Comparative Pharmacokinetics of Apalcillin and Piperacillin

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The pharmacokinetics of apalcillin and piperacillin, each administered intravenously as a single 2-g dose, were compared in 10 volunteers in a randomized study of crossover design using bioassay and high-pressure liquid chromatographic procedures. The concentrations of both penicillins in serum were determined over a period of 12 h and in urine over 24 h. Concentrations of apalcillin and piperacillin at the end of the 15-min infusion were similar; however, at 8 h, concentrations of piperacillin were below measurable levels, whereas concentrations of apalcillin were still measurable at 10 h. Pharmacokinetic parameters were calculated according to a two-compartment open model. The area under the curve and the half-life for apalcillin were longer than for piperacillin. On the other hand, renal clearance of piperacillin was substantially greater than that of apalcillin. Of the apalcillin excreted via the kidneys, approximately one-fifth was eliminated as two microbiologically inactive penicillic acid derivatives. The nonrenal clearance of apalcillin was 79% of total clearance. Binding of apalcillin to serum protein was almost twice that of piperacillin.

Apalcillin and piperacillin are two relatively new broad-spectrum penicillins with similar antibacterial activities in vitro (3, 10, 18–20, 23, 25). The results of a preliminary pharmacokinetic study (6) suggested that apalcillin had a longer biological half-life and a lower level of renal excretion than other acylureido-penicillins. This led to the current comparison of the pharmacokinetics of apalcillin and piperacillin in which 2-g doses were administered to 10 human volunteers in a cross-over study.

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MATERIALS AND METHODS

Human volunteers. Ten healthy test subjects (five females and five males) participated in the study after providing informed written consent in accordance with Federal Republic of Germany regulations. They ranged in age from 23 to 44 years (mean, 30.4 years), in weight from 48 to 84 kg (mean, 68.1 ± 12.1 kg), and in body surface area from 1.48 to 2.05 m² (mean, 1.8 ± 0.2 m²). None had a known allergy to β-lactam antibiotics. Results of history, prestudy physical examinations, electrocardiogram, and laboratory investigations (liver and renal function, complete blood count, and urinalysis) were normal. None had been treated with any antimicrobial agent during 4 weeks preceding the study period.

Doseage. Aplacillin (lot 10417, supplied by Sumitomo Chemical Co., Osaka, Japan, via Dr. Karl Thomas Co., Biberach, West Germany) and piperacillin (lot L188, supplied by Lederle Laboratories, Pearl River, N.Y., via Cyanamid/Lederle Co., Munich, West Germany) were administered intravenously in a dosage of 2,000 mg/50 ml of physiological sodium chloride via a constant infusion over 15 min (perfusion pump; Braun Co., Melsunger, West Germany). The study was of cross-over design with a time interval of 2 weeks between the two arms.

Sampling. Blood samples (8 to 10 ml each) for assay of antibiotic concentrations in serum were drawn from the antecubital vein before the first dose of each antibiotic, at the end of infusion, and 5, 10, 20, 30, 45, 60, and 90 min and 2, 3, 4, 6, 8, 10, and 12 h later. Samples were allowed to clot at room temperature for 30 min and then centrifuged for collection of serum. Samples taken up to the 6-h bleedings were assayed immediately. Samples acquired at 8 to 12 h were stored at −70°C and assayed within 48 to 72 h. Urine samples collected before dosage and at 0 to 3, 3 to 6, 6 to 12, and 12 to 24 h thereafter were analyzed within 24 h of collection.

Microbiological assay. The microbiological assay was performed by agar diffusion (cup plate method) as modified by Reeves and Bywater (21). Serum and urine assays were performed with antibiotic medium no. 2 (Difco Laboratories, Detroit, Mich.) using Bacillus subtilis ATCC 6633 as the test strain for high concentrations (>5 μg/ml) and Sarcina lutea ATCC 9341 for low concentrations (<5 μg/ml). The details of the bioassay have been described previously (14, 15).

Pooled normal human serum (pH fixed at 7.4) was used as a diluent for serum specimens; 0.5 M Sörensen phosphate buffer (pH 7.0) was used for urine samples and standards. Apalcillin-sodium (lot T-13; activity titer, 869 μg/mg) and piperacillin-sodium (lot 161 12; activity titer, 952 μg/mg) were used for the standards. Serum and urine samples were assayed in triplicate, and five standards were used on each plate. The coefficients of variability of the bioassay were between 5.3 and 10.8% within the range of measurement. The lowest levels determinable with the present method were 0.3 μg/ml in serum for apalcillin and piperacillin, 0.1 μg/ml in buffer for apalcillin, and 0.3 μg/ml in buffer for piperacillin.

HPLC method. Details of the high pressure liquid chromatography (HPLC) of apalcillin have been described previously (4). Basically, the method consisted of deproteinization of serum followed by separation of the protein-free supernatant by a reversed-phase column (Lichrosorb RP18). Urine was diluted with buffer before chromatography. An isocratic elution mode was used for the determination of apalcillin only. For the separation of both the penicillic acids and the parent compound, a gradient method of elution was applied. Column effluents were monitored at 254 or 310 nm. The detection limits were 0.5 and 1.5 μg/ml. Within-batch precision (coefficient of variation) varied from 1.1% (concentration, 185.3 μg/ml) to 10.2% (concentration, 7.8 μg/ml).

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**Protein binding.** Binding of the test compounds to protein was determined at concentrations ranging from 5 to 200 µg/ml with the micropartition MPS-1 method for separation of free from protein-bound solutes (Amicon GmbH, Witten, West Germany). Separations were done at 22°C; incubations were done at 37°C.

**Pharmacokinetic calculations.** In the present study, a two-compartment open model was assumed (9, 22). The model equation is:

\[ C_p(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]

where \( C_p(t) \) is the serum concentration (in micrograms per milliliter) at time \( t \) (in minutes); and \( A, B, \alpha, \) and \( \beta \) are hybrid constants for calculation of the secondary pharmacokinetic constants. The pharmacokinetic parameters were determined by the least-squares method and by minimizing the sum of squared relative deviations (11). The fitting was performed by nonlinear regression analysis using a digital computer (IBM 1800 and Control Data Cyber 172) (11, 22). All calculated pharmacokinetic parameters were normalized to an average body weight of 70 kg. The effect of infusion time was considered as advised by Loo and Riegelmann (16). Other pharmacokinetic parameters considered were: \( k_{12}, k_{21} \) (per minute), the transfer constants between the two compartments; \( k_{10} \) (per minute), the elimination constant; \( t_{1/2a} \) and \( t_{1/2b} \) (in minutes), biological half-lives; \( V_1 \) and \( V_2 \) (l/100 kg body weight), apparent volumes of distribution in blood \( (C_1) \) and the tissue compartment \( (C_2) \); \( V_{dss} \) (liters/100 kg of body weight), total apparent volume of distribution at steady state, calculated as

\[ V_{dss} = \frac{k_{12} + k_{21}}{k_{21}} \cdot V_1 \]

\( V_d \) area (l/100 kg of body weight) was calculated as

\[ V_d \text{ area} = V_{dss} + \frac{k_{12} - \beta}{k_{21}} \cdot V_1 \]

(9). AUC\( _A \) and AUC\( _B \) (h \cdot µg/ml), areas under the curve during \( \alpha \) and \( \beta \) phases, and AUCC\( _{tot} \) (h \cdot µg/ml), total area under the curve, were calculated as

\[ \text{AUCC}_{tot} = \frac{B}{\beta} + \frac{A}{\alpha} \]

Total body clearance was expressed in milliliters per minute per 1.73 m\(^2\) by the formula

\[ \text{Cl}_B = \frac{\text{dose}}{\text{AUCC}_{tot} \cdot 60 \cdot 1.73 \cdot \text{BSA}} \]

where the dose is in milligrams and BSA is the body surface area. Renal clearance was calculated as \( \text{Cl}_R = \text{Cl}_B \cdot F_{el} \), where \( F_{el} \) is the fraction of the administered dose excreted in urine (22).

**Statistical evaluation.** The distribution being nonnormal for some of the variables, the Wilcoxon signed rank test was used to analyze the significance of differences. All hypotheses were tested at the \( P = 0.05 \) level of significance.

**RESULTS**

The concentrations of apacillin and piperacillin in serum after application of 2.0-g doses are shown in Fig. 1. The mean concentration of apacillin in serum 5 min after completing the intravenous infusion was 218.6 ± 39.6 µg/ml, with a decrease to 59.2 ± 14.8 µg/ml after 1 h, to 7.1 ± 5.6 µg/ml after 4 h, and to 0.9 ± 0.7 µg/ml after 8 h. At 10 h after the end of infusion, only 4 of 10 volunteers had levels above the detection limit. Mean serum concentrations of these individuals were 0.3 ± 0.3 µg/ml. There were no differences between the apacillin serum concentrations determined by HPLC and bioassay (4). No metabolites of apacillin could be detected in serum.

The peak concentration of piperacillin in serum at the end of infusion was 201.3 ± 32.2 µg/ml. Concentrations decreased to 41.4 ± 10.2 µg/ml after 1 h, to 5.0 ± 3.0 µg/ml after 4 h, and to 1.0 ± 0.7 µg/ml after 6 h, a more rapid decline than for apacillin. At 8 h, only 3 of the 10 volunteers had measurable piperacillin serum concentrations (0.3 µg/ml).

Pharmacokinetic parameters calculated on the basis of an two-compartment open model (Table 1) showed that apacillin had a slower distribution phase \( (t_{1/2a} = 15.4 \pm 8.7 \text{ min}) \) and a longer elimination half-life \( (t_{1/2b} = 70.8 \pm 14.3 \text{ min}) \) than piperacillin \( (t_{1/2a} = 3.2 \text{ min}; t_{1/2b} = 55.5 \pm 6.9 \text{ min}) \). The total areas under the curve \( (\text{AUCC}_{tot}) \) were significantly \( (P < 0.05) \) higher for apacillin than for piperacillin.

Renal elimination of both penicillins occurred mainly within the first 3 h after application. At 18.4 ± 2.5% of the applied dose, the 24-h urine recovery rate of apacillin was relatively low in comparison to the rate of 71.2 ± 14.4% for piperacillin. Counting metabolites of apacillin, 36.4% of the apacillin applied was found in urine (18.3% apacillin, 6.9% metabolite A\(_1\), and 11.2% metabolite A\(_2\)) (Fig. 2). The metabolites A\(_1\) and A\(_2\) were identified as stereoisomeric penicilloic acids. Renal clearance of apacillin, calculated as 29 ± 6 ml/min, was considerably lower than renal piperacillin clearance (Table 1). The high total and nonrenal clearances of apacillin underscore the fact that a significant amount of this substance will be eliminated by extrarenal mechanisms. Protein binding in serum was determined by ultrafiltration at concentrations between 5 and 200 µg/ml.
Mean serum protein binding was 86% for apalcillin and 48% for piperacillin. Tolerance of both substances in this single-dose kinetic study was good, and no abnormalities were registered in any volunteer.

**DISCUSSION**

The more recent antipseudomonal penicillins (ticarcillin, azlocillin, mezlocillin, piperacillin, and apalcillin) show no relevant differences in biological half-life (t1/2B) and apparent volume of distribution (7, 17). Apalcillin (t1/2B = 76 min) and ticarcillin (t1/2B = 72 min) have somewhat longer biological half-lives than the acylureido-penicillins (mezlocillin and azlocillin) (t1/2B = 40 to 60 min). Still, the apparent volume of distribution of these penicillins in the extracellular space of the human organism is relatively constant (16 to 25% of body weight) (1, 2, 12, 13). Even so, these five penicillins, especially apalcillin and piperacillin, clearly differ with regard to elimination modalities and metabolism. Seventy-four to eighty-nine percent of piperacillin is eliminated renally in the active form, 56 to 73% thereof being excreted via tubuli (24). Accordingly, renal clearance is high, and extrarenal clearance is low, amounting to only 10 to 20% of total clearance (2, 7, 24). Our piperacillin data (half-lives, clearances, distribution volumes, etc.) are in good agreement with those previously determined (2, 8, 24). Small deviations in some of the parameters can be explained by the dose-dependent kinetics of piperacillin (2).

In contrast to piperacillin, only 18 to 20% of apalcillin is eliminated renally in the active form, whereas inactive metabolites (penicilloic acids) make up another 18%. This correlates well with the low renal clearance of only 29 ml/min and the high extrarenal clearance of 110 ml/min, which amounts to 79% of the total clearance of active apalcillin.

A high proportion of extrarenal elimination of apalcillin takes place via bile (Brogard, Apalcillin Workshop, paper 12). After administration of 1 g, concentrations averaging 3,860 μg/ml were found in the biliary common duct bile of patients undergoing cholecystectomy. However, this biliary elimination as active apalcillin, amounting to 12% of a 1.0-g dose (Brogard, Apalcillin Workshop, paper 12), together with renal elimination, is not sufficient to account for all of the apalcillin administered. The identification of metabolites in the human bile would certainly provide a more comprehensive understanding of the excretion mechanisms. There seems to be no significant enterohepatic recycling of apalcillin (12).

Unlike other recent penicillins, apalcillin is mainly eliminated via the liver. Why this is so is still unclear. Some of the characteristics of apalcillin, such as its high molecular weight of 522, its high serum protein binding of more than 80%, and the presence of bulky aromatic rings in the apalcillin molecule, may explain the increased biliary elimination of this substance (5, 26).

Due to the longer biological half-life, the mean total AUC of apalcillin was 25% higher than that of piperacillin. Clinical studies will have to demonstrate whether these kinetic advantages will lead to more favorable therapeutic results with the same dosage and whether the apalcillin dosage can be reduced without loss of efficacy as compared to piperacillin.

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LITERATURE CITED