effect in the test systems; for this reason, plasma antithrombin III measurements were performed on all subjects. In comparing base-line with day-7 samples, we found a drop in antithrombin III activity as follows: group A,

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TABLE 1. Platelet function after apalcillin administration

| Test | No. abnormal/no. evaluable | | | | | |
|---|---|---|---|---|---|---|
| | Group A | | Group B | | Group C | |
| | Day 4 | Day 7 | Day 4 | Day 7 | Day 4 | Day 7 |
| Bleeding time | 0/6 | 1/6 | 0/7 | 1/6 | 0/7 | 1/6 |
| Aggregation (ADP) | 1/6 | 1/6 | 5/7 | 5/6 | 7/7 | 6/6 |
| Aggregation (epinephrine) | 0/6 | 1/6 | 3/7 | 2/6 | 5/7 | 2/6 |
| Aggregation (arachidonic acid) | 0/6 | 1/6 | 1/7 | 1/6 | 2/7 | 5/6 |

16 to 34%, mean 23%; group B, 26 to 52%, mean 39%; group C, 22 to 52%, mean 33%.

Finally, one patient in group B developed a diffuse maculopapular rash on day 4 of the study, and drug administration was discontinued. In addition, two volunteers in group B and four in group C developed rashes on day 7 or 8 of the study. All of the rashes faded within 7 days of discontinuation of the antibiotic.

## DISCUSSION

This study confirms that apalcillin, like other semisynthetic penicillins, can cause platelet dysfunction by inducing abnormalities in platelet aggregation. As previously reported (3, 7), the most sensitive indicator of platelet dysfunction was reduced aggregation to an ADP stimulus. The degree of abnormality produced by apalcillin appeared similar to that found with piperacillin and mezlocillin.

The changes in prothrombin consumption, thrombokinetic fibrinogen concentration, and plasma antithrombin III activity had not previously been observed in normal volunteers receiving semisynthetic penicillins. Modification of the electroimmunodiffusion pattern and activity of antithrombin III has been described in uremic patients achieving extremely high levels of antibiotic in their blood (1). The maximum level of apalcillin measured 30 min after administration during our study was only 508 μg/ml of blood.

The mechanism and the clinical significance of the reduced antithrombin III activity remain uncertain. It is clear that antithrombin III reductions in the hereditary state or induced by oral contraceptives of the magnitude noted in this study can predispose patients to venous thrombosis (5, 8). Because apalcillin also produces a mild functional platelet defect, perhaps the tendency toward thrombosis might be counteracted.

The high incidence of observed skin rashes also merits comment. Only one volunteer reacted to skin tests with penicillin G and benzylpenicilloyl-polylysine apalcillin, and then only to the apalcillin derivative. Further clinical experience with apalcillin is required to clarify the incidence and the significance of this observation.

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