Studies of Interaction of a Low-Molecular-Weight Heparinoid (Org 10172) with Cloxacillin and Ticarcillin in Healthy Male Volunteers

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Pharmacokinetic and pharmacodynamic interactions between Org 10172 (intravenous bolus injection of 3,250 anti-Xa units), which is a low-molecular-weight heparinoid, cloxacillin (500 mg orally four times daily for 3 days), and ticarcillin (4,000 mg intravenously four times daily for 2 days) were evaluated in two separate studies with healthy male volunteers (n = 18). Both cloxacillin and ticarcillin caused a significant increase in elimination half-life of anti-Xa activity, i.e., from 31 ± 10 to 54 ± 23 h and from 27 ± 6 to 42 ± 13 h, respectively (P < 0.05). Ticarcillin decreased clearance (11%) and increased apparent volume of distribution (35%) (P < 0.05), while for cloxacillin, these differences did not reach statistical significance. These changes in disposition of Org 10172 by the penicillins were not accompanied by important pharmacodynamic changes as evaluated by coagulation tests, platelet aggregation, and bleeding time. Cloxacillin appeared to influence blood coagulation (prolongation of the activated partial thromboplastin time and shortening of thrombin time; P < 0.05) and facilitated thrombin-induced platelet aggregation, which coincided with a shorter bleeding time during the combined treatment in comparison with the time during treatment with Org 10172 alone (P < 0.05). In conclusion, the disposition of Org 10172 was slightly changed by cloxacilllin and ticarcillin, and, unexpectedly, cloxacillin appeared to have mild procoagulant effects.

Org 10172, a low-molecular-weight (4,000 to 10,000) heparinoid, is a mixture of sulfated glycosaminoglycuronans derived from porcine intestinal mucosa (20). In contrast to standard heparin (molecular weight, 4,000 to 30,000), Org 10172 has little effect on coagulation tests and platelet function. Furthermore, its elimination from plasma is slower than that of heparin. Its main therapeutic advantage may be that it causes less enhancement of bleeding than heparin at comparable antithrombotic doses, as has been found in animal experiments (15, 20). Clinical experiments demonstrated that Org 10172 is an effective agent in the prophylaxis of thromboembolic diseases (3, 9). Furthermore, in patients with an increased bleeding risk, Org 10172 appeared to be a safe prophylactic anticoagulant (13, 26). It can be expected that concomitant use of Org 10172 and other medication may frequently occur, and drug interactions may be potentially dangerous. Changes in pharmacokinetics and/or pharmacodynamics of Org 10172 may lead to an increased bleeding risk or to inadequate prophylaxis of thromboembolic conditions. Studies of interactions with compounds which may affect hemostasis by themselves are especially important.

Synthetic penicillanic acid derivatives are expected to be combined with an anticoagulant when a patient with deep venous thrombosis has a concomitant infection requiring penicillin treatment. Penicillins may affect hemostasis by several independent mechanisms. They may inhibit platelet function (6) and are able to induce circulating immunoglobulin G-type antibodies against clotting factor VIII (16). Especially during short-term penicillin treatment, the inhibition of platelet function may be important.

Penicillins are eliminated predominantly by the kidneys (8), and after Org 10172 administration, anti-Xa activity is detectable in urine (14). Therefore, a kinetic interaction at the renal level is also possible.

The purpose of this investigation was to determine potential interactions of Org 10172 with cloxacillin and with ticarcillin.

MATERIALS AND METHODS

Subjects. Eighteen healthy male volunteers (ages, 18 to 27 years; body weights, 60 to 89 kg) participated in the studies after informed consent was obtained. None of the volunteers reported previous allergic reactions to penicillins, and none had a condition known to be associated with an increased bleeding risk (hypertension, gastrointestinal ulcers or erosions, the use of antiplatelet drugs, or acquired or congenital hemorrhagic diathesis). Drugs other than those under investigation were not administered to or taken by any volunteer during and 2 weeks prior to the study. Volunteers were especially instructed to avoid the use of salicylates. The investigational protocol was approved by the Ethics Committee of Leiden University Hospital.

Drugs. Cloxacillin sodium (Orbenin; Beecham, Heppignies, Belgium) in 500-mg capsules and ticarcillin sodium (Ticar; Beecham) were supplied by the pharmacy of Leiden University Hospital. Ticarcillin was dissolved in sterile water (200 mg/ml). Org 10172 (Lomoparan) was supplied by Organon International B.V., Oss, The Netherlands, as injection fluid containing 1,250 anti-Xa and 57 anti-IIa units of Org 10172 per ml (CP 084126, 0.6 ml per ampoule; CP 084127, 1 ml per ampoule).

Study design. Two separate studies designed as open

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randomized crossover trials were performed. In study A, the effect of oral cloxacillin treatment on the pharmacokinetics and pharmacodynamics of Org 10172 was studied with six volunteers. On occasions 2 weeks apart, volunteers received (i) a single intravenous dose of 3,250 anti-Xa units of Org 10172 (the prophylactic dose of Org 10172 is 750 anti-Xa units two times daily subcutaneously, and the therapeutic dose of Org 10172 has not been defined yet) and (ii) 3,250 anti-Xa units of Org 10172 intravenously in combination with cloxacillin (500 mg four times daily for 3 days), with cloxacillin treatment started 24 h before administration of Org 10172.

In study B, the effect of intravenous ticarcillin treatment on the pharmacokinetics and pharmacodynamics of Org 10172 and vice versa were studied. On three occasions all 1 week apart, 12 volunteers received (i) 3,250 anti-Xa units of Org 10172 intravenously; (ii) 3,250 anti-Xa units of intravenous Org 10172 in combination with intravenous ticarcillin (4,000 mg four times daily for 2 days), with ticarcillin treatment started immediately before administration of Org 10172; and (iii) a single intravenous injection of 4,000 mg of ticarcillin. In study B, the order of treatments was randomized by four 3 x 3 Latin squares. All treatments started at 9:00 a.m., and the volunteers were allowed light physical activities.

**Blood sampling.** Blood samples were collected via a cannula (Venflon 18G) which was inserted into a convenient vein of the forearm contralateral to the arm in which Org 10172 and ticarcillin were given. The cannulas were kept patent by intermittent injections of saline. Twelve hours after Org 10172 administration, blood samples were taken by separate venipunctures. For the amidolytic assays of anti-Xa, antithrombin, and thrombin generation-inhibiting (TGI) activities and coagulation tests, 9 parts of venous blood were collected in 1 part of 3.8% sodium citrate (total, 10 ml) in siliconized glass vacuum tubes (Vacutainer; Becton Dickinson, Plymouth, United Kingdom) for study A and in plastic tubes (Sarstedt, Eindhoven, The Netherlands) for study B. Blood (10 ml) was collected in glass vacuum tubes for the determination of cloxacillin and in plastic tubes for determination of ticarcillin, with all tubes containing heparin (143 USP units). Plasma samples were separated immediately by centrifugation (6,000 x g for 6 min) and stored at -30°C until analysis. For the platelet function tests, 20 ml of free-flowing blood was taken with a Wasserman needle (bore, 1.0 mm) and collected in 0.1 volume of sodium citrate - 2H2O (3.8%) in distilled water in plastic tubes. The platelet aggregation tests in platelet-rich plasma were performed immediately after blood sampling.

**Sampling schedule.** Blood samples for the amidolytic assays were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6.5, 8, 10, 13, 16, 24, 28, 32, 36, 48, 56, and 72 h following administration of Org 10172 (antithrombin and TGI activities were measured for up to 24 h). Following ticarcillin administration (during the combination treatment only, after the first ticarcillin injection), collection times were 0, 5, 15, 30, 45, 60, 90, 120, 150, 180, and 240 min, and following the ingestion of the fifth cloxacillin tablet, collection times were 0, 1, 24, and 48 h. Blood samples for the coagulation tests were collected at 0, 0.25, 1, 6.5, and 24 h after the administration of Org 10172, and in study A, samples for the platelet aggregation studies were collected at 0, 0.25, and 1 h after the administration of Org 10172. In study B, following Org 10172 and ticarcillin treatments, collection times for the platelet studies were 0, 0.5, 2, and 4 h. In study A, bleeding times were determined prior to and 15 min after administration of Org 10172. In study B, this was done at 30 min after the administration of Org 10172, of ticarcillin, and of the combination.

**Assays.** The coagulation tests and activated partial thromboplastin time (APTt), prothrombin time, thrombin time, and Thrombotest determinations were done by standard procedures (4).

**Bleeding times** were determined by the method of Mielke et al. (21) using the Simplate-II device (General Diagnostics, Inc., Morris Plains, N.J.). The tourniquet was inflated to 40 mm Hg (1 mm Hg = 133.22 Pa), and two incisions (5 mm long and 1 mm deep) were made on the volar aspect of the forearm. The bleeding time was taken as the mean of two individual measurements. In case the individual measurements of one bleeding-time determination were more than 8 min apart, the mean value was not taken and these data were not used for statistical analysis.

**Platelet aggregation** was measured in a Payton aggregometer (Payton Associated Ltd., Scarborough, Ontario, Canada) at 37°C. Aggregation in platelet-rich plasma (400,000 platelets per ml) was induced by collagen (equine tendon collagen; Hormon Chemie, Munich, Germany), thrombin (bovine; gift of C. M. Jackson, Blood Services, American Red Cross, Detroit, Mich.), and ADP (Boehringer, Mannheim, Germany). Two concentrations of thrombin were used: 15 U/ml for predrug samples and 40 U/ml for postdrug samples. The concentration of collagen was always 5 μg/ml in study A and 5, 10, or 20 μg/ml in study B (one concentration was used for each individual subject). In study A, normal aggregation was seen in pretreatment samples in all subjects, using 5 μg of collagen per ml. In study B, this was not the case, and the collagen concentration was therefore titrated per individual. The ADP concentration used (1.5 μM) causes a first-phase and a submaximal second-phase aggregation curve in control platelet-rich plasma. The aggregation results were recorded on paper and expressed as the increase in optical density by measuring the difference (in millimeters) between the baseline (before addition of the agonist) and the top of the aggregation curve.

Chromogenic amidolytic assays were used to measure plasma anti-Xa (25), antithrombin (18), and TGI (23) activities. The activities of each plasma sample were measured in at least 2 dilutions (each dilution in duplicate) against a calibration curve for the batch of Org 10172 used in the study. The penicillins did not interfere in any of the three assays.

The levels of cloxacillin and ticarcillin in plasma were determined by means of high-pressure liquid chromatography with UV detection, using flucloxacillin and carbenicillin as internal standards, respectively (modifications of the method described in reference 17). The within- and between-day reproducibilities for high and low cloxacillin concentrations (n = 4) did not exceed 3.7%, and they were below 1% for ticarcillin (n = 12). The detection limits were 0.04 μg/ml for cloxacillin and 15 μg/ml for ticarcillin. Org 10172 did not interfere in either assay.

**Data analysis.** Pharmacokinetic parameters for Org 10172 based on plasma anti-Xa and antithrombin activities and for ticarcillin were calculated by using a two-compartment open pharmacokinetic model (except for antithrombin activity during study B, for which a one-compartment model supplied the best fit). The curve-fitting procedure for Org 10172 was performed with anti-Xa and antithrombin concentrations after subtraction of their respective baseline levels. The parameters of the biexponential function characterizing the model were estimated by using a weighted least-squares nonlinear regression computer program, ELSMOS (11).
TABLE 1. Effects of cloxacillin and ticarcillin treatments on pharmacokinetic parameters of Org 10172*  

<table>
<thead>
<tr>
<th>Study and drug*</th>
<th>Anti-Xa</th>
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<tbody>
<tr>
<td></td>
<td>CL (ml/min)</td>
<td>t_{1/2a} (h)</td>
</tr>
<tr>
<td>A (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>4.0 (1.2)</td>
<td>31 (10)</td>
</tr>
<tr>
<td>O + C</td>
<td>3.4 (0.8)</td>
<td>54 (23)</td>
</tr>
<tr>
<td>B (n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>7.3 (1.2)</td>
<td>27 (6)</td>
</tr>
<tr>
<td>O + T</td>
<td>6.5 (1.4)</td>
<td>42 (13)</td>
</tr>
</tbody>
</table>

* Data are presented as means (standard deviations). CL, clearance; t_{1/2a}, half-life at B phase; V, volume of distribution; AUC, area under the curve.

a O, Org 10172; C, cloxacillin; T, ticarcillin.

b P < 0.05 for Org 10172 versus Org 10172 plus ticarcillin.

c P < 0.05 for both studies.

(study A), and a Siphar software package (Simed, Créteil, France) (study B) [error model variance = v(1)(Y calculated) (23)]. Various pharmacokinetic parameters (clearance, elimination half-life, and apparent volume of distribution) were calculated from the coefficients and exponents of the fitted functions according to conventional techniques. Satisfactory curve fits for TGI activities could not be obtained; therefore, only the areas under the curve calculated by the trapezoidal rule without extrapolation to infinity were determined.

Statistical analysis was performed by using repeated-measures analysis of variance. Data were analyzed with the SPSS/PC+ Version 3.0 (SPSS Inc., Chicago, Ill.) statistical package.

RESULTS

During study A, four volunteers developed bruising (<20 cm²) at venipuncture sites during treatment with Org 10172 alone and with combined drugs. During study B, the treatment order was changed in four volunteers. During their first treatment (in two subjects, ticarcillin alone; in one subject, Org 10172 alone; and in one subject, combined drugs), the intended collagen concentration (5 μg/ml) appeared to be too low to obtain normal platelet aggregation in pretreatment samples. For this reason, these treatments were repeated at the end of the experiment after adjustment of the collagen concentration. Several volunteers complained of moderate pain in the forearm during ticarcillin infusion. One of the volunteers developed a thrombophlebitis at the site where ticarcillin had been infused and was therefore excluded from treatment with ticarcillin alone. The data for this volunteer were not used for the analysis of the pharmacokinetics of ticarcillin or for bleeding-time and platelet aggregation studies. All adverse events resolved spontaneously.

Pharmacokinetics. In study A, the mean elimination half-life of plasma anti-Xa activity after administration of Org 10172 alone was 31 ± 10 h and increased to 54 ± 23 h after coadministration of cloxacillin (P < 0.05; Table 1), while clearance and volume of distribution remained unchanged (P > 0.05). Pharmacokinetic data for Org 10172 as evaluated from plasma antithrombin and TGI activities were unchanged during the treatment with combined drugs (P > 0.05). Plasma cloxacillin levels at 1 h after the ingestion of 500 mg of cloxacillin ranged from 0.23 to 6.30 μg/ml.

There was a similar prolongation in elimination half-life of plasma anti-Xa activity, from 27 ± 6 to 42 ± 13 h in study B when Org 10172 was combined with ticarcillin (P < 0.05) (Table 1). During the treatment with combined drugs, clearance of plasma anti-Xa activity was decreased (P < 0.05) and apparent volume of distribution was increased (P < 0.05). Disposition of Org 10172 estimated from plasma antithrombin activity was not influenced by ticarcillin.

The elimination half-life (mean ± standard deviation) of ticarcillin was decreased from 66 ± 12 h to 61 ± 11 min during coadministration of Org 10172 (P < 0.05), while the clearance and distribution volume remained unchanged (P > 0.05). The clearance of ticarcillin was 164 ± 30 ml/min when it was given alone and 166 ± 31 ml/min during the combination treatment. For the apparent volume of distribution, these values were 0.20 ± 0.03 and 0.19 ± 0.03 liters/kg of body weight.

Hemostatic parameters. In both studies, Org 10172, whether administered alone or combined with cloxacillin or ticarcillin, showed only minor effects on all coagulation tests (Table 2, study A). At 6.5 h after Org 10172 administration, these effects had almost subsided. At several points, the APTT and thrombin time were increased and reduced, respectively, to a limited extent by the combination of Org 10172 and cloxacillin (P < 0.05). In study B, no changes were observed in APTT and Thrombotest during treatment with the combination of Org 10172 and ticarcillin (data not shown).

Aggregation by thrombin prior to Org 10172 administration (at 0 min) was higher after cloxacillin intake (P < 0.05; Table 3). There was a moderate decrease in collagen-induced platelet aggregation compared with pretreatment aggregation at 15 min and 1 h, both during treatment with the combination of Org 10172 and cloxacillin and during treatment with Org 10172 alone. There was no statistically significant difference in effects between treatment with the combination of Org 10172 and cloxacillin and treatment with Org 10172 alone. In the experiments with ADP as inducer, no effects of the drugs used were seen. Except in a very few cases, intra- and interindividual variabilities in platelet response were low.

In study B, none of the treatments had an effect on collagen-, thrombin-, and ADP-induced platelet aggregation as evaluated by average changes in optical density (Table 3). Pretreatment values of thrombin-induced platelet aggregation are not given, since the thrombin concentration used for these samples often produced no or only slight platelet aggregation. Compared with study A, the intra- and interindividual platelet aggregation responses for all agonists were more marked. Low aggregation values were occasionally observed for all agonists and were often associated with a low platelet count in platelet-rich plasma. These low re-
INTERACTIONS OF Org 10172 WITH PENICILLINS

In the present study, no serious interactions between Org 10172 and cloxacillin or ticarcillin were detected. Both cloxacillin and ticarcillin caused an increase in elimination half-life of plasma anti-Xa activity (74 and 56%, respectively) following intravenous administration of Org 10172 (P < 0.05). In study B (ticarcillin), this increase appeared to be due to the combination of a decrease in clearance (11%) and an increase in apparent volume of distribution (35%) of anti-Xa activity (P < 0.05). Although in study A a similar tendency was observed, this study probably lacked statistical power to detect these differences. The changes in disposition of Org 10172 brought about by ticarcillin were not associated with increased effects of the combination treatment on the hemostatic system as evaluated by coagulation tests, platelet aggregation studies, and bleeding time. Unexpectedly, there were decreased effects on thrombin time and bleeding time and increased effects on the APTT during treatment with the combination of Org 10172 and cloxacillin. These findings are unlikely to be due to an interaction between Org 10172 and the penicillin, since these differences were in all cases already present prior to the administration of Org 10172. It is more likely that cloxacillin has mild procoagulant effects. This, however, cannot be directly inferred from this study, since for each hemostatic parameter there is only one observation point at which the effects of cloxacillin alone could be evaluated. Furthermore, the findings on thrombin-induced platelet aggregation and bleeding time during treatment with cloxacillin alone and with the combination of Org 10172 and the penicillin, respectively, are rather puzzling, since cloxacillin was expected to inhibit platelet function and prolong the bleeding time (27). Obviously, the present studies were not designed to evaluate possible effects of penicillins on hemostasis, and further research is needed to evaluate the exact nature of the effects of cloxacillin on the hemostatic systems.

Penicillins are cleared almost completely by the kidneys (2), and anti-Xa activity can be detected in urine after intravenous administration of Org 10172 (14). The decrease in clearance of plasma anti-Xa activity brought about by ticarcillin therefore may be due to an interaction at the renal level. This could be evaluated only by determination of the
amount of anti-Xa activity excreted in urine after the administration of Org 10172 in the absence and presence of ticarcillin, but such a determination was not made in this study. It is well known that several anionic compounds compete with each other for tubular secretion. Since Org 10172 is an anionic mucopolysaccharide, the interaction between Org 10172 and the penicillins could have been the result of competition between fractions of Org 10172 and the penicillins for tubular secretion. The mechanism of the change in apparent volume of distribution of plasma anti-Xa activity brought about by ticarcillin is not clear. Ticarcillin is bound for only 65% to plasma proteins (2), which makes it unlikely that the changes in volume of distribution were caused by changes in plasma protein binding. Although for cloxacinil the plasma protein binding is 95%, cloxacinil did not influence the distribution volume of plasma anti-Xa activity.

The kinetics of plasma antithrombin and TGI activities were not influenced by the penicillins. This suggests that the changes in anti-Xa kinetics reflect a separate effect of both penicillins on the fraction of Org 10172 with a high affinity for antithrombin III (antithrombin activity is mediated by heparin cofactor II).

The clinical implications of the changes in disposition of Org 10172 are probably limited. The decrease in clearance of plasma anti-Xa activity was relatively small. Although relationships between antithrombotic effect and anti-Xa activity have been found in animal experiments (15), this was not confirmed in humans (24). The consequences of the 35% increase in apparent volume of distribution of anti-Xa activity are not clear. For heparin-like drugs, the endothelium is considered the “effect compartment” (1, 19). Changes in binding of fractions of these compounds to the endothelium therefore might have clinical implications. However, for Org 10172, a change in endothelial binding is unlikely, since the fractions of Org 10172 with high affinity for antithrombin III did not bind to the vessel wall in an in vitro model (Langerdorff formation; 14).

The pharmacokinetics of Org 10172 measured by both plasma anti-Xa and antithrombin activities were quite different in studies A and B. In study B, the mean clearance of plasma anti-Xa activity was almost double that in study A (7.3 ± 1.2 versus 4.0 ± 1.2 ml/min), a difference for which we have no explanation. Antithrombin activity had a much shorter elimination half-life in study B (2.9 ± 0.8 versus 7.5 ± 3.8 h). Several factors may have contributed to this difference in antithrombin activity. In study B (ticarcillin), plasma antithrombin activity could be described satisfactorily by monoeponential kinetics, while in study A, a two-compartment model was found to give the best fit. Plasma antithrombin activity levels are quite low during the elimination phase and are close to the detection limit of the assay even after an intravenous injection of 3,250 anti-Xa units of Org 10172. This may have contributed to a high variability in the elimination half-life of antithrombin activity.

In the present studies, mean terminal half-life of plasma anti-Xa activity after treatment with Org 10172 alone was substantially longer (approximately 29 h) than had been reported by others (18 h in 42 patients) (5). An explanation for this difference may be the fact that we monitored anti-Xa activity for 72 h instead of only 48 h, as Bradbrook et al. did (5).

The slight decrease in elimination half-life of ticarcillin during treatment with the combination of Org 10172 and ticarcillin has no clinical relevance. It is possibly a spurious finding, since both the clearance and the apparent volume of distribution of ticarcillin remained unchanged. Pharmacoki-
The parameters formed by Brown and adrenalin-induced platelet aggregation. They observed that a dose level of 300 mg/kg of ticarcillin per kg per day was the level above which profound impairment of platelet function occurred. Our study showed that a bolus injection of a relatively high dose of ticarcillin did not influence platelet aggregation acutely, as evaluated by the agonist’s collagen, thrombin, and ADP. This result corresponded well with the unchanged bleeding time. Studies with mice have also demonstrated that the platelet effect of ticarcillin is observed only after four large subcutaneous doses (each dose, 320 mg/kg) of this compound daily for 2 days. There is no explanation for the relatively late effects of ticarcillin on platelet function.

In conclusion, coagulation tests, platelet aggregation studies, and bleeding time revealed no important interactions at the pharmacodynamic level, although minor changes in the disposition of Org 10172 caused by ticarcillin and cloxacillin were observed. These results were, however, observed after relatively short periods (<2 days) of penicillin treatment and cannot be extrapolated to concomitant administration for longer periods. The possible mild procoagulant effects of cloxacillin on the hemostatic system need to be explored further.

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REFERENCES