Pharmacodynamic study of β-lactams alone and in combination with β-lactamase inhibitors against Pseudomonas aeruginosa possessing an inducible β-lactamase

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Objectives: The antimicrobial efficacies of β-lactams alone and in combination with β-lactamase inhibitors were investigated by applying a rabbit tissue cage model against a strain of Pseudomonas aeruginosa with an inducible AmpC (iAmpC) β-lactamase.

Methods: Two sterilized golf Wiffle balls were surgically implanted in the rabbit dorsal cervical area. After 4 weeks, Wiffle balls had filled with tissue cage fluid (TCF), in which 2 mL of 10⁶ cfu/mL of the test isolate were inoculated. To achieve the same T > MIC as in humans, 400 mg/kg of the β-lactams alone and in combination was administered twice a day via subcutaneous injection. The dosing regimens were as follows: piperacillin alone, 4 g piperacillin/0.5 g tazobactam; ticarcillin alone, 3 g ticarcillin/0.1 g clavulanate; and 3 g ticarcillin/0.3 g clavulanate.

Results: The changes in bacterial counts (log cfu/mL) after the 3 day treatments were as follows: 1.03 ± 0.97 (control), –1.31 ± 0.61 (piperacillin), –2.81 ± 0.53 (4 g piperacillin/0.5 g tazobactam), –1.61 ± 0.68 (ticarcillin), –3.42 ± 0.75 (3 g ticarcillin/0.1 g clavulanate) and –1.65 ± 1.47 log cfu/mL (3 g ticarcillin/0.3 g clavulanate). AmpC induction by high-dose clavulanate was observed in rabbit TCF, and was confirmed by the in vitro induction study.

Conclusions: The study indicated that tazobactam significantly enhanced the antibacterial activity of piperacillin against iAmpC P. aeruginosa; clavulanate had synergy with the antibacterial activity of ticarcillin at low concentration, but had no effect on ticarcillin at high concentration due to AmpC induction by clavulanate.

Keywords: piperacillin, tazobactam, ticarcillin, clavulanate, β-lactamase inhibitor, tissue cage model, β-lactamase induction

Introduction

The presence of inducible chromosomal β-lactamases, most notably AmpC β-lactamase, is a species-specific characteristic of some Gram-negative bacteria, i.e. Enterobacter, Serratia, Pseudomonas, Citrobacter and Proteus species. These organisms are virulent nosocomial pathogens and are difficult to treat. AmpC β-lactamases are usually present at a low level, but after exposure to some β-lactams, i.e. certain cephalosporins and extended-spectrum penicillins, the enzymes in these bacterial species are induced, causing these drugs to lose their efficacy.

Interestingly, clavulanate, a β-lactamase inhibitor, can induce AmpC β-lactamase in vitro in clinical isolates of Pseudomonas aeruginosa, Enterobacter cloacae, Serratia marcescens and Citrobacter freundii.¹–³ Clavulanate has synergy with the antibacterial activity of β-lactams by competing for β-lactamase and hence protecting β-lactams from attack. Clavulanate also antagonizes the antibacterial activity of β-lactams by inducing β-lactamase production.

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The enzyme-inducing capacity of clavulanate is concentration dependent. In one in vitro study, clavulanate at 2 or 4 mg/L had neither an antagonistic nor a synergistic antimicrobial effect in combination with ticarcillin against P. aeruginosa possessing an inducible β-lactamase. Antagonism of ticarcillin by clavulanate was observed when MICs of ticarcillin increased in the presence of 1–32 mg/L clavulanate. In an in vitro pharmacokinetic model, antagonism of ticarcillin against P. aeruginosa with inducible β-lactamase were observed in some tests with regimens simulating the clinically relevant concentrations achieved with a 3.1 g dose of ticarcillin/clavulanate (3 g ticarcillin plus 0.1 g clavulanate), and in all tests with regimens simulating clinically relevant concentrations achieved by a 3.2 g dose of ticarcillin/clavulanate (3 g ticarcillin plus 0.2 g clavulanate). No enzyme induction by tazobactam was observed. Moreover, tazobactam enhanced the activity of piperacillin against all tested strains of P. aeruginosa, despite β-lactamase inducibility of the organism in this in vitro model.

Although β-lactamase induction by clavulanate and the resultant antagonism of ticarcillin and other β-lactams have been extensively studied in vitro, there are insufficient in vivo data available on enzyme induction by clavulanate. It remains unclear whether β-lactamases could be induced by clavulanate in vivo, with the consequent antagonism of ticarcillin activity. One study in mice showed that clavulanate did not antagonize the efficacy of ticarcillin against β-lactamase-inducible strains of P. aeruginosa, E. cloacae, C. freundii or S. marcescens, but no drug concentration data were presented in this study. As stated above, the β-lactamase induction level depends on the clavulanate level.

The current study was designed to investigate over a 3 day treatment period the in vivo antimicrobial activities of β-lactams alone and in combination with β-lactamase inhibitors against P. aeruginosa possessing an inducible AmpC (iAmpC) β-lactamase. To understand the impact of β-lactamase inhibitors on the antimicrobial activities of β-lactams against this organism, AmpC β-lactamase induction was studied in vitro and in vivo.

Materials and methods

Bacterial strain and antibiotics

One clinical bacterial strain of P. aeruginosa, PSA246, which has been shown to possess iAmpC expression in vitro after exposure to clavulanate, was used in this study. The stock strain was frozen at −80°C in skimmed milk. Before each experiment the stock bacteria were subcultured twice onto a trypticase soy agar plate with 5% sheep’s blood (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) and incubated at 35°C overnight to ensure bacterium purity. Piperacillin/tazobactam and ticarcillin/clavulanate analytical standards for the in vitro test and HPLC assay were obtained from Wyeth Laboratories (Pearl River, NY, USA) and GlaxoSmithKline, respectively. For all in vivo studies, commercially available intravenous preparations of 4 g piperacillin (Pipracil; Lederle Piperacillin, Carolina, Puerto Rico), 4 g piperacillin/0.5 g tazobactam (Zosyn; Lederle Piperacillin), 4 g ticarcillin (Ticar; SmithKline Beecham Pharmaceuticals, Philadelphia, PA, USA) and 3 g ticarcillin/0.1 g clavulanate (Timentin; SmithKline Beecham Pharmaceuticals) were purchased from their respective manufacturers. In addition to the commercial products, the combination of 3 g ticarcillin and 0.3 g clavulanate was used for this study, which was made by adding 0.2 g clavulanate analytical standard to one vial of 3 g ticarcillin/0.1 g clavulanate.

In vitro susceptibility testing

MICs of piperacillin, piperacillin/tazobactam, ticarcillin and ticarcillin/clavulanate for P. aeruginosa PSA246 and a reference strain of P. aeruginosa (ATCC 27853) were determined in duplicate using the broth microdilution method with cation-supplemented Mueller–Hinton broth and a bacterial inoculum of ∼5 × 10^6 cfu/mL, according to NCCLS guidelines.

Rabbit tissue cage model

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Hartford Hospital, Hartford, CT, USA. Female New Zealand white rabbits, weighing ∼4 kg, were used in the present study. All animals received water and food ad libitum. The animal vendor (Covance Research Products, Denver, PA, USA) implanted the golf Wiffle balls in the animals. Wiffle balls are 2 mm thick, 4 cm diameter hollow plastic balls with an even distribution of 26 0.5 cm diameter holes. The procedure for placing the Wiffle balls into the animals as follows: rabbits were anesthetized, and two sterilized Wiffle balls were implanted subcutaneously on the rabbit dorsal cervical surface and tacked down on the dorsal musculature with a loosely tied suture of 3-0 Nylon. The skin incision was closed in two layers with absorbable sutures. During the 4 week surgical recovery period, including a 1 week quarantine at the Hartford Hospital Animal Laboratory, the Wiffle balls filled with fluid, the so-called tissue cage fluid (TCF). This fluid served as a bacterial growth medium.

Antibiotic assay

HPLC methods were developed and validated to determine simultaneously the piperacillin/tazobactam (C. Li, D. Xuan, M. Ye, D. P. Nicolau and C. H. Nightingale, unpublished results) and ticarcillin/clavulanate concentrations, respectively, in rabbit serum and TCF. The concentration ranges of the standard curves were 1–100 mg/L for piperacillin, tazobactam and ticarcillin, and 0.2–2 mg/L for clavulanate in rabbit serum and TCF. The relative standard deviations and relative errors of the inter- and intra-assay of these four HPLC methods were <7.3%. Samples containing concentrations above the quantification limits were diluted with tested drug-free rabbit serum or TCF.

Pharmacokinetic/pharmacodynamic study

β-Lactams are time-dependent antibiotics, i.e. their bacterial killing efficacies are associated with the time for which drug concentrations exceed the MIC. To achieve a similar time above the MIC (T > MIC) in rabbits as in humans for piperacillin (Pipracil, 4 g every 6 h), piperacillin/tazobactam (Zosyn, 4.5 g every 6 h), ticarcillin (Ticar, 3 g every 6 h) and ticarcillin/clavulanate (Timentin, 3.1 g every 6 h), the dose regimens were as follows: 400 mg/kg piperacillin alone or in combination with tazobactam (4 g piperacillin/0.5 g tazobactam), and 400 mg/kg ticarcillin alone or in combination with clavulanate (3 g ticarcillin/0.1 g clavulanate). In addition, to achieve a similar peak concentration of clavulanate in rabbit TCF as in human serum for another clinically used combination of 3 g ticarcillin/0.2 g clavulanate (Timentin, 3.2 g every 6 h), the dose regimen of 400 mg/kg ticarcillin in the combination of 3 g ticarcillin/0.3 g clavulanate was also applied in this study. After sample size analysis, six rabbits for each dose regimen were used, creating a total of six groups, including one control group that did not receive any treatment. Before infection, the rabbit TCF in each Wiffle ball was sampled to test whether it was sterile, then rabbits that had been implanted with Wiffle balls for 4 weeks were infected by percutaneous injection of 2 mL of PSA246 (10^6 cfu/mL) into each Wiffle ball. After 24 h of organism incubation in the Wiffle balls, the rabbits received antibiotic treatment for 3 days by subcutaneous injection twice a day.
**β-Lactams and β-lactamase inhibitors in vivo**

Blood (1 mL) was collected using the marginal ear vein bleeding technique. The sampling time points were as follows: 0 h (prior to dose), and 0.25, 0.5, 1, 2, 4, 6, 8, and 12 h after the first dose. Blood samples were centrifuged, separated and frozen at −80°C until analysis. The bacterial density within the two Wiffle balls of each rabbit was monitored over the 72 h treatment period by aspirating TCF (400 µL) from each Wiffle ball. The sampling time points were as follows: 0 h (prior to the initiation of antimicrobial therapy), and 2, 4, 6, 8, 12, 24, 36, 48, 60 and 72 h after the initiation of therapy. A 10-fold dilution series in saline was made using 100 µL TCF from each Wiffle ball, and a 10 µL sample of each dilution was placed on blood agar plates, followed by incubation for 24 h. To determine the concentration profiles of the antibiotics in TCF during the first dosing interval, TCF samples from one Wiffle ball were stored at −80°C until analysis.

**Induction of β-lactamase**

It is important to elucidate the mechanism of the impact of clavulanate on the antimicrobial activity of ticarcillin in vivo, and to confirm that the antagonism in vivo and in vitro arise for the same reason: AmpC β-lactamase induction. For comparative purposes, tazobactam was also used to test β-lactamase-induction capacity. An HPLC method was developed (see below) to measure AmpC β-lactamase activity, and applied to monitor changes in AmpC β-lactamase activity in the rabbit TCF during the first dosing interval.

**In vitro induction**

PSA246 was subcultured onto a blood agar plate and incubated at 35°C overnight; five colonies were inoculated into 5 mL of cation-adjusted Mueller–Hinton broth at 35°C. After a 2 h incubation, tazobactam or clavulanate was added. The final concentrations were as follows: 0 h (prior to the induction), and 2, 4, 6, 8, 12, 24, 36, 48, 60 and 72 h after addition of AMP. The sample was washed once with phosphate buffer (0.1 M, pH 7), and lysed by 15 cycles of 15 s sonication at 30°C, the enzyme reaction was terminated by heating in a boiling water bath for 30 min. The precipitated proteins were removed by centrifugation at 10,000 rpm for 20 min. The supernatant was injected directly onto an HPLC system to analyse the residual substrate. The assay was carried out with a Phenomenex Prodigy ODS (3) column (10 µm, 240 × 4.6 mm) (Phenomenex, Torrance, CA, USA), coupled with a Bondapak C18 Guard-Pak pre-column (Waters, Milford, MA, USA). The column was maintained at room temperature; the mobile phase consisted of 40:60 (v/v) acetonitrile/phosphate buffer (0.014 M, pH 2.4). The flow rate of the mobile phase was 1 mL/min, and the eluate was monitored at 254 nm. This external standard method was validated before running unknown samples. β-Lactamase activity in PSA246 after induction in vitro and in vivo was evaluated by assessing the enzymatic reaction rate constant, which is the reaction rate constant of cefalothin at the initial concentration of 250 mg/L degraded by β-lactamase at 37°C.

**Data analysis**

Non-compartmental analysis (WinNonlin, version 3.3; Pharsight Corporation, Mountain View, CA, USA) was used to evaluate the concentration profiles of antibiotics in rabbit serum and TCF during the first dose interval. The following parameters were estimated: the maximum concentration (C_max), the time to reach the maximum concentration (T_max), the area under the concentration-time curve (AUC_0→∞), the area under the concentration-time curve (AUC_0→∞) and the mean residence time (MRT). Bacterial density change–time curves were plotted for each dosing regimen.

In the β-lactamase induction study, enzymatic activity was estimated as the enzyme reaction rate constant. The substrate degradation at enzyme reaction follows first-order kinetics. When the natural logarithm of the residual substrate concentration is plotted against time, the slopes of these lines are equal to the rate constant of the enzyme reaction (units: mg/L/min), designated the apparent β-lactamase activity. Owing to different bacterial densities at different time points, the β-lactamase activity ratio was used to estimate the enzyme activity change, which was calculated by Equation 1:

\[
\text{Ratio} = \frac{\text{Enzyme activity (t h)}}{\text{Enzyme activity (0 h)}} \times \frac{\text{Bacterial density (0 h)}}{\text{Bacterial density (t h)}}
\]

Equation 1

Student’s t-test was used to determine whether there was a statistical difference in the pharmacokinetic parameters between piperacillin in combination with tazobactam, and piperacillin alone. A one-way ANOVA was employed to test for a statistical difference in the pharmacokinetic parameters of ticarcillin among the three different dose regimens. For the pharmacodynamic study, a one-way ANOVA was employed to determine whether there was a statistical difference in bacterial killing between the control group and the β-lactam alone or in combination with β-lactamase inhibitor groups. For the enzyme induction study, a one-way ANOVA was used to determine whether there was a statistical difference between these groups in vitro. *P* values <0.05 were considered statistically significant (S-Plus 2000; Mathsoft, Inc., Seattle, WA, USA).

**Results**

**Susceptibility of the tested isolate**

MICs of piperacillin, piperacillin/tazobactam, ticarcillin and ticarcillin/clavulanate for PSA246 were 16, 8/4, 32 and 32/2 mg/L, respectively. The MICs of all antibiotics and their combinations were <64 mg/L, i.e. PSA246 was susceptible to these two β-lactams and their combinations.

**Antibiotic concentration profiles in rabbit serum and TCF**

Figures 1–4 show the concentration–time profiles of piperacillin alone, 4 g piperacillin/0.5 g tazobactam, ticarcillin alone, 3 g ticarcillin/0.1 g clavulanate and 3 g ticarcillin/0.3 g clavulanate in rabbit serum and TCF. The mean pharmacokinetic parameters are summarized in Table 1. There was no statistical difference in the pharmacokinetic parameters of piperacillin with or without tazobactam, or ticarcillin...
with and without clavulanate, in either serum or TCF. These data suggest that tazobactam and clavulanate do not alter the absorption and disposition of piperacillin and ticarcillin in rabbits.

Antimicrobial efficacy

The antimicrobial activities of these five dose regimens were evaluated by assessing the bacterial density change within the Wiffle ball during the 3 day treatment period. After 24 h incubation in rabbit TCF, the recovery of PSA246 was $\sim 5 \times 10^5$ cfu/mL immediately prior to antibiotic treatment. All of the bacterial time–kill curves are shown in Figures 5 and 6. For the untreated control group, log bacterial density in TCF increased 1.03 ± 0.97 cfu/mL (mean ± S.D., n = 12) after 72 h. For the treatment groups, log changes of the bacterial density in rabbit TCF after 3 days of therapy were as follows (mean ± S.D.): $-1.31 \pm 0.61$ cfu/mL (n = 12) for piperacillin alone group; $-2.81 \pm 0.53$ cfu/mL for the combination 4 g piperacillin/0.5 g tazobactam; $-1.60 \pm 0.63$ cfu/mL for ticarcillin alone; $-3.42 \pm 0.75$ cfu/mL for the combination 3 g ticarcillin/0.1 g clavulanate; and $-1.65 \pm 1.47$ cfu/mL for the combination 3 g ticarcillin/0.3 g clavulanate.

There was a statistical difference in the bacterial density change in rabbit TCF between the untreated control group and all five treatment groups 3 days after the initiation of therapy. Bacterial killing between piperacillin with and without tazobactam was found to be statistically significantly different ($P < 0.001$); tazobactam synergized the antimicrobial activity of piperacillin against PSA246 in rabbit TCF (Figure 5). However, there was a complex scenario for the combination of ticarcillin with clavulanate. There was a statistically significant difference in bacterial killing between 3 g ticarcillin alone and in combination with 0.1 g clavulanate ($P < 0.001$), and no statistically significant difference in bacterial killing between 3 g ticarcillin alone and in combination with 0.3 g clavulanate ($P = 0.93$). In this case, clavulanate at low levels enhanced the antimicrobial activity of ticarcillin against PSA246, but clavulanate at high levels did not enhance the antimicrobial activity of ticarcillin. The MICs of the antibiotics for the isolate recovered from rabbit TCF after 3 days of treatment were tested again, and they were the same as before drug exposure, indicating that resistant bacteria were not selected.

$\beta$-Lactamase induction

The in vitro enzyme induction by $\beta$-lactamase inhibitors was conducted in Mueller–Hinton broth at 37°C. Bacteria grew from $1.26 \times 10^7$ to $7.08 \times 10^8$ cfu/mL during the 12 h period, and there was no difference in bacterial growth between the four groups. Figure 7 shows the kinetics profiles of $\beta$-lactamase activity versus time. $\beta$-Lactamase in PSA246 remained at a low level in the blank Mueller–Hinton broth and piperacillin at 15 mg/L during the 12 h incubation, and there was no statistically significant difference between these two groups. However, compared with the enzyme level in the control group at 8 h, $\beta$-lactamase activity increased 38- and 200-fold at clavu-
β-Lactams and β-lactamase inhibitors in vivo

The data indicate that the higher concentration of clavulanate resulted in increased enzyme induction. For the in vivo study, the β-lactamase activity in PSA246 recovered from rabbit TCF prior to treatment was detectable, but at a low level. However, the enzyme activity became undetectable due to the reduction of bacterial density during the first dosing interval after the initiation of the therapy in the groups treated with piperacillin alone, the combination of 4 g piperacillin/0.5 g tazobactam, ticarcillin alone and the combination of 3 g ticarcillin/0.1 g clavulanate. Except in two rabbits with low bacterial densities (log cfu/mL <4), in rabbits (n = 4) treated with the combination of 3 g ticarcillin/0.3 g clavulanate, β-lactamase activities of PSA246 were detectable in TCF during the first dosing interval. AmpC activity ratios (mean ± S.D.), calculated using Equation 1, were as follows: 24.9 ± 20.7, 50.3 ± 60.8, 130.1 ± 118.1, 152.7 ± 146.8 and 182.0 ± 73.3, at 2, 4, 6, 8 and 12 h.
after exposure to 3 g ticarcillin/0.3 g clavulanate, respectively. These in vivo data suggest that AmpC induction increased with increasing clavulanate exposure.

Discussion

In the current study, the 3 day therapeutic efficacy data indicated that tazobactam potentiated the antimicrobial activity of piperacillin against this strain of iAmpC P. aeruginosa in this well-defined infection model. This in vivo result confirmed the synergy between tazobactam and piperacillin against the same bacterial strain in the previous in vitro study conducted by Lister et al. No AmpC β-lactamase induction was observed after the bacteria were exposed to tazobactam in vitro or in vivo. The concentration–time profile of tazobactam in rabbit TCF (Figure 2b) shows that tazobactam levels are >1 mg/L during the entire dosing interval. This indicates that the synergy between piperacillin and tazobactam is the result of the lack of β-lactamase induction and the protection of piperacillin from β-lactamase inactivation.

AmpC β-lactamase induction was observed after the bacteria were exposed to clavulanate in vitro and in vivo. This strain of P. aeruginosa produced much more β-lactamase when it was exposed to 15 mg/L clavulanate in vitro than when it was exposed to 5 mg/L clavulanate. This phenomenon indicates that the capacity of clavulanate to induce AmpC β-lactamase is concentration dependent. As a result, the higher the level of clavulanate, the more AmpC β-lactamase was induced. AmpC was induced in the TCF of the rabbits treated with 0.3 g clavulanate in combination with 3 g ticarcillin; the enzyme activity increased >100-fold 12 h after drug exposure. However, no AmpC activity was measured in the TCF of rabbits treated with 0.1 g clavulanate in combination with 3 g ticarcillin. Unlike the in vitro study, in which clavulanate concentrations were fixed, clavulanate in rabbit TCF followed a kinetic profile, i.e. there were different concentrations at different times. Under these conditions, the in vivo observations indicated that AmpC β-lactamase in PSA246 could be induced by clavulanate exposure (i.e. AUC) of 83 mg·h/L, with the maximum concentration at 13.2 mg/L.

Table 1. Pharmacokinetic parameters of antimicrobials in rabbit serum and TCF

<table>
<thead>
<tr>
<th>Drugs in rabbit serum</th>
<th>4 g PIP</th>
<th>4 g PIP/0.5 g TAZ</th>
<th>3 g TIC</th>
<th>3 g TIC/0.1 g CLA</th>
<th>3 g TIC/0.3 g CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (mg/L)</td>
<td>150.72 (33.98)</td>
<td>158.75 (22.02)</td>
<td>33.14 (5.34)</td>
<td>229.09 (80.66)</td>
<td>289.63 (96.38)</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>1.17 (0.41)</td>
<td>1.50 (0.71)</td>
<td>1.10 (0.55)</td>
<td>1.33 (0.52)</td>
<td>1.50 (0.58)</td>
</tr>
<tr>
<td>AUC_{0→∞} (mg·h/L)</td>
<td>502.07 (71.15)</td>
<td>548.96 (35.20)</td>
<td>102.64 (13.83)</td>
<td>856.50 (256.13)</td>
<td>962.02 (202.33)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>2.57 (0.29)</td>
<td>2.58 (0.33)</td>
<td>2.06 (0.16)</td>
<td>3.38 (1.08)</td>
<td>2.67 (0.58)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs in rabbit TCF</th>
<th>4 g PIP</th>
<th>4 g PIP/0.5 g TAZ</th>
<th>3 g TIC</th>
<th>3 g TIC/0.1 g CLA</th>
<th>3 g TIC/0.3 g CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (mg/L)</td>
<td>49.84 (12.68)</td>
<td>49.28 (16.36)</td>
<td>14.21 (6.53)</td>
<td>46.73 (16.99)</td>
<td>72.05 (15.30)</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>3.33 (1.03)</td>
<td>3.60 (0.89)</td>
<td>2.80 (1.10)</td>
<td>4.00 (1.26)</td>
<td>4.00 (0.00)</td>
</tr>
<tr>
<td>AUC_{0→∞} (mg·h/L)</td>
<td>423.63 (74.70)</td>
<td>444.74 (52.28)</td>
<td>93.24 (19.93)</td>
<td>713.16 (251.06)</td>
<td>775.78 (153.09)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.21 (1.42)</td>
<td>8.47 (2.96)</td>
<td>5.95 (1.99)</td>
<td>12.35 (2.84)</td>
<td>9.50 (2.04)</td>
</tr>
</tbody>
</table>

Table 1. Pharmacokinetic parameters of antimicrobials in rabbit serum and TCF

The numbers represent mean (S.D.).
The results of this pharmacodynamic study of ticarcillin and clavulanate in immunocompetent rabbits were complicated. Comparing bacterial killing by ticarcillin alone, 0.1 g clavulanate in combination with 3 g ticarcillin seemed to increase the antimicrobial activity of ticarcillin against this strain of iAmpC \( \beta \)-lactamase-inducing \( \beta \)-lactamase (L). As tazobactam enhanced the antimicrobial activity of piperacillin against this strain of iAmpC, the combination of ticarcillin and clavulanate mainly acted as a \( \beta \)-lactamase inhibitor balanced the AmpC induced by clavulanate and that clavulanate had no effect on the antimicrobial activity of ticarcillin in the same bacterial strain. Based on the enzyme induction data, we postulated that at the dose of 0.3 g clavulanate, the effect of clavulanate as a \( \beta \)-lactamase inhibitor balanced the AmpC induced by clavulanate, and that clavulanate mainly acted as a \( \beta \)-lactamase inhibitor at 0.1 g dose. This may explain why there was no influence of high-dose clavulanate on the antimicrobial activity of ticarcillin, and why there was a synergy of low-dose clavulanate with ticarcillin. To some extent, the observed bacterial killing by ticarcillin in combination with clavulanate in the immunocompetent rabbit contradicts the antagonism of clavulanate to ticarcillin in \textit{in vitro} study reported in the study by Lister et al.\textsuperscript{7} Such contradiction was probably due to the dose and species of the immunocompetent animals tested in this study.

Overall, the antimicrobial efficacy data indicated that piperacillin/tazobactam had some advantages over ticarcillin/clavulanate against this strain of \textit{P. aeruginosa} with its iAmpC. As a \( \beta \)-lactamase inhibitor, tazobactam inactivated \( \beta \)-lactamases and potentiated piperacillin against this bacterium. Since the concentration range of tazobactam in human skin, appendix and intestinal mucosa was 6.3–14.5 mg/L after a single dose of 4 g piperacillin/0.5 g tazobactam via intravenous infusion over 30 min,\textsuperscript{12} it may be clinically significant that the synergy of piperacillin by tazobactam was observed in rabbit TCF.

The most noteworthy observation of this study was that clavulanate could induce AmpC \( \beta \)-lactamases in \textit{P. aeruginosa} in animals, and that the AmpC induction level was related to clavulanate concentrations. As an enzyme inducer, clavulanate induced AmpC \( \beta \)-lactamase production and failed to enhance the activity of ticarcillin against the tested strain of iAmpC \textit{P. aeruginosa}. In clinical settings, the \( \beta \)-lactamase-inducing capacity of clavulanate needs to be defined at clinically relevant concentrations. Clavulanate is well distributed in human tissues, and ticarcillin does not affect the distribution of clavulanate to body tissue or vice versa.\textsuperscript{13} Clavulanate concentrations reached 17.8 and 32.5 µg/mg in human spongiosa and corticalis bone, respectively, 45–85 min after prophylactic administration of 5 g ticarcillin/0.2 g clavulanate.\textsuperscript{14} Another human study showed that the concentration of clavulanate in the thread fluid was similar to the corresponding serum values, and the concentrations of clavulanate in the blister fluid and lymph were higher than those in serum 1 h after dosing.\textsuperscript{15} Emergence of resistant \textit{P. aeruginosa} isolates has been reported in several clinical trials during therapy with ticarcillin/clavulanate combinations, including cases of clinical treatment failure,\textsuperscript{16–18} but no detailed information on AmpC \( \beta \)-lactamase induction were presented. Further clinical research on combinations of ticarcillin/clavulanate is needed to fully answer this question. The pharmacodynamic results of the combinations of ticarcillin/clavulanate in this study suggest that there is some risk of clavulanate antagonizing ticarcillin when used against \textit{P. aeruginosa} strains with an inducible \( \beta \)-lactamase.

In conclusion, there is synergy between tazobactam and piperacillin, as tazobactam enhanced the antimicrobial activity of piperacillin against this strain of iAmpC \textit{P. aeruginosa}. At low concentrations, clavulanate increased the antimicrobial activity of ticarcillin against this strain of iAmpC \textit{P. aeruginosa}, but had no effect on the antimicrobial activity of ticarcillin at high concentrations. Moreover, an increase in \( \beta \)-lactamase activity in this iAmpC strain of \textit{P. aeruginosa} caused by clavulanate at high concentration was observed in rabbits. One should be cautious when treating serious \textit{P. aeruginosa} infections with high-dose clavulanate-containing regimens.

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References


