Introduction

Streptococcus pneumoniae is a major cause of otitis media and is traditionally susceptible to penicillins and cephalosporins. Therefore, \( \beta \)-lactams have been widely used in the clinical treatment of this disease.\(^1\)\(^-\)\(^3\) Since penicillin-intermediately resistant \( S. \) pneumoniae (PISP) was isolated in Australia in 1967,\(^4\) the emergence of PISP and penicillin-resistant \( S. \) pneumoniae (PRSP) has been reported in many countries and frequencies of PISP and PRSP have been increasing year by year.\(^5\)\(^-\)\(^9\) Furthermore, reports have indicated that isolates are commonly resistant not only to penicillins but also to cephalosporins, macrolides and tetracyclines, and others are resistant only to cephalosporins with an oxyimino group.\(^10\)\(^-\)\(^12\)

High frequencies of both PISP and PRSP have been observed in juvenile and elderly patients. Therefore, it was felt necessary to study the optimum antibiotic and dosing route against experimental otitis media with PISP or PRSP; the comparative efficacy of several drugs was investigated in Mongolian gerbils and chinchilla.\(^13\)\(^-\)\(^15\) However, only one or two drugs were compared in these previous reports, and few studies have compared several drugs at the same time. In addition, few researchers have discussed the effect of in vitro activity and pharmacokinetics on the drug efficacy in models with otitis media at the same time.

We previously reported the dual requirements of an increased dosage of oral cephalosporin and prolonged treatment period to cure otitis media caused by PISP in guinea-pigs, and also showed that clinical efficacy reflected both the in vitro activity and pharmacokinetic properties of the antimicrobial.\(^16\) However, the appearance of PRSP has become important clinically. For example, Sato & Mimura reported that oral cephalosporins such as cefaclor were not effective and iv administration of antibiotics was required for treatment of otitis media with PRSP.\(^17\)

The present study, we evaluated efficacies of five parenteral \( \beta \)-lactam antibiotics with various degrees of antipneumococcal activity in experimental otitis media caused by PRSP in guinea-pigs. We also investigated the effects of in vitro activities and pharmacokinetics of the drugs on the efficacies.

Materials and methods

Bacterial isolate

The clinical isolate used in this study was penicillin-resistant \( S. \) pneumoniae D-1051 (MIC of penicillin G, 1.56 mg/L).

Antibiotics

The antibiotics used in this study were cefotiam (Takeda Chemical Industries, Osaka, Japan), ceftazidime (Nippon Glaxo, Tokyo, Japan), cefotaxime (Hoechst Japan, Tokyo, Japan), ceftiraxone (Nippon Roche K.K., Tokyo, Japan) and piperacillin (Toyama Chemical Co., Tokyo, Japan).

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Animals

Male Hartley guinea-pigs (bodyweight 250-300 g) were purchased from Japan SLC (Shizuoka, Japan). Animals were housed in regulation cages and were given free access to food and water.

In vitro studies

MICs were determined by the two-fold agar dilution method using Heart Infusion Agar (Eiken Chemical Co., Tokyo, Japan) supplemented with 5% defibrinated sheep blood. Bacteria stored in skim milk were spread on the blood agar and incubated at 37°C for 18 h. The bacteria grown on agar were suspended in Brain Heart Infusion Broth (Eiken Chemical Co., Tokyo, Japan), and then further incubated at 37°C for 6 h. The cells were inoculated on agar plates containing a two-fold dilution of antibiotics using a microplanter (Sakuma, Tokyo, Japan). The final inoculum was $10^4$ cfu per spot. The MICs were determined after incubation at 37°C for 18 h.

Efficacy experiment

Four or five guinea-pigs were used for each dosage group. Animals were anaesthetized with diethyl ether, and inoculated bilaterally with 50 μL of the bacterial suspension ($5.4 \times 10^8$ cfu/mL) in the tympanic cavity through the tympanic membrane. Treatment was initiated 1 day after infection and antimicrobial agent at 50 mg/kg was administered iv bd for 3 days. Control animals were infected in the same manner but did not receive any antimicrobial agent. At 24 h after the final administration of antimicrobials, the animals were anaesthetized with diethyl ether and killed by bleeding from the abdominal artery. The middle ear bullae (containing the tympanic cavity and auditory tube) were removed, opened and suspended in saline. The suspension was diluted in saline and cultured on blood agar. Colonies were counted after incubation at 37°C for 18 h.

Pharmacokinetic experiment

Animals with otitis media caused by S. pneumoniae D-1051 were given an iv dose of cefotiam, ceftazidime, cefotaxime, ceftriaxone or piperacillin at 100 mg/kg, 1 day after infection. At 5, 15, 30, 60 and 120 min after antimicrobial administration, a serum sample was taken and middle ear mucosa (MEM) was removed from the guinea-pigs. Each group comprised three animals. MEM was homogenized in 0.066 M phosphate buffer (pH 7.0) and then centrifuged at 5000 g for 10 min. Serum and MEM concentrations of cefotiam, ceftazidime, cefotaxime, ceftriaxone and piperacillin were measured by a bioassay method using Klebsiella pneumoniae ATCC10031, Proteus mirabilis ATCC21100, Micrococcus luteus ATCC9341, Escherichia coli NIHJ JC-2 and Micrococcus luteus ATCC9341, respectively. The detection limits were as follows: ceftiam, cefotaxime and piperacillin, 0.39 mg/L; ceftazidime, 0.1 mg/L; ceftriaxone, 0.78 mg/L.

Statistical analysis

Data were analysed for statistical significance by use of Tukey’s test. A $P$ value of $<0.05$ was considered to be significant.

Results

In vitro data

The MICs of cefotaxime, ceftriaxone and piperacillin for S. pneumoniae D-1051 were 1.56 mg/L, and superior to those of cefotiam (6.25 mg/L) and ceftazidime (12.5 mg/L).

Efficacy experiment

Figure 1 shows the viable cell counts in the middle ear after the administration of the antimicrobial at 50 mg/kg bd for

![Figure 1](image-url)
Therapeutic effects on experimental otitis media

3 days to guinea-pigs with otitis media caused by S. pneumoniae D-1051. The bacterial cell count in the untreated group was $6.49 \log_{10} \text{cfu/middle ear}$. Bacterial cell counts in the groups treated with cefotiam and ceftazidime were $6.14$ and $3.84 \log_{10} \text{cfu/middle ear}$, respectively, and these decreases were not statistically significant in comparison with the value for the untreated group. On the other hand, the values in the groups treated with cefotaxime, ceftriaxone and piperacillin were $<3.34$, $<2.59$ and $<2.68 \log_{10} \text{cfu/middle ear}$, respectively. These decreases were statistically significant in comparison with the value for the untreated group ($P < 0.01$).

Pharmacokinetic experiment

Figure 2 shows serum and M E M concentrations and the Table shows the pharmacokinetic variables of the parenteral $\beta$-lactams after administration of 100 mg/kg to guinea-pigs with otitis media caused by S. pneumoniae D-1051. The highest concentration ($C_{\text{max}}$) and the area under the concentration-time curve from time zero to infinity (AUC$_{\text{0-\infty}}$) in M E M were in the order ceftazidime $>$ piperacillin $>$ cefotiam $>$ ceftriaxone $>$ cefotaxime and ceftazidime $>$ ceftriaxone $>$ piperacillin $>$ cefotaxime $>$ cefotiam, respectively. In addition, the times above MIC in M E M were in the order cefotaxime $>$ ceftriaxone $>$ piperacillin $>$ ceftazidime $>$ cefotiam.

Discussion

S. pneumoniae is a major pathogen of acute otitis media. Recently, the increase of PISP and PRSP has become a clinical problem. Until now, it has been reported that an increased dosage of penicillins is effective in the clinical treatment of otitis media caused by PISP and PRSP. However, the reports indicated that iv administration of $\beta$-lactams was needed to treat such infections because oral administration was not completely effective.$^{17-19}$ In the present study, we evaluated the efficacies of some parenteral $\beta$-lactams on an experimental model of otitis media caused by PRSP and also investigated the effects of in vitro antipneumococcal activity and pharmacokinetic profiles of the antimicrobials on the therapeutic efficacies. The following antimicrobials were used: cefotiam, ceftazidime, cefotaxime, ceftriaxone and piperacillin; these were chosen because of their different in vitro antipneumococcal activities and pharmacokinetic profiles.

Previously, we used the guinea-pig model of otitis media

<table>
<thead>
<tr>
<th>Drug</th>
<th>$C_{\text{max}}$ (mg/L)</th>
<th>AUC$_{0-\infty}$ (mg·h/L)</th>
<th>$t_{1/2}$ (h)</th>
<th>$C_{\text{max}}$ (µg/g)</th>
<th>AUC$_{0-\infty}$ (mg·h/L)</th>
<th>$t_{1/2}$ (h)</th>
<th>Time above MIC (h)</th>
<th>M/S*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotiam</td>
<td>246.7</td>
<td>84.7</td>
<td>0.19</td>
<td>115.1</td>
<td>47.4</td>
<td>0.36</td>
<td>1.37</td>
<td>0.56</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>335.6</td>
<td>225.7</td>
<td>0.45</td>
<td>194.7</td>
<td>172.2</td>
<td>0.63</td>
<td>2.43</td>
<td>0.76</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>242.9</td>
<td>179.4</td>
<td>0.33</td>
<td>31.9</td>
<td>52.0</td>
<td>1.31</td>
<td>5.41</td>
<td>0.29</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>370.8</td>
<td>270.3</td>
<td>0.62</td>
<td>97.2</td>
<td>122.7</td>
<td>0.80</td>
<td>4.94</td>
<td>0.45</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>247.8</td>
<td>103.7</td>
<td>0.19</td>
<td>131.1</td>
<td>79.8</td>
<td>0.52</td>
<td>3.04</td>
<td>0.77</td>
</tr>
</tbody>
</table>

* A U C in mucosa/A U C in serum.

Table. Pharmacokinetic variables of $\beta$-lactam antibiotics after iv injection of 100 mg/kg in guinea-pigs with acute otitis media caused by S. pneumoniae D-1051 (PR SP)
by inoculation of penicillin-susceptible S. pneumoniae (PSSP) or PISP into the middle ear through the tympanic membrane to evaluate the efficacies of oral cephalosporins. This model with S. pneumoniae D-1051 (PRSP) could also be used to evaluate the efficacy on otitis media because $10^5$–$10^7$ cells/ear persisted for at least 1 week (data not shown).

In the otitis media model, cefotaxime, ceftriaxone and piperacillin reduced the viable cell counts in the middle ear close to the detection limit, following iv doses of 50 mg/kg bd for 3 days. On the other hand, administration of cefotaxime and ceftazidime did not lead to a significant decrease in the viable cell counts at the same dosage. All the drugs were expected to give concentrations over MICs in MEM after iv administration. However, cefotaxime had only a short time above MIC, because of its inferior antipneumococcal activity and its short $t_{1/2}$, resulting in no therapeutic effect. Ceftazidime had a longer time above MIC than cefotaxime because of its superior distribution to MEM despite having the lowest antipneumococcal activity of the five antimicrobials. This longer time above MIC seemed to contribute to the reduction in viable cell counts in some cases. Cefotaxime, ceftriaxone and piperacillin had a long time above the MIC owing to their low MICs, resulting in good efficacy. These results indicate that the therapeutic effects of parenteral $\beta$-lactams on otitis media caused by PRSP reflect both the in vitro antipneumococcal activity and the distribution to MEM.

In conclusion, cefotaxime, ceftriaxone and piperacillin, on account of having a longer time above their MICs in the MEM, showed greater efficacy than cefotaxim and ceftazidime against the otitis media model with PRSP.

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**Reference**


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