Pharmacokinetics and Distribution of Ticarcillin-Clavulanic Acid (Timentin) in Experimental Animals

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The pharmacokinetics and distribution of ticarcillin and clavulanic acid were studied in rats and rabbits after intravenous coadministration of the compounds. The elimination half-lives for ticarcillin and clavulanic acid were similar in both rats (ticarcillin, 0.22 h; clavulanic acid, 0.24 h) and rabbits (ticarcillin, 0.38 h; clavulanic acid, 0.31 h). Both compounds distributed widely throughout rat tissues, and the patterns of distribution were similar to those observed for other β-lactams. Values for penetration into rat pleural, peritoneal, and subcutaneous fluids calculated from the equation (AUCfluid/AUCserum) × 100, where AUC is the area under the concentration-time curve, were between 83 and 93% for ticarcillin and 86 and 103% for clavulanic acid. Values for penetration into tissue cages in rabbits were 139% ± 45% for ticarcillin and 109% ± 22% for clavulanic acid. The penetration of clavulanic acid into rabbit cerebrospinal fluid was higher (P < 0.05) (4.0% ± 0.61%) than that of ticarcillin (1.3% ± 0.53%). Overall, the results show that ticarcillin and clavulanic acid distribute readily throughout body tissues and fluids and predict that the penicillin and β-lactamase inhibitor would be present together at sites of infection.

Ticarcillin is active against a broad range of gram-positive and -negative bacteria, including Pseudomonas aeruginosa. It shows moderate stability in the presence of the Richmond class I β-lactamases but is inactivated by most other β-lactamases encountered in clinical isolates. In the presence of the β-lactamase inhibitor clavulanic acid, however, the spectrum of activity is extended to include many bacterial strains resistant to ticarcillin alone (5, 7, 15).

In the treatment of infections, the inhibitor should display distribution characteristics similar to ticarcillin to achieve effective concentrations of both compounds at infected sites. In the experiments described here, the pharmacokinetics and distribution properties of ticarcillin and clavulanic acid were characterized after intravenous coadministration of the two compounds to rats and rabbits. The doses administered in the experiments were calculated on the basis of dose/surface area (11) to be comparable with a recommended dose of ticarcillin-clavulanic acid for humans (3 g of ticarcillin with 100 mg of clavulanic acid; 3.1 g of Timentin [Beecham Pharmaceuticals, Worthing, England]).

MATERIALS AND METHODS

Compounds. Disodium ticarcillin and potassium clavulanate were used, and all doses were adjusted to contain the pure free-acid equivalents. The compounds were obtained from Beecham Pharmaceuticals. Sterile water was the diluent for all doses.

Animals. Albino male CFY rats weighing 250 to 350 g were purchased from Hacking and Churchill Ltd., Huntingdon, England. Dutch strain rabbits weighing 2 to 2.5 kg were purchased from Ranch Rabbits, Sussex, England.

Pharmacokinetic and protein binding studies. Ticarcillin-clavulanic acid was administered to rats as an intravenous bolus dose of 300 mg of ticarcillin per kg with 10 mg of clavulanic acid per kg. Groups of four rats each were killed at intervals after treatment. Blood samples were taken, and sera were separated for assay.

Three rabbits received an intravenous bolus dose of 150 mg of ticarcillin per kg with 5 mg of clavulanic acid per kg. Blood samples were taken from the marginal ear veins at intervals after treatment, and sera were separated for assay.

The blood samples removed from the rats at 5, 45, and 90 min and from the rabbits at 10 and 60 min were used for determination of the binding of ticarcillin and clavulanic acid to serum proteins. Portions of the samples were kept for bioassay, and the remaining volumes of sera were centrifuged through a Centrifree micropartition system (Amicon, Gloucestershire, England) to collect ultrafiltrates containing unbound fractions of ticarcillin and clavulanic acid.

Pharmacokinetic analysis was based on lines of best fit for data conforming to an open one-compartment model; a curve-stripping procedure was used for the analysis of data conforming to an open two-compartment model. Lines were fitted and analyzed with an Apple 11+ microcomputer. The program was a modification of that described by Nielsen-Kudsk (12).

Distribution in rat tissues. After administration of an intravenous bolus dose of 300 mg of ticarcillin per kg with 10 mg of clavulanic acid per kg, groups of rats were killed and exsanguinated at 15, 30, and 60 min. Blood samples were collected, and sera were separated for assay. Selected tissues were removed, rinsed briefly in Sorensen buffer (0.1 M, pH 6.5), patted dry, and weighed. All tissues, other than bone, were homogenized in buffer (20% [wt/vol]) by using a Colworth Stomacher 80 (Seward Medical, London, England). Bone marrow was removed from the shafts of the long bones by repeated washing with 1 ml of buffer. The bone matrix was homogenized in buffer (20% [wt/vol]) by using an Ultra-Turrax 18/10 (Janke and Kunkel KG, Staufen, Federal Republic of Germany). All homogenates were centrifuged at 5°C, and the supernatants were assayed.

Penetration into rat pleural, peritoneal, and subcutaneous fluids. Groups of rats were killed at 5, 15, 30, 45, 60, 90, and 120 min after administration of an intravenous bolus dose of...
FIG. 1. Concentrations of ticarcillin (TIC) and clavulanic acid (CA) in rat and rabbit sera after coadministration as an intravenous bolus dose. Each point is a mean ± standard deviation for three rabbits or four rats.

300 mg of ticarcillin per kg with 10 mg of clavulanic acid per kg. Blood samples were collected, and sera were separated for assay. Preweighed filter paper disks (diameter, 6 mm; Mast Laboratories, Merseyside, England) were inserted immediately into the pleural and peritoneal cavities and between the skin and underlying muscle fascia. Four disks were used for each site, two for the assay of clavulanic acid concentrations and two for ticarcillin assays. The disks were left in place for 2 min, replaced in sealed tubes, and reweighed to measure the amounts adsorbed on the disks.

Samples of pleural, peritoneal, and subcutaneous fluids taken at 5, 15, 30, and 45 min were diluted for assay by the addition of 0.1 ml of buffer (phosphate-buffered saline, 0.1 M, pH 7.2) to each tube, and the eluted samples were tested microbiologically by the hole-in-plate agar diffusion assay. Samples taken at 60, 90, and 120 min were not eluted from the disks and were assayed by placing the disks on the surface of the agar. Standard solutions were applied to blank disks at the appropriate volumes. The performance of the well and disk assays was similar.

Penetration into rabbit tissue cages. Tissue cages were constructed from silicone rubber tubing (Silastic tubing; Jencons Scientific Ltd., Leighton Buzzard, England). Each cage measured 6 by 1 cm, and 40% of the total surface area was perforated (perforations 3 mm in diameter). Five tissue cages were implanted subcutaneously in each rabbit and left in situ for 6 weeks, at which time the cages were excised in fibrous tissue and contained approximately 2 ml of fluid, the protein content of which was 5.3 g% (70% of that in rabbit serum). The total leukocyte count was 657 cells per mm³. Ticarcillin-clavulanic acid was administered as an intravenous bolus dose of 155 mg/kg (150 mg of ticarcillin per kg with 5 mg of clavulanic acid per kg). At intervals after treatment, blood samples were removed and sera were separated for assay. The tissue cages were sampled in rotation by percutaneous puncture over a period of 24 h.

Penetration into rabbit CSF. Rabbits were anesthetized by an intravenous bolus dose of sodium pentobarbital (Sagatal; May & Baker, Dagenham, England) at 32 mg/kg, and light anesthesia was maintained throughout the experiment. Ticarcillin-clavulanic acid was administered into the marginal ear vein as a bolus dose of 775 mg/kg (750 mg of ticarcillin per kg with 25 mg of clavulanic acid per kg). The high dose was used to produce detectable concentrations in cerebrospinal fluid (CSF) at all time intervals to obtain a more complete concentration profile for both compounds. Blood samples were taken over a period of 6 h from a cannula implanted in the external carotid artery. Sera were separated for assay. Consecutive CSF samples were removed from the cisterna magna at intervals after treatment.

Microbiological assay. For microbiological assay, all samples were kept at 4°C until tested within 2 to 4 h after collection. The large-plate agar diffusion assay was used. Concentrations of ticarcillin were assayed with P. aeruginosa NCTC 10701. The enzyme inhibition assay with Klebsiella pneumoniae NCTC 11228 as the test organism (9) was used for clavulanic acid. Samples were assayed in duplicate against standard solutions over the concentration ranges 1.56 to 50 μg/ml for ticarcillin and 0.08 to 5.0 μg/ml for clavulanic acid. The correlation coefficients for the regression lines of the standard solutions were not less than 0.997. The coefficients of variation for the assays were between 2.8 and 3.3% for ticarcillin and between 6.1 and 9.4% for clavulanic acid. Serum samples were assayed against standard solutions prepared in the appropriate animal serum. When dilutions were required to bring the expected concentrations within the concentration range of the assay, dilutions of the serum samples were made into phosphate-buffered saline and assayed against standards prepared in the appropriate dilution of serum. Rabbit tissue cage fluid was assayed against standards prepared in a 1 in 3 dilution of rabbit serum in phosphate-buffered saline. Standards prepared in phosphate-buffered saline were used to assay dilutions of tissue cage fluid, rabbit CSF, and rat pleural, peritoneal, and subcutaneous fluids. Tissue homogenate supernatants were assayed against standards prepared in Sorensen buffer (pH 6.5) because preliminary studies had indicated there was <10% difference between the assay results for the solutions prepared in the supernatants or the required dilutions of the supernatants and those prepared in buffer.

RESULTS

Pharmacokinetic studies. The concentrations of ticarcillin and clavulanic acid measured in rat and rabbit sera after coadministration are shown in Fig. 1. In the rats, the concentrations of the two compounds declined in parallel and data were fitted to an open one-compartment model. In the rabbits, the concentrations of clavulanic acid declined monoexponentially and were fitted to an open one-compartment model, whereas ticarcillin concentrations declined biexponentially, and data were fitted to an open two-compartment model. The pharmacokinetic parameters are shown in Table 1. In both animal species the elimination half-lives of ticarcillin and clavulanic acid were similar, but the values were higher in the rabbit. There were differences in the apparent volumes of distribution and clearance of the two compounds, with values for clavulanic acid generally higher than those for ticarcillin in both species.

Protein binding studies. Concentrations of clavulanic acid ranged from 1.8 to 19.2 μg/ml in rabbit serum samples taken at 10 and 60 min; in rat serum samples, concentrations at 5, 45, and 60 min ranged from 0.23 to 19.5 μg/ml. The binding
Pharmacokinetic parameters of ticarcillin and clavulanic acid in rats and rabbits after coadministration as an intravenous bolus dose

<table>
<thead>
<tr>
<th>Animal and compound*</th>
<th>$t_{1/2}$ (h)</th>
<th>$V$ (liter/kg)</th>
<th>CL (liters/h per kg)</th>
<th>AUC ($\mu$g · h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.22</td>
<td>0.25</td>
<td>0.78</td>
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</tr>
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<td>0.44</td>
<td>1.26</td>
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<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.38 ± 0.03</td>
<td>0.12 ± 0.01c</td>
<td>0.42 ± 0.03</td>
<td>362.40 ± 30.9</td>
</tr>
<tr>
<td>CA</td>
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<td>0.21 ± 0.02</td>
<td>0.45 ± 0.03</td>
<td>11.0 ± 0.76</td>
</tr>
</tbody>
</table>

* Parameters for rats were calculated from means for four animals at each time point. Values for rabbits are means for three animals with standard deviations.

$\alpha$, Half-life; $V$, volume of distribution; CL, clearance; AUC, area under the concentration-time curve.

T, Ticarcillin; CA, clavulanic acid.

$c$, Volume of distribution at steady state.

of clavulanic acid to serum proteins in these samples was not dependent upon concentration, and means with standard deviations were therefore calculated. The binding of clavulanic acid in rabbits was 21.1% ± 4.4%. In rats, values ranged from 0 to 14.0% with a mean of 3.5%; a standard deviation was not calculated for these results because there was no binding in some samples. These values were similar to or lower than those observed previously for clavulanic acid in rats (13).

Concentrations of ticarcillin in rabbit serum samples taken at 10 and 60 min ranged from 20.7 to 830 $\mu$g/ml. The binding of ticarcillin was not dependent upon concentration, and the mean with standard deviation was 49.6% ± 1.4%. In rats, however, there was some evidence that protein binding was concentration dependent. The concentrations of ticarcillin in the 5-min samples were between 889 and 737 $\mu$g/ml, and binding ranged widely from 18.8 to 39.9%, whereas in the 45- and 90-min samples, concentrations were between 9.4 and 133 $\mu$g/ml and values for binding were generally slightly higher and more consistent, ranging from 35.1 to 45.1% (mean with standard deviation, 41.7% ± 3.4%).

Distribution in rat tissues. The ticarcillin and clavulanic acid concentrations in rat tissues are shown in Fig. 2. The highest concentrations for both compounds were in the liver and kidneys. The concentrations of both compounds in the small intestine gradually increased over the 1-h period, whereas in other tissues, the concentrations tended to decline at rates similar to those in serum. Ticarcillin and clavulanic acid penetrated to a similar extent into tissues other than the small intestine and liver. For example, the concentrations of clavulanic acid in lungs over 1 h averaged 40% of those in serum, compared with 35% for ticarcillin, and concentrations of clavulanic acid in bone marrow averaged 56%, compared with 54% for ticarcillin. The percent penetration values in the remaining tissues ranged from 7 to 20% for clavulanic acid and from 5 to 18% for ticarcillin.

Penetration into rat tissue fluids. The concentrations of ticarcillin and clavulanic acid in rat pleural, peritoneal, and subcutaneous fluids reached rapid equilibrium with those in serum (Fig. 3). The areas under the concentration-time curves (AUCs) for ticarcillin in the fluids ranged between 83 and 93% of those in serum, values which were similar to those for clavulanic acid (86 to 103%).

Penetration into rabbit tissue cages. Both ticarcillin and

FIG. 2. Concentrations of ticarcillin and clavulanic acid in rat kidney (△), liver (□), lung (○), bone marrow (△), spleen (○), small intestine (■), skeletal muscle (○), bone matrix (○), and serum (●) after coadministration as an intravenous bolus dose. Each point is the mean for two rats.

FIG. 3. Concentrations of ticarcillin and clavulanic acid in rat sera and extravascular fluids after coadministration as an intravenous bolus dose (300 mg of ticarcillin per kg plus 10 mg of clavulanic acid per kg). Each point is the mean for two rats.
Clavulanic acid penetrated into subcutaneously implanted tissue cages (Fig. 4). Concentrations in the tissue fluid retained within the cages were maintained considerably longer than in serum, and the compounds were detectable at 24 h after treatment. The peak concentrations of clavulanic acid in tissue cage fluid tended to occur slightly earlier than those of ticarcillin, but overall the extent of penetration, expressed as the (AUC$_{\text{fluid}}$/AUC$_{\text{serum}}$) × 100, was similar for the two compounds: 139% ± 45% for ticarcillin and 109% ± 22% for clavulanic acid.

**Penetration into rabbit CSF.** The concentrations of ticarcillin and clavulanic acid in rabbit serum and CSF are shown in Fig. 5. The peak concentrations of both compounds in CSF occurred at 1 h after treatment, and levels were maintained longer than in serum. The elimination half-life for ticarcillin in CSF was 1.12 ± 0.1 h, compared with 0.52 ± 0.05 h in serum; for clavulanic acid the half-lives were 1.68 ± 0.46 h in CSF and 0.25 ± 0.05 h in serum. The extent of penetration of clavulanic acid was greater than for ticarcillin, and the AUC for clavulanic acid in CSF was 4.0% ± 0.61% of that in serum, compared with a value of 1.3% ± 0.53% for ticarcillin ($P < 0.05$; paired t test).

**DISCUSSION**

The pharmacokinetic experiments indicated that ticarcillin and clavulanic acid were eliminated more rapidly from rats than rabbits, but in each species the elimination half-lives of the compounds were similar, approximately 0.23 h in rats and 0.35 h in rabbits. The elimination half-lives of ticarcillin and clavulanic acid are also comparable in humans, although elimination is slower and the half-lives are about 1 h (3; D. Jackson, T. C. G. Tasker, D. Staniforth, R. Horton, A. T. Murray, and A. Swaisland, Proc. 13th Int. Congr. Chemother., p. 55/43-55/45, 1983). The doses of ticarcillin and clavulanic acid used in the experiments reported here approximated, in terms of body surface area, a dose in humans of 3 g of ticarcillin with 100 mg of clavulanic acid. Body surface area is a function of body weight to the two-thirds power (6, 11), and its use in adjusting doses may be regarded as a means to allow for species differences in physiology and metabolism. The influence of body size on pharmacokinetics is through its correlation with blood flow and clearance (12), and pharmacokinetic parameters for a number of compounds including β-lactam antibiotics have been shown to correlate with body size (either body surface area or body weight) for a wide range of mammalian species including humans (4, 6, 11, 17). By definition, the AUC is solely a function of clearance, and it is therefore of interest that the AUCs for ticarcillin and clavulanic acid in rat and rabbit sera approximated those observed in humans after a dose of 3 g of ticarcillin with 100 mg of clavulanic acid. Studies by Jackson et al. (8) found AUC values of 450 (294 to 580) µg · h/ml for ticarcillin and 8.3 (6.1 to 12.6) µg · h/ml for clavulanic acid. Similar results were observed by Bennett et al. (3). These observations imply that dosing on the basis of body surface area indeed compensated for species differences in clearance of the two compounds.

The binding of ticarcillin and clavulanic acid to serum proteins in rats and rabbits was low to moderate and was similar to that observed in humans, 45% (16) and 22% (7), respectively. Although there were small differences between the binding of ticarcillin and clavulanic acid, the extent of binding would not be expected to significantly influence their distribution (2, 14). The compounds readily penetrated subcutaneously implanted tissue cages in rabbits, and both compounds were detected in rat pleural, peritoneal, and subcutaneous fluids. On the basis of values calculated by expressing extravascular fluid AUC measurements as percentages of serum AUC measurements, the extent of penetration was similar for ticarcillin and clavulanic acid, suggesting comparable diffusion characteristics. The profiles of concentrations in tissue cage fluid for both compounds were consistent with the compact fibrous sheath surrounding the implant and the relatively large volume of fluid within

**FIG. 4.** Concentrations of ticarcillin and clavulanic acid in rabbit sera and tissue cage fluid (TCF) after coadministration as an intravenous bolus dose. Each point is the mean ± standard deviation for six rabbits.

**FIG. 5.** Concentrations of ticarcillin and clavulanic acid in rabbit sera and CSF after coadministration as an intravenous bolus dose. Each point is the mean ± standard deviation for three rabbits.
constituting a diffusional barrier to a rapid achievement of equilibrium between concentrations in the fluid and serum. In rat extravascular fluids, however, for which the surface-area-to-volume ratio is large (18), equilibrium was rapidly attained and concentrations in the fluids paralleled those in serum. These results suggest therefore that ticarcillin and clavulanic acid diffuse together into sites in the body for which the diffusional pathways can vary considerably in length. Small differences were apparent however in the penetration of the compounds into rabbit CSF, and clavulanic acid was seen to penetrate to a greater extent than ticarcillin.

The rat tissue distribution experiments showed that the pattern of distribution of ticarcillin and clavulanic acid was similar to that after administration of the two compounds in the ratio of 15:1 (10) and was not unlike that seen for other β-lactam compounds in rats (1). The concentrations in tissues generally depended upon vascularity and whether excretory processes were present. It is thought that the lower levels of clavulanic acid in liver tissue were due to the biliary pathway being a minor route of elimination for this compound (9).

The pharmacokinetic and distribution experiments reported here demonstrated that after coadministration, ticarcillin and clavulanic acid distribute widely throughout animal tissues and body fluids. Their pharmacokinetics were essentially compatible, and the results suggest that the penicillin and inhibitor would be present together at sites of infection.

LITERATURE CITED