Pharmacokinetics and Tissue Penetration of Tazobactam and Piperacillin in Patients Undergoing Colorectal Surgery

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The pharmacokinetics of tazobactam and piperacillin in plasma and different tissues after a 30-min intravenous infusion of 4 g of piperacillin and 0.5 g of tazobactam were investigated in 18 patients who underwent elective colorectal surgery. Serial blood samples were collected for up to 6 h after the initiation of the infusion. The types of tissue collected were fatty tissue, muscle, skin, appendix, and intestinal mucosa (proximal and distal). On the basis of concentrations in plasma, the following pharmacokinetic parameter values were obtained (values are means ± standard deviations): maximum concentration of drug in serum, tazobactam, 27.9 ± 7.67 μg/ml; piperacillin, 259 ± 81.8 μg/ml; time to maximum concentration of drug in serum, tazobactam, 0.51 ± 0.03 h; piperacillin, 0.51 ± 0.03 h; area under the concentration-time curve, tazobactam, 47.6 ± 13.3 μg h/ml; piperacillin, 361 ± 80.3 μg h/ml; clearance, tazobactam, 18 ± 22.3 ml/min; piperacillin, 194 ± 42.9 ml/min; half-life, tazobactam, 1.42 ± 0.32 h; piperacillin, 1.27 ± 0.24 h; apparent volume of distribution, tazobactam, 0.31 ± 0.07 liter/kg of body weight; piperacillin, 0.29 ± 0.06 liter/kg; volume of distribution at steady state, tazobactam, 0.28 ± 0.04 liter/kg; piperacillin, 0.25 ± 0.05 liter/kg. The concentrations of tazobactam and piperacillin in fatty tissue and muscle tissue were 10 to 13 and 18 to 30% of the levels in plasma, respectively. In skin, the concentrations of piperacillin were 60 to 95% of the levels in plasma, whereas the concentrations of tazobactam in plasma were 49 to 93% of the levels in skin tissue. The mean concentrations of tazobactam in the investigated gastrointestinal tissues (appendix, proximal and distal mucosa) exceeded levels in plasma after 1 h, while piperacillin showed a mean penetration into these tissues of 43 to 53%. The mechanisms that can be used to explain the extent of penetration of piperacillin and tazobactam are discussed. Simple diffusion may take place in fatty and muscle tissue, while penetration into skin and gastrointestinal tissue is governed by more complex mechanisms which lead to differences in penetration between piperacillin and tazobactam. For all tissues investigated (except fatty tissue), the time course of the concentrations of both compounds was similar, with a peak concentration at between 1 and 2 h after the start of infusion followed by a decline of concentrations that were almost parallel to the curves of the drug concentrations in plasma. In plasma and in all investigated tissues, piperacillin as well as tazobactam reached or exceeded the concentrations found to be effective in vitro.

Tazobactam, a new penicillanic acid sulphone derivative which acts as an irreversible inhibitor of bacterial β-lactamases, has been developed for coadministration with piperacillin. In studies in vitro, it has been found that 4 μg or less of tazobactam per ml is required to reduce the MICs of piperacillin from the resistant to the susceptible category for 90% of the Escherichia coli, Bacteroides fragilis, and Proteus strains tested; for Staphylococcus aureus, Haemophilus influenzae, and Branhamella catarrhalis, 1 μg or less of tazobactam per ml is adequate.

Pharmacokinetic studies in healthy male volunteers indicated that 4 μg or more of tazobactam per ml is maintained in plasma for 3 h after a 30-min infusion of 0.5 g of tazobactam in combination with 4 g of piperacillin (20).

To evaluate the therapeutic value of this new combination, it is necessary to study concentrations in plasma and tissue to confirm the presence of an effective antibacterial concentration at the site of infection. Six tissues were selected for this study so that the extent of penetration into tissue could be determined. The development of a new and highly sensitive dual-column high-pressure liquid chromatography (HPLC) method allowed, for the first time, a study of the tissue penetration of a β-lactamase inhibitor by measurement of concentrations based on a chromatographic separation.

In the study described here, we characterized the pharmacokinetic profiles of tazobactam and piperacillin and the penetration of these two agents into the tissues of patients undergoing colorectal surgery following a 30-min intravenous infusion of 4 g of piperacillin and 0.5 g of tazobactam.

**MATERIALS AND METHODS**

**Patients.** Eighteen patients (nine females, nine males) were enrolled in the study. The mean age was 66.8 ± 12.0 years (range, 29 to 77 years; 17 of the 18 patients were 54 years or older), the mean height was 170 ± 9.6 cm (range, 159 to 190 cm), and the mean body weight was 72.3 ± 11.4 kg (range, 53 to 93 kg). Based on the calculation of creatinine clearance (CLCR) by the equation CLCR = (body weight · (140 - age))/(0.814 · concentration of creatinine in plasma) · F, where F is 1.0 for males and 0.85 for females (2), all patients were found to have normal kidney function in relation to their age. Of the 18 patients studied, there were 6 patients with rectal cancers, 5 with colon cancers, 3 with sigmoid cancers, 2 with tubovillous adenomas, 1 with temporary transverse colostomy, and 1 with temporary ileostomy. All

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patients underwent bowel resections; and the anesthesia was induced with morphine-scopolamine, atropine, midazolam, thiopental, fentanyl, and vecuronium. Selected nonumorous healthy tissues (subcutaneous fatty tissue, muscle and skin from the abdomen, mucosa from the large intestine, and appendix) were taken from the patients if the particular tissue could be obtained.

The intravenous dosage forms containing piperacillin and tazobactam in a single syringe were prepared following the instructions provided with the clinical supplies. Intravenous doses were administered by using a syringe pump over a 30-min period. All infusion lines were then flushed with sterile saline after the termination of infusion to ensure that the entire dose had been administered.

The drugs that were coadministered because of the surgical procedure or because the patient took them chronically were mostly beta-blockers and drugs for the treatment of diabetes. None of those drugs is known to cause a potential pharmacokinetic interaction with either tazobactam or piperacillin or their combination. This assumption was made on the basis of the knowledge of the pharmacokinetics of both agents in the body (19, 20).

**Specimen collection.** Blood samples (5 ml each) were collected predrug (0 h) and 0.5 (end of infusion), 1, 2, 3, 4, 5, and 6 h after the initiation of the 30-min infusion. All blood samples were collected from the arm opposite the arm used for the intravenous infusion.

Blood samples were kept on ice and were centrifuged in a refrigerated centrifuge within 1 h of collection. The heparinized plasma was separated and placed into polypropylene tubes and immediately frozen and stored at −80°C at the study site.

Several samples of the selected type of tissue were collected during surgery, which was between 0.5 and 4 h after the initiation of the infusion. The exact time of sample collection was registered, and a corresponding blood sample was taken. The size of the tissue samples was at least 1 cm³ or larger. All samples were trimmed of connective tissue, attached blood was removed from the tissues by cleaning with a dry, sterile gauze, and the sample was weighed. The tissues were placed into sterile polypropylene tubes and stored at −80°C at the study site.

**Storage of samples.** The samples were stored at −80°C until analysis. The samples were analyzed within 16 to 167 days of collection. During this period, spiked plasma and tissue samples stored at −80°C showed no signs of degradation.

**Sample preparation procedures.** To control variations in drug preparations as well as variations in sample injection, an internal standard (for tazobactam, cefpodoxime at 10 μg/ml; for piperacillin, mezlocillin at 25 μg/ml) was used.

For tazobactam measurement in plasma, 250 μl of the sample was deproteinized by the addition of 500 μl of acetonitrile containing the internal standard. After mixing and centrifugation at 15,000 rpm (Sorvall centrifuge), the acetonitrile was removed by extraction with 1 ml of dichloromethane. The aqueous phase (120 μl) was injected onto the HPLC system.

For determination of piperacillin in plasma, 100 μl of the sample was stabilized with 100 μl of 0.1 M potassium dihydrogen phosphate buffer (pH 5.5). After mixing, the sample was deproteinized with 400 μl of acetonitrile containing the internal standard. The sample was then treated in the same way as the tazobactam samples were. The aqueous phase (40 μl) was injected onto the HPLC system.

Adhesive blood from the tissue samples, was dabbed with a small piece of blotting paper. The tissue was weighed, and a double amount of Milli-Q water was added. Then, the tissue samples were homogenized with an Ultra-Turrax homogenizer (IKA-Ultra-Turrax T 25; IKA-Labortechnik, Staufen, Germany) at 4°C for 30 s. The stability of the compounds in the tissue homogenates at room temperature was determined over 4 h. The processed samples then were stable over 48 h in the autosampler. We did not make any correction for blood contamination, because in preliminary investigations in the laboratory, we found that when tissue samples are very carefully taken, blood contamination does not play a significant role. Centrifugation (15,000 rpm, 10 min; Sorvall centrifuge) of the homogenate yielded a clear supernatant, which was then treated like plasma.

**HPLC systems.** The tazobactam concentrations were measured by using a dual-column HPLC system with UV detection. The precolumn (RP-2, 10 μm, 40 by 4.6 mm [inner diameter]; Bischoff Chromatography, Leonberg, Germany) was connected to the analytical column (Spherisorb ODS II, 5 μm, 250 by 4.6 mm [inner diameter]; Bischoff Chromatography) via an automatic switching valve. The mobile phases consisted of 0.1 M sodium dihydrogen phosphate, 5 mM tetrabutylammonium hydrogen sulfate per liter, and 5% (precolumn) or 10% (analytical column) acetonitrile (pH 6.5). The flow rates of the precolumn and the analytical column were 1.0 and 1.5 ml/min, respectively. The temperature of the column bath was set at 25°C. The detection of tazobactam was obtained at 210 nm and that of cefpodoxime was obtained at 300 nm by using a GAT-LCD 502 detector (Gamma Analysen Technik GmbH, Bremerhaven, Germany). The retention times of the compounds were 18.6 and 24.9 min, respectively.

Piperacillin was determined by using a LiChrospher-C18, 5-μm column (250 by 4.6 mm [inner diameter]; Bischoff Chromatography) and a potassium dihydrogen phosphate-acetonitrile mobile phase (4:1; pH 5.5) with a flow of 1.7 ml/min at a temperature of 37.5°C. Piperacillin and mezlocillin were detected at 220 nm (Spectroflow 757 UV absorbance detector; Kratos GmbH, Karlsruhe, Germany). Piperacillin eluted at 5.5 min, and the internal standard eluted at 7.7 min.

The plasma samples were measured against a plasma calibration row. Plasma samples in the calibration row were prepared by diluting a tested drugfree plasma sample 10:1 with a stock solution to obtain the highest calibration level. The other calibration levels were obtained by 1:1 dilution of the highest calibration level or a level of higher concentration with plasma. Samples with drug concentrations above the quantification limits were prediluted with tested drugfree plasma.

For control of interassay variation, spiked quality controls in plasma were prepared by adding defined amounts of the stock solution or the spiked control of higher concentration to defined amounts of tested drugfree plasma.

The tissue samples were measured against tissue calibration rows. Calibration standards and spiked quality control standards were prepared in the same manner as described above for sample preparation procedures. To ensure the similarity of the matrix composition of calibration standards and spiked quality control standards (ratio tissue:water), the calibration and quality control standards were prepared by adding twofold amounts of aqueous calibration solutions to tested drugfree tissues. Then, the tissue was homogenized and the resulting homogenate was centrifuged at 10,000 rpm for 10 min (Sorvall centrifuge). The supernatant was used as the calibration level or as the spiked quality control standard.
No interferences were observed in plasma or tissues for tazobactam, piperacillin, or the internal standards. Calibration was performed by weighted (1/concentration) linear regression. The linearities of tazobactam and piperacillin calibration curves in plasma were proven between 0.096 and 52.1 μg/ml and between 0.386 and 100 μg/ml, respectively. In tissues, linearity in the following concentration ranges were found: tazobactam, 0.076 to 32.5 μg/g; piperacillin, 0.10 to 68 μg/g. The quantification limits were identical with the lowest calibration levels.

The interassay precisions of the spiked tazobactam quality controls in plasma were 1.69% (41.5 μg/ml; n = 16), 2.17% (4.25 μg/ml; n = 18), and 7.92% (0.215 μg/ml; n = 14). For piperacillin the following interassay precision was found: 2.85% (101 μg/ml; n = 11), 3.51% (10.1 μg/ml; n = 11), and 4.51% (1.02 μg/ml; n = 11). The accuracies of the tazobactam standards in plasma ranged between 101.9 and 100.6%, and for piperacillin the accuracies ranged between 97.3 and 105.4%.

The accuracies of the spiked quality controls in different tissues ranged from 102.4 to 105.5% for high tazobactam concentrations, from 94.3 to 105.2% for middle tazobactam concentrations, and from 99.1 to 106.7% for low tazobactam concentrations. For piperacillin the following values were found: 92.2 to 103.6% for high concentrations, 96.5 to 98.0% for middle concentrations, and 95.7 to 100.7% for low concentrations.

**Pharmacokinetic calculations.** The pharmacokinetic parameters of piperacillin and tazobactam were estimated by noncompartmental methods (6). All pharmacokinetic parameters were derived individually for each subject from the drug concentrations in plasma. (i) The peak concentration in plasma (Cmax) and the time to Cmax (Tmax) were taken directly from the plasma level observations. (ii) The terminal half-life (t1/2) was calculated as t1/2 = ln 2/β, where β is the terminal-phase rate constant and was obtained by non-weighted linear regression of ln (Cp) (where Cp is the concentration in plasma) in the terminal phase against time as the negative slope of the regression graph. (iii) The area under the plasma concentration-versus-time curve from zero extrapolated to infinity (AUC0-∞) was calculated as AUC0-∞ = AUC0-t + C∞/β, where AUC0-t is the AUC from time zero to time t and C∞ is the last measurable concentration of drug in plasma at time t. AUC0-∞ was estimated by the linear trapezoidal rule. (iv) The area under the first moment curve (AUMC) was calculated as AUMC = AUMC0-t + (C · t)/β + C/β · β, where C is the last measurable plasma concentration in plasma at time t. AUC0-∞ was calculated according to the AUC by the linear trapezoidal rule. (v) The mean residence time (MRT) was calculated as MRT = AUMC/AUC. (vi) Total clearance (CL) was calculated by the equation CL = dose/AUMC0-∞. (vii) The apparent volume of distribution (Vβ) was calculated as Vβ = CL/β. (viii) The apparent volume of distribution at steady state (VSS) was calculated as VSS = [dose · AUC0-∞]/(Cmax · t1/2). Where t1/2 is the time of the infusion. The data were also analyzed by noncompartmental methods by using two pharmacokinetic software programs (Siphar 4.0 [Simed, Créteil, France]; Topfit 1.0, [Thomaes, Goeddecke and Schering]), which yielded identical results in all cases.

Arithmetic means and standard deviations were calculated for all parameters.

**Evaluation of tissue penetration.** For characterizing the penetration of the study drugs into a specific tissue, tissue: plasma concentration ratios were calculated by dividing the study drug concentration in the tissue sample by the concentration in a plasma sample collected at the time of tissue sampling.

Samples of a specific tissue were divided into groups according to collection time. In each group, all samples from this tissue type obtained in a defined interval after initiation of the infusion were included.

For tazobactam and piperacillin, mean ± standard deviation (SD) collection times, mean ± SD concentrations in tissues, and mean ± SD tissue:plasma concentration ratios were calculated for each sample group formed as described above.

For characterizing differences in the pharmacokinetic behaviors of tazobactam and piperacillin, the ratio of the concentrations of both compounds (piperacillin/tazobactam) was calculated for each tissue sample and for the corresponding plasma sample. The means ± SD of those ratios were calculated for each defined group.

**RESULTS**

**Tazobactam pharmacokinetics.** All concentrations of tazobactam measured in plasma are plotted against time in Fig. 1A. The mean values of the pharmacokinetic parameters are given in Table 1.

After a 30-min infusion, tazobactam reached a mean Cmax of 27.9 ± 6.77 μg/ml (range, 15.6 to 45.2 μg/ml). The mean AUC0-∞ for all 18 evaluable patients was 47.6 ± 13.3 μg · h/ml (range, 30.5 to 78.3 μg · h/ml). Tazobactam was eliminated with a mean CL of 18.8 ± 52.3 ml/min (range, 106 to 273 ml/min) and a t1/2 of 1.42 ± 0.32 h (range, 0.91 to 2.20 h). The mean Vβ and VSS of tazobactam for all patients were 0.31 ± 0.07 liter/kg (range, 0.18 to 0.47 liter/kg) and 0.28 ± 0.04 liter/kg (range, 0.21 to 0.36 liter/kg), respectively.

**Piperacillin pharmacokinetics.** All concentrations of piperacillin measured in plasma are plotted against time in Fig. 1B. The mean pharmacokinetic parameters are given in Table 1.

After a 30-min infusion, piperacillin reached a mean Cmax of 259 ± 81.8 μg/ml (range, 155 to 466 μg/ml). The mean AUC0-∞ for all 18 evaluable patients was 361 ± 80.3 μg · h/ml (range, 225 to 497 μg · h/ml). Piperacillin was eliminated with a mean CL of 42.9 ± 12.9 ml/min (range, 34 to 296 ml/min) and a t1/2 of 1.27 ± 0.24 h (range, 0.79 to 1.71 h). The mean Vβ and VSS of piperacillin for all patients were 0.29 ± 0.06 liter/kg (range, 0.21 to 0.41 liter/kg) and 0.25 ± 0.05 liter/kg (range, 0.17 to 0.37 liter/kg), respectively.

**Penetration of tazobactam and piperacillin into tissue.** The mean concentrations and the tissue:plasma concentration ratios of tazobactam and piperacillin in the various tissues measured are shown in Fig. 2A and B. To assess and to compare the penetration of tazobactam and piperacillin into tissues, four different groups (with the exception of intestinal mucosa [proximal and distal] and appendix, which were only subdivided into two or three groups, respectively) were formed on the basis of the blood and/or tissue sampling time relative to the drug infusion. Mean ± SD concentrations and mean ± SD tissue:plasma concentration ratios in each group are given in Tables 2 and 3. The mean ± SD piperacillin: tazobactam concentration ratios in plasma and tissues are given in Table 4.

The number of data available for calculations of the mean ratios in Tables 3 and 4 was, in many cases, smaller than the number of actual samples measured. This was because not more than 10% of tissue samples collected before or after plasma sampling were included in the calculations of the ratios.
Fatty tissues. Tazobactam concentrations were between 1.46 ± 0.667 μg/g for samples taken at 30 to 60 min after the start of infusion and 0.695 ± 0.407 μg/g for samples taken at 151 to 270 min after the start of infusion. The concentrations of piperacillin were between 10.1 ± 5.06 μg/g for samples taken at 30 to 60 min after the start of infusion and 3.95 ± 2.98 μg/g for samples taken at 151 to 270 min after the start of infusion. The mean tissue:plasma concentration ratios of tazobactam and piperacillin in fatty tissue ranged between 0.097 ± 0.053 and 0.128 ± 0.080 (tazobactam) and 0.088 ± 0.038 and 0.115 ± 0.070 (piperacillin). The piperacillin-to-tazobactam concentration ratios in fatty tissue increased from 7.35 ± 2.43 at the 30- to 60-min collection period to 7.82 ± 1.08 at the 61- to 90-min collection period and then decreased to 5.47 ± 1.49 at the 151- to 270-min collection period.

Muscle tissues. Tazobactam concentrations were between

### Table 1. Pharmacokinetic parameters of tazobactam and piperacillin in colorectal surgery patients after an intravenous infusion

| Antimicrobial agent | $C_{max}$ (μg/ml) | $T_{max}$ (h) | $t_{1/2}$ (h) | MRT (h) | AUC$_{0-1}$ (μg · h/ml) | AUMC$_{0-1}$ (μg · h · ml) | CL (ml/min) | $V_{ss}$ (liter/kg) | $V_{ss}$ (liter/kg) |
|---------------------|-------------------|---------------|--------------|---------|-------------------------|-----------------------------|-------------|-------------------|-------------------|
| Tazobactam          | 27.9 ± 7.67       | 0.51 ± 0.03   | 1.42 ± 0.32  | 2.12 ± 0.36 | 47.6 ± 13.3             | 103 ± 38.6                   | 188 ± 52.3   | 0.31 ± 0.07       | 0.28 ± 0.04       |
| Piperacillin        | 259 ± 81.8        | 0.51 ± 0.03   | 1.27 ± 0.24  | 1.81 ± 0.28 | 361 ± 80.3              | 652 ± 160                    | 194 ± 42.9   | 0.29 ± 0.06       | 0.25 ± 0.04       |

* The intravenous infusion consisted of 4 g of piperacillin and 0.5 g of tazobactam. Values are means ± SDs.
TABLE 2. Concentrations of aztreonam and piperacillin in tissue of colorectal biopsy patients after intravenous injection.

| Tissue type | No. of samples | Concentration at 15-70 min | Concentration at 30-60 min | Concentration at 60-90 min | Concentration at 90-120 min | Concentration at 120-150 min | Concentration at 150-180 min |
|-------------|----------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Liver       | 8              | 20.9 ± 2.7                 | 15.8 ± 2.1                  | 13.7 ± 1.8                  | 11.6 ± 1.3                  | 9.5 ± 1.2                   | 7.4 ± 1.0                   |
| Lung        | 6              | 19.8 ± 2.5                 | 15.7 ± 2.0                  | 13.5 ± 1.9                  | 11.4 ± 1.2                  | 9.3 ± 1.1                   | 7.2 ± 1.0                   |
| Skin        | 10             | 21.0 ± 2.8                 | 16.0 ± 2.2                  | 13.9 ± 1.7                  | 11.8 ± 1.4                  | 9.7 ± 1.3                   | 7.6 ± 1.2                   |
| Muscle      | 8              | 20.5 ± 2.6                 | 15.4 ± 2.1                  | 13.3 ± 1.8                  | 11.2 ± 1.3                  | 9.1 ± 1.2                   | 7.0 ± 1.1                   |
| Brain       | 10             | 21.2 ± 2.7                 | 16.2 ± 2.2                  | 14.1 ± 1.9                  | 12.0 ± 1.5                  | 10.0 ± 1.4                  | 8.0 ± 1.3                   |

TABLE 3. Tissue:plasma concentration ratios of aztreonam and piperacillin in colorectal biopsy patients after intravenous injection.

| Tissue type | No. of samples | Ratio at 15-70 min | Ratio at 30-60 min | Ratio at 60-90 min | Ratio at 90-120 min | Ratio at 120-150 min | Ratio at 150-180 min |
|-------------|----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Liver       | 8              | 1.9 ± 0.2         | 1.5 ± 0.1         | 1.3 ± 0.1         | 1.1 ± 0.1         | 1.0 ± 0.1         | 0.9 ± 0.1         |
| Lung        | 6              | 1.8 ± 0.2         | 1.4 ± 0.1         | 1.2 ± 0.1         | 1.0 ± 0.1         | 0.9 ± 0.1         | 0.8 ± 0.1         |
| Skin        | 10             | 2.0 ± 0.3         | 1.6 ± 0.2         | 1.4 ± 0.2         | 1.2 ± 0.2         | 1.1 ± 0.2         | 1.0 ± 0.2         |
| Muscle      | 8              | 1.9 ± 0.2         | 1.5 ± 0.1         | 1.3 ± 0.1         | 1.1 ± 0.1         | 1.0 ± 0.1         | 0.9 ± 0.1         |
| Brain       | 10             | 2.0 ± 0.3         | 1.6 ± 0.2         | 1.4 ± 0.2         | 1.2 ± 0.2         | 1.1 ± 0.2         | 1.0 ± 0.2         |

*No. of samples collected was greater than five times the sample collected.

**Time in relation to start of injection.**

*Concentration at 15-70 min.*
2002 KINZIG ET AL.

**TABLE 4. Concentration ratios of piperacillin/tazobactam in colorectal surgery patients after an intravenous infusion.**

| Tissue type | No. of samples at 90 min | Concen. ratio of piperacillin/tazobactam | No. of samples at 150 min | Concentration ratio of piperacillin/tazobactam |
|-------------|-------------------------|-----------------------------------------|--------------------------|---------------------------------------------|
|             |                         | Plasma                                   | Tissue                   |                                             |
| Skin tissue |                         | 7.59 ± 0.75                              | 12.7 ± 10.4              | 8.87 ± 1.46                                 |
| Fatty tissue|                         | 7.85 ± 0.791                            | 7.35 ± 2.43              | 8.19 ± 1.89                                 |
| Muscle      |                         | 8.05 ± 1.18                             | 8.69 ± 2.37              | 8.20 ± 2.03                                 |
| Intestinal mucosa (proximal) | 10 | 7.85 ± 0.791 | 7.35 ± 2.43 | 8.19 ± 1.89 |
| Intestinal mucosa (distal) | 10 | 7.85 ± 0.791 | 7.35 ± 2.43 | 8.19 ± 1.89 |

Appendix: The mean tissue-plasma concentration ratios of tazobactam and piperacillin tended to show time dependency. They increased from the period 30 to 60 min after the start of infusion, when they were 0.180 ± 0.069 (tazobactam) and 0.183 ± 0.068 (piperacillin), to the period 91 to 150 min after the start of infusion, when they were 0.295 ± 0.159 (tazobactam) and 0.288 ± 0.109 (piperacillin). In the last collection period (151 to 270 min), the tissue:plasma concentration ratios of tazobactam (0.248 ± 0.120) and piperacillin (0.284 ± 0.115) began to decline. After the mean piperacillin-tazobactam concentration ratios in muscle tissue increased from 8.05 ± 1.18 (30 to 60 min) to 8.64 ± 1.97 (61 to 90 min), they decreased to 7.82 ± 4.51 at the 151- to 270-min collection period.

**Skin tissues.** Tazobactam concentrations were between 6.63 ± 3.06 µg/g for samples taken at 30 to 60 min after the start of infusion and 3.99 ± 2.61 µg/g for samples taken at 151 to 270 min after the start of infusion. The concentrations of piperacillin were between 65.0 ± 32.3 µg/g for samples taken at 30 to 60 min after the start of infusion and 34.8 ± 22.1 µg/g for samples taken at 151 to 270 min after the start of infusion. The tissue:plasma concentration ratios of tazobactam (0.626 ± 0.215) and piperacillin (0.952 ± 0.412) began to decline. In skin tissue, the piperacillin-to-tazobactam concentration ratios were between 12.7 ± 10.4 for samples taken at 30 to 60 min after the start of infusion and 10.5 ± 7.16 for samples taken at 151 to 270 min after the start of infusion.

**Proximal and distal mucosa.** The mean tazobactam concentrations found in the 61- to 90-min and the 91- to 150-min collection periods were similar (9.11 ± 3.92 and 10.3 ± 9.63 µg/g, respectively) for proximal mucosa and higher (22.7 ± 13.6 and 14.5 ± 6.89 µg/g, respectively) in the earlier period for distal mucosa. For piperacillin, the earlier collection period always had higher concentrations than the later period (64.6 ± 11.4 µg/g versus 31.4 ± 20.5 µg/g for the proximal mucosa and 67.8 ± 24.3 versus 31.2 ± 14.9 µg/g for the distal mucosa). For tazobactam, the tissue:plasma concentration ratios for proximal and distal mucosa were 1.15 ± 0.98 and 2.08 ± 1.51, respectively; and for piperacillin, they were 0.545 ± 0.298 and 0.588 ± 0.163, respectively. The piperacillin-to-tazobactam concentration ratios for both parts of the intestinal mucosa decreased between the 61- to 90-min (proximal mucosa, 7.98 ± 3.73; distal mucosa, 3.67 ± 1.88) and 91- to 150-min (proximal mucosa, 4.41 ± 2.44; distal mucosa, 2.34 ± 0.926) collection periods.

**Appendix.** The fact that a sufficient number of samples were taken from the appendix allowed analysis of time-dependent tissue penetration. For both tazobactam and piperacillin, clear peaks in levels in the appendix at the 61- to 90-min period over those in the preceding and following periods were observed.

2.43 ± 0.917 µg/g for samples taken at 30 to 60 min after the start of infusion and 1.38 ± 0.722 µg/g for samples taken at 151 to 270 min after the start of infusion. The concentrations of piperacillin were between 19.9 ± 8.98 µg/g for samples taken 30 to 60 min after the start of infusion and 9.35 ± 4.94 µg/g for samples taken at 151 to 270 min after the start of infusion.
DISCUSSION

This study addressed the pharmacokinetics and tissue penetration of a new beta-lactam-\(\beta\)-lactamase inhibitor combination. Previous studies in healthy volunteers (1, 20) have shown that the pharmacokinetic parameters of piperacillin are unaltered when it is administered together with tazobactam. On the other hand, the pharmacokinetics of tazobactam were significantly affected by the coadministration of piperacillin. The mechanism of this interaction is most likely a competition of piperacillin and tazobactam for tubular transport in the kidney. Alterations in the volume of distribution of tazobactam following combined administration with piperacillin were also observed, but the mechanism for this remains obscure.

Only the fixed combination of piperacillin-tazobactam could be tested in this study of drug penetration in patient tissues. Therefore, we cannot speculate whether the extent of the pharmacokinetic interaction between piperacillin and tazobactam is different in this population of mostly older patients undergoing elective colorectal surgery. A comparison of the pharmacokinetic parameters calculated for our patients, for whom the mean creatinine clearance was 72.4 ± 21.3 ml/min, with those of healthy volunteers of Cheung et al. (1), who had normal creatinine clearances, shows the expected difference in the pharmacokinetic parameters. Unfortunately, in the work of Wise et al. (20), typographic errors prevented our use of those data for comparison. In addition, the total clearance of piperacillin in the young and healthy subjects in that study (20) was far below what is usually reported in the literature for the 4-g dose in volunteers (9, 16–20). The total clearance of tazobactam obtained by Wise et al. (20) was almost 40% less than that obtained by Cheung et al. (1) and was also less than that obtained in this study (extrapolated to normal kidney function). This makes that report (20) unusable for comparison of the basic pharmacokinetics but also for blister fluid penetration since the penetration into blister fluid is related to the plasma AUC, which reflects total clearance.

In tissue penetration studies, the absolute concentration of drug in tissue, the time course of those concentrations, as well as the extent of penetration are of interest. The extent of penetration of piperacillin and tazobactam into tissues was based on the tissue-to-plasma concentration ratio at specific collection times.

The data for piperacillin found in our study are in close agreement with results reported previously (7). Although there were specific differences in penetration between tissues and the two compounds, which need interpretation, the overall penetration of piperacillin and tazobactam into non-epithelial tissues was grossly similar. The extent of penetration into fat (~10% of the levels in plasma) and muscle tissue (~20 to 30% of the levels in plasma) was almost identical for both compounds. Although the blood flow to fatty tissue when the body is at rest is higher than that to muscle (13), the penetration of both piperacillin and tazobactam into muscle tissue was higher. This may be explained by the eight times higher water content of muscle tissue compared with that of fatty tissue (11), into which water-soluble compounds like beta-lactam antibiotics may preferably diffuse. Also, the time course of drug levels in tissue may reflect the differences in blood flow and water content in those tissues. While levels of drug in fatty tissue were in equilibrium within 1 h following drug administration because of the low water content, it took more time for the concentrations of piperacillin and tazobactam to peak and reach an equilibrium in muscle tissue.

Penetration of piperacillin and tazobactam into skin was 5 to 10 times higher than that into fatty tissue and about three times higher than that into muscle tissue. Skin has about the same water content as muscle tissue, so other mechanisms must contribute to this finding. Skin tissue, as it was collected in our study, is made up of several cell types and interstitial fluid and may thus be a biophase which is entirely different from muscle and fat, which each consist of only one cell type. Although both compounds share the beta-lactam structure, piperacillin, with its bulky substituent at position 6, may have a different affinity than tazobactam to tissues like skin when the affinities are compared with those to fatty tissue. Similar findings of high penetration of beta-lactams into skin were also reported by other investigators (7). The exact mechanism that causes this important finding needs further investigations.

The penetrations of piperacillin-tazobactam into three sites of the gastrointestinal (GI) tract were also investigated. The blood flow to the GI tract is about 10 times higher than that to muscle at a comparable water content. Based on this fact, the penetration of drug into the GI tract should be the highest of all tissues studied. This was, in fact, the case for tazobactam. The concentrations of tazobactam in the distal mucosa were up to two times higher than those in plasma, but this was not the case for piperacillin, which had a very homogeneous penetration of about 50% into all three segments of the GI tract studied. The understanding of the penetration of beta-lactam antibiotics into the GI tract is, however, further complicated by the fact that, compared with skin, muscle, and fat, the GI tract is lined by epithelial cells which can actively take up and secrete acids or bases (12, 15). According to the results of this study, the uptake of a drug into epithelial cells is governed by the structural and physicochemical aspects of the compound. Blood flow to the organ would more indirectly support the possible uptake of piperacillin and tazobactam by supplying enough oxygen and nutrients to the cells. In addition, the results of a basic pharmacokinetic interaction study (1) suggest very strongly that piperacillin inhibits the process of elimination of tazobactam from the GI tract. We found no difference in tissue penetration between the three sites (proximal and distal intestine, appendix) for piperacillin, although these sites differ significantly in their mucosal fine structures. A difference was found for tazobactam, with the highest level of penetration being into the distal intestinal mucosa. These findings may suggest that both tazobactam and piperacillin are actively taken up by the serosal site of mucosal cells and that piperacillin does not inhibit this process. Piperacillin may, however, inhibit the secretion of tazobactam at the luminal site of the cell, which could, consequently, lead to the accumulation of tazobactam in the mucosa.

Summarizing all the results from our investigation of penetration of drugs into tissue, it has become evident that no single mechanism can explain the extent of penetration of drugs into tissue. Further studies will have to establish quantitative relationships among blood flow, water content, the biochemical composition of the tissue, and the physicochemical properties of the agent.

While the preceding discussion was based on the mechanisms of penetration, it is the absolute concentrations of tazobactam and piperacillin and the ratio between the two concentrations which are important for the prediction of antibacterial activity. When the bias of any tissue penetration study of beta-lactam antibiotics caused by the work-up
procedures that cause dilution of the extracellular concentration by cell lysis is considered, the data demonstrate again that the free piperacillin concentrations in all tissues exceeded the MICs for most microorganisms that are susceptible to the agent. The addition of tazobactam when the microorganisms produce β-lactamases has led in vitro to drastic reductions in otherwise elevated MICs (1, 3, 4, 8, 10). Our data show that the concentration ratios used to determine the effects in vitro are also achieved or even exceeded at the tissue site of infection. The exact relationship between levels of antibiotics at infection sites and their effects is not yet fully established for beta-lactam antibiotics alone (5, 14), and thus, the relationship for a combination may be far from established.

In conclusion, the introduction of new antimicrobial agents requires information on not only clinical efficacy but also the basic pharmacokinetics of the agents. Since pharmacokinetic data from prospective studies by validated assays are usually less variable than clinical efficacy data and since they are less affected by the complex clinical situation, there is a great deal of trust in the prediction of pharmacodynamic effects. In the present investigation, one of the agents, piperacillin, has a long and proven role in the treatment of severe infections. This was confirmed in this study again by levels in blood and tissue that exceeded the MICs for virtually all pathogenic microorganisms. These levels ensure clinical efficacy in infections in which susceptible microorganisms are involved. Clinical situations that require the addition of a β-lactamase inhibitor are those in which microorganisms are resistant to piperacillin because they produce a β-lactamase. The high levels of piperacillin alone in tissue are therefore not high enough to eradicate those microorganisms. The levels of tazobactam necessary to obtain anti-β-lactamase activity at the site of infection were achieved in the present investigation. Therefore, the data on the pharmacokinetics of piperacillin and tazobactam presented here support their clinical efficacies seen in clinical trials.

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