

Study of the Effects of Ticarcillin on Blood Coagulation and Platelet Function

CLARENCE H. BROWN III,* ETHAN A. NATELSON, MAJOR W. BRADSHAW, CLARENCE P. ALFREY, JR., AND TEMPLE W. WILLIAMS, JR.

Department of Medicine, The Methodist Hospital and Baylor College of Medicine, Houston, Texas 77025*

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Ticarcillin is a new semisynthetic penicillin similar to carbenicillin. Since carbenicillin has been shown to inhibit platelet function to such an extent that bleeding may accompany its use, an investigation of the effects of ticarcillin on hemostasis was made. The drug was administered to 17 human volunteers for periods of 3 to 10 days in intravenous doses of 100, 200, or 300 mg/kg per day (7 to 21 g/day). Serial studies of blood coagulation and platelet function indicated that coagulation was unaffected by ticarcillin but that platelet function became defective in all subjects. Abnormal platelet function was evidenced by lengthening of bleeding time (17 of 17 volunteers), depressed adenosine diphosphate-induced platelet aggregation (17 of 17), defective collagen-induced aggregation (15 of 17), and abnormal epinephrine-induced aggregation (10 of 17). Prothrombin consumption, due to reduced platelet procoagulant activity, was significantly decreased with a dose of 300 mg/kg per day. Comparison of results from this study with those from an earlier carbenicillin study revealed that ticarcillin at 300 mg/kg per day produces the same defects in hemostasis as does carbenicillin at 300 mg/kg per day, but that lower doses (100 or 200 mg/kg per day) of ticarcillin result in only a mild defect in platelet function. If the effective dose of ticarcillin is proven to be lower than the doses of carbenicillin currently employed for treatment of certain gram-negative infections, bleeding should not be a frequent complication of ticarcillin administration, when the drug is given to patients with normal renal function.

Ticarcillin (disodium α -carboxyl-3-thienylmethyl penicillin, BRL-2288) is a new semisynthetic penicillin with an antibacterial spectrum similar to that of carbenicillin (disodium α -carboxylbenzylpenicillin). Studies (2, 9) indicate that not only is ticarcillin more effective in vitro than carbenicillin against *Pseudomonas* sp., but also that its serum half-life is slightly greater than that of carbenicillin. For these reasons, the daily dose of ticarcillin necessary for effective treatment of certain gram-negative bacterial infections may prove to be less than the 20- to 40-g dose level recommended for carbenicillin (8, 9).

Several studies (1, 3, 6, 7, 10) have indicated that patients receiving carbenicillin may exhibit a bleeding disorder. Most, but not all, of those patients had renal insufficiency and exceedingly high serum levels of carbenicillin. We (3) have reported that the hemostatic defect produced by carbenicillin is the result of its effect on platelets and is manifested by prolongation of the bleeding time and abnormalities in platelet aggregation, prothrombin consumption, and clot retraction. Other investigators (4,

5, 7) have also noted an inhibitory effect of carbenicillin (as well as other penicillin compounds) on platelets. Because of the similarities between ticarcillin and carbenicillin, we carried out a study to evaluate the effects of this new agent on hemostasis.

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MATERIALS AND METHODS

Subjects. Seventeen human volunteers were obtained through the Texas Department of Corrections and admitted to the General Clinical Research Center of The Methodist Hospital. 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 ticarcillin were not administered to any volunteer during the study and no aspirin-containing medications were given for the month prior to the study.

Signed informed consent was obtained from all volunteers. Six of the 17 volunteers received ticarcillin for a full 10-day period to evaluate the potential cumulative effect of ticarcillin on hemostasis. Four additional volunteers were entered on the 10-day protocol but drug was discontinued in these subjects after either 5 days (two volunteers) or 6 days (two volunteers). One of these subjects voluntarily withdrew from the study and the other three were withdrawn when their bleeding times exceeded 15 min, the maximum value for bleeding time allowed by the study protocol. The remaining seven volunteers received ticarcillin for 3-day periods.

Ticarcillin administration. Ticarcillin (BRL-2288, Beecham-Massengill Pharmaceuticals, Bristol, Tenn.) was reconstituted in sterile water to the desired concentration, diluted in 100 ml of 5% dextrose in water solution, and infused over a 1-h period every 4 h. Daily doses of ticarcillin were 100, 200, or 300 mg/kg of body weight (7 to 21 g/day). Serum levels of ticarcillin were obtained approximately 2 h after infusion of drug in volunteers receiving 200 or 300 mg/kg per day. The large plate microbiological assay with *Pseudomonas aeruginosa* (ATCC 25619) (NCTC 10490) as the test organism was used for this purpose.

Coagulation and platelet function tests. Blood coagulation and platelet function were studied by methods previously described (3). These included platelet count, template bleeding time, prothrombin time, activated partial thromboplastin time, thrombin time, plasma fibrinogen, fibrinogen/fibrin degradation products, prothrombin consumption, i.e., serum prothrombin time (a measure of platelet activity; to be distinguished from the standard prothrombin time which measures the activity of plasma coagulation factors II, V, VII, and X), thrombin-induced platelet-rich plasma clot retraction, kaolin-induced platelet factor 3 availability, and platelet aggregation. Platelet aggregation was stimulated in a Bryston Aggregometer (Bryston Mfg., Ltd., Scarborough, Ont.) with collagen, epinephrine, and adenosine diphosphate (ADP). The primary aggregation response to ADP was quantified by determining the relative light transmissions of platelet-rich plasma (LT_{PRP}) and platelet-poor plasma (LT_{PPP}) and after completion of the primary wave of aggregation (LT_{10}) by the following equation: $(LT_{10} - LT_{PRP}) + (LT_{PPP} - LT_{PRP})$ (3). In all aggregation studies, unless indicated otherwise, the final concentrations of ADP and epinephrine were 2 and 20 μ M, respectively. The final concentration of collagen used in aggregation studies was the greatest dilution that gave maximum aggregation response with control platelets.

Results were statistically analyzed by the Student *t* test.

RESULTS

Tests of plasma coagulation including prothrombin time, partial thromboplastin time, and plasma fibrinogen were not significantly altered by the administration of ticarcillin to human volunteers (Fig. 1). Other measures of coagulation and/or fibrinolysis, i.e., thrombin

time and fibrinogen/fibrin degradation products, also were unchanged by ticarcillin.

With respect to the effect of this drug on platelets, no significant changes were observed in platelet count, platelet-rich plasma clot retraction, or kaolin-induced platelet factor 3 availability; however, significant alterations in bleeding time, serum prothrombin times, and platelet aggregation occurred.

All volunteers experienced lengthening of bleeding time during drug administration (Fig. 2). After 3 days of ticarcillin administration, 9 of the 17 volunteers exhibited bleeding times greater than 6 min, the upper limit of normal for that test. The longest bleeding times were seen in subjects receiving the largest dose of ticarcil-

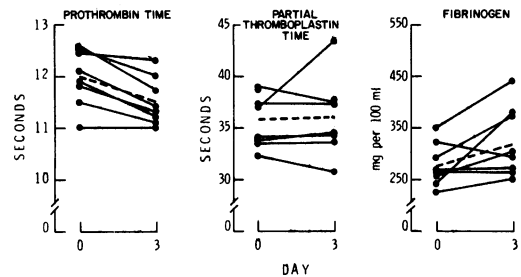


FIG. 1. Effects of ticarcillin on plasma coagulation in human volunteers receiving 300 mg/kg per day for 3 days. Broken lines represent mean values. The differences between mean values before and after 3 days of ticarcillin administration are not statistically significant for any of these tests.

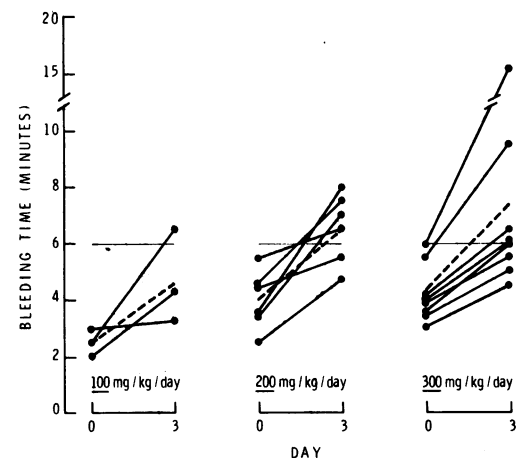


FIG. 2. Bleeding times in volunteers receiving ticarcillin at doses of 100, 200, and 300 mg/kg per day for 3 days. Horizontal lines represent the upper limit of normal for this test. The differences between mean values before and after 3 days of ticarcillin administration are significant only at 200 and 300 mg/kg per day ($P < 0.01$).

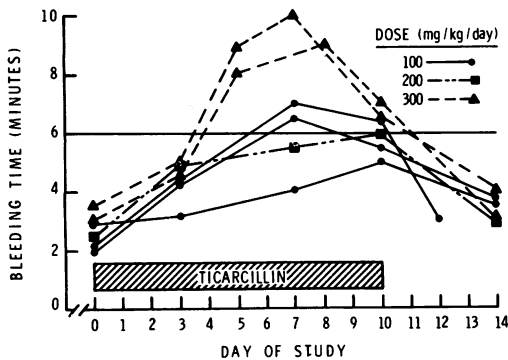


FIG. 3. Serial bleeding times in volunteers receiving ticarcillin for 10 days. The solid horizontal line at 6 min represents the upper limit of normal for this test.

lin (300 mg/kg per day). Three volunteers whose studies after 3 days of drug administration are included in the 3-day infusion data received ticarcillin for periods of 5 or 6 days. Drug was discontinued at that time because of bleeding times in excess of 15 min. Two of these volunteers received 300 mg/kg per day for 5 days and one received ticarcillin at a dose of 200 mg/kg per day for 6 days.

In six subjects who completed a 10-day course of drug, bleeding times peaked between 7 and 10 days after initiation of drug administration and returned to base-line levels by approximately 4 days after drug was stopped (Fig. 3). The longest bleeding times in these six volunteers occurred in those receiving the largest dose of ticarcillin. A curious and unexplained observation was that bleeding times in four of these six volunteers (two receiving 100 mg/kg per day and two receiving 200 mg/kg per day) began to fall after peak values were reached, but before ticarcillin was discontinued.

Serum prothrombin times were significantly shortened after 3 days of ticarcillin at a dose of 300 mg/kg per day (Fig. 4). Lower doses of drug did not alter this test. All abnormal serum prothrombin times were correctable by adding Platelin (General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, N.J.), a platelet phospholipid substitute, to the clotting mixture, indicating that abnormally short times were a reflection of reduced platelet procoagulant activity. Unlike bleeding times, serum prothrombin times did not become progressively abnormal with prolongation of the drug infusion period.

Platelet aggregation became abnormal in all volunteers studied. In 17 of 17 subjects, ADP-induced aggregation was abnormal by day 3 of drug administration. At that time, abnormalities in epinephrine-induced aggregation were

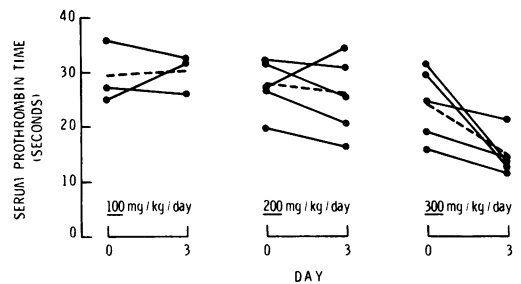


FIG. 4. Serum prothrombin times in volunteers receiving ticarcillin at doses of 100, 200, and 300 mg/kg per day for 3 days. Mean values are indicated by the broken lines. The difference between the mean values on day 0 and day 3 is significant only at 300 mg/kg per day ($P < 0.05$).

apparent in 10 of 17 volunteers and abnormalities in collagen-induced aggregation in 15 of 17 volunteers. Defects in ADP-induced aggregation consisted of a loss of secondary aggregation to a concentration ($2 \mu\text{M}$) of ADP that produced secondary aggregation with control platelets and a depression of primary aggregation. Although loss of secondary aggregation occurred in all subjects, regardless of dose of ticarcillin, depression of primary aggregation was dose-related (vide infra). Complete aggregation of ticarcillin-affected platelets could be achieved by employing ADP concentrations 10 times that required for complete aggregation of control platelets. Abnormal epinephrine-induced aggregation was characterized by a loss of secondary aggregation and often a depression in primary aggregation. Defective aggregation in response to collagen consisted of an increase in the latency period between addition of collagen and onset of aggregation, as well as a decrease in the magnitude of the aggregation response. A typical aggregation study is shown in Fig. 5.

Abnormalities in ADP-induced aggregation, the most sensitive test for detecting the effects of ticarcillin on platelets, were usually apparent within 24 h of drug administration (the earliest that studies were performed) and, as stated above, were always present by day 3 of drug infusion, regardless of dose. As with prothrombin consumption, there did not appear to be a cumulative effect of ticarcillin on platelet aggregation; and, recovery in ADP-induced aggregation occurred in most volunteers by 7 to 10 days after drug was stopped. One subject, however, received ticarcillin for 6 days at a dose of 300 mg/kg per day and exhibited abnormal aggregation for more than 2 weeks after cessation of drug. This volunteer was one of those whose drug infusion was discontinued because of a bleeding time in excess of 15 min.

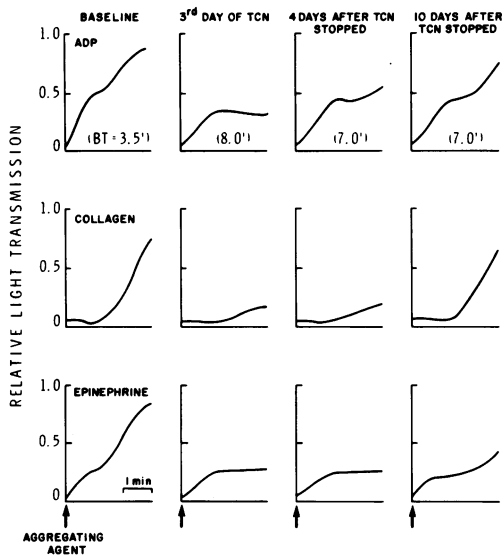


FIG. 5. Platelet aggregation study in a volunteer receiving ticarcillin (TCN), 200 mg/kg per day for 3 days. Note that on day 3 of drug administration, the bleeding time (BT) was 8 min and aggregation responses to ADP, collagen, and epinephrine were abnormal. Defective aggregation to each of these agents was apparent 4 days after drug was stopped, but only epinephrine-induced aggregation was abnormal 10 days after cessation of drug. Concentrations of aggregating agents used are noted in text.

Although the prevalence, time of onset, or duration of aggregation defects were not correlated with dose of drug, the profoundness of the aggregation abnormality was. This was especially noted with the primary aggregation response to ADP, that is, the initial wave of aggregation following the addition of ADP. Table 1 shows results of an analysis of this measure of platelet function. Primary aggregation was reduced only in volunteers receiving 300 mg/kg per day, not in volunteers receiving 100 or 200 mg/kg per day.

Serum levels of ticarcillin 2 h after drug administration ranged from 20 to 36 $\mu\text{g}/\text{ml}$ in volunteers receiving 200 mg/kg per day and from 20 to 41 $\mu\text{g}/\text{ml}$ in volunteers receiving 300 mg/kg per day. There was no correlation between platelet function abnormalities and serum concentrations of ticarcillin.

In this study untoward bleeding was not observed in any volunteer.

DISCUSSION

This study reveals that ticarcillin, administered intravenously to normal human volunteers in doses of 100 to 300 mg/kg per day (7 to 21 g/day), results in defective platelet function, the severity of which is dose related. Qualitative

TABLE 1. Primary platelet aggregation to ADP in volunteers receiving ticarcillin

Dose (mg/kg per day)	No. studied	Degree of primary aggregation (mean \pm SD) ^a	
		Baseline	Day 3 of drug
100	3	0.50 \pm 0.07	0.47 \pm 0.13
200	6	0.49 \pm 0.07	0.41 \pm 0.13
300	8	0.48 \pm 0.11	0.27 \pm 0.05 ^b

^a SD, Standard deviation.

^b Significantly less than baseline value ($P < 0.001$).

platelet defects are evidenced by prolongation of bleeding time, reduced prothrombin consumption, and abnormal platelet aggregation. Ticarcillin, in doses employed in this study, does not affect the plasma coagulation system.

Our previous study (3) of the effects of carbenicillin on hemostasis produced results very similar to these with ticarcillin. Like carbenicillin, ticarcillin causes a defect in platelet function, the most sensitive test for which is ADP-induced platelet aggregation, that can be detected soon after the initiation of drug administration and that usually lasts for approximately 1 week after the drug has been stopped. This prolonged effect indicates that platelets exposed to ticarcillin are permanently affected by the drug. Furthermore, since some subjects exhibited platelet function defects for up to 2 weeks after drug was stopped, the possibility that megakaryocytes are affected by the drug and therefore produce defective platelets cannot be dismissed.

With the exception of clot retraction which became strikingly abnormal in most volunteers given carbenicillin but not in volunteers given ticarcillin, the character of the hemostatic defect with these two drugs is identical. This similarity is to be expected in view of the proposed mechanism of platelet inhibition by these and other penicillin compounds. Our data and those of Cazenave et al. (4) suggest that penicillin coats platelet, as it does red cells, blocking receptor sites on the platelet membrane where stimulants of platelet function exert their effects. The result of such an interference is that the platelet release reaction is blocked and secondary aggregation does not occur.

Unlike the carbenicillin study (3), we saw no untoward bleeding in volunteers given ticarcillin. It should be noted, however, that no subject in the present study underwent a surgical or other traumatic procedure and all subjects who bled in the carbenicillin study had experienced some degree of trauma.

TABLE 2. Comparison of effects of ticarcillin and carbenicillin on platelet function after 3 days of drug administration

Drug (mg/kg per day)	No. studied	Bleeding time (min) ^a	Degree of primary aggregation to ADP ^a	Serum prothrombin time (s) ^a
Ticarcillin				
100	3	4.6 ± 1.6	0.47 ± 0.13	30.1 ± 3.5
200	6	6.6 ± 1.3 ^b	0.41 ± 0.13	25.5 ± 7.4
300	8	7.3 ± 3.5 ^b	0.27 ± 0.05 ^{b, c}	14.9 ± 3.8 ^{b, c}
Carbenicillin				
300	5	7.9 ± 5.2 ^b	0.23 ± 0.06 ^{b, c}	20.0 ± 4.2
400	6	6.9 ± 6.4 ^b	0.27 ± 0.08 ^{b, c}	17.0 ± 3.3 ^{b, c}
Normal Values ^d	28	3.7 ± 1.2	0.48 ± 0.08	24.3 ± 5.5

^a Mean ± standard deviation.

^b Significantly different from normal ($P < 0.02$).

^c Significantly different from values for ticarcillin at 200 mg/kg per day ($P < 0.05$).

^d Determined from base-line values in 28 volunteers included in both the ticarcillin and carbenicillin studies.

A cumulative effect of ticarcillin on platelet function was noted with respect to bleeding time but not with tests for platelet aggregation or prothrombin consumption. An explanation for the disparity is not apparent but may be related to the sensitivity of these different tests of platelet function. The observation that bleeding time may not reach its maximum level for approximately a week after institution of ticarcillin administration should be kept in mind when the drug is utilized in the therapy of infection.

Since one of the aims of this study was to generate information that could be used to compare the effects on hemostasis of ticarcillin and carbenicillin (when the two drugs were administered in doses considered to be equivalent in effectiveness against *Pseudomonas* sp.), we tabulated, for the purpose of comparison, the results of both studies. These results are shown in Table 2. Bleeding times measured 3 days after drug was started were significantly and similarly prolonged by 200 or 300 mg of ticarcillin per kg per day and 300 or 400 mg of carbenicillin per kg per day. Ticarcillin (100 mg/kg per day) caused only slight prolongation of bleeding time. After 3 days of 300 mg of either drug per kg per day, primary aggregation to ADP was significantly less than normal and significantly less than the values obtained with ticarcillin at 200 mg/kg per day. Serum prothrombin times followed this same pattern except that with carbenicillin, at 300 mg/kg per day, an insignificant reduction in serum prothrombin time was noted. These comparative analyses indicate that a dose level of 300 mg of

either drug per kg per day is the level above which profound derangements in platelet function occur.

If doses of ticarcillin below 300 mg/kg per day prove to be efficacious in the therapy of severe infection, the use of this new agent in patients without impaired renal function or an underlying bleeding disorder is not likely to be associated with a significant incidence of bleeding. However, when the drug is given to patients with compromised renal function (who therefore will possess platelet function defects and perhaps reduced platelet production related to uremia), careful attention must be given to the dosage regimen since the drug will be cleared from the circulation more slowly. Also, patients with underlying hemostatic defects, especially patients with thrombocytopenia, may be at an increased risk of hemorrhage when this drug is administered. When severe infection in such patients can be effectively treated with ticarcillin, the clinician will be required to weigh the risk of bleeding secondary to ticarcillin therapy against the risk of inadequate treatment of the infection should the drug be withheld.

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