CP6679, a new injectable cephalosporin with broad spectrum and potent activities against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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**Abstract** The antibacterial activities of CP6679, a new injectable cephalosporin with a broad antibacterial spectrum, were compared with those of other cephalosporins. CP6679 had stronger in-vitro activity than ceftazidime and cefpirome against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci, and *Pseudomonas aeruginosa*. Its activity against MRSA was eight times stronger than that of cefpirome, and it showed high binding affinity for penicillin-binding protein 2’ of MRSA. Furthermore, the antibacterial activity of CP6679 against ceftazidime-resistant and imipenem-resistant *P. aeruginosa* was eight times stronger than that of ceftazidime and four times stronger than that of imipenem. In addition to its in-vitro activities, CP6679 showed the highest efficacy among all cephalosporins tested in murine models of systemic infection induced by MRSA or *P. aeruginosa*. It was more effective than vancomycin and cefpirome against respiratory tract infections induced by MRSA in mice.

**Key words** CP6679 · Antibacterial activity · MRSA · *Pseudomonas aeruginosa*

**Introduction**

Nosocomial infections caused by opportunistic pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* constitute a serious problem in all medical fields, because these pathogens have acquired resistance to various antimicrobial agents and they spread within the hospital population. In general, cephalosporins have been widely used in infection therapy because of their broad spectrum antibacterial activity and lower frequency of side effects. However, their efficacy against MRSA and *P. aeruginosa* is still insufficient to eradicate infections caused by these pathogens. We have searched for cephalosporin derivatives with potent activity against both MRSA and *P. aeruginosa* and found a new injectable cephalosporin, CP6679 (Fig. 1), with strong antibacterial activities against both pathogens. In this study, we evaluated the in-vitro and in-vivo activities of CP6679 against several species of bacteria, including MRSA and *P. aeruginosa*, and compared these activities with those of other cephalosporins and vancomycin.

**Materials and methods**

**Antibacterial agents**

CP6679, cefozopran, and cefoselis were prepared at the Pharmaceutical Research Center of Meiji Seika Kaisha (Yokohama, Japan). The other antibacterial agents were obtained from commercial sources as follows: ceftazidime (GlaxoSmithKline, Tokyo, Japan), cefpirome (Shionogi Pharmaceutical, Osaka, Japan), cefotaxime (Chugai Pharmaceutical, Tokyo, Japan), imipenem (Banyu Pharmaceuticals, Tokyo, Japan), benzylpenicillin (Meiji Seika Kaisha, Tokyo, Japan), and vancomycin (Shionogi Pharmaceutical).

**Bacterial strains**

We used reference bacterial strains from the culture collection maintained in our laboratory. Clinical isolates of various pathogens were obtained from several hospitals in Japan. The interpretive categories [break point minimum inhibitory concentrations (MICs)] of methicillin resistance of staphylococci, penicillin resistance of *Streptococcus pneumoniae*, and ceftazidime or imipenem resistance of *P. aeruginosa* provisionally established were as follows:
methicillin, \( \geq 12.5 \mu g/ml \); benzylpenicillin, \( \geq 0.1 \mu g/ml \); ceftazidime, \( \geq 12.5 \mu g/ml \); and imipenem, \( \geq 12.5 \mu g/ml \).

**Determination of minimum inhibitory concentrations (MICs)**

The MICs were determined by the twofold agar dilution method with Sensitivity Disk Agar N (Nissui Pharmaceutical, Tokyo, Japan). The bacteria were grown overnight in Sensitivity Test broth (Nissui) at 35°C. The culture as diluted with buffered saline containing gelatin to a final concentration of 10^6 cfu/ml. The bacterial suspensions were delivered by an inoculator (Sakuma Seisaku, Tokyo, Japan), with an inoculum size of 10^4 cfu/spot, on agar plates. The agar plates were incubated for 18 h at 35°C. The MIC was defined as the lowest concentration of the compound that prevented visible growth.

**Binding affinity for penicillin-binding protein**

The binding affinities of the test compounds for penicillin-binding protein 2’ (PBP2’) prepared from the MRSA isolate, *S. aureus* M-12 EHR, were determined by the fluorography of membrane proteins labeled in a competition assay with [14C]benzylpenicillin (Amersham Pharmacia Biotech, Buckinghamshire, UK). The binding of each compound for PBP2’ was quantified by scanning densitometry of the fluorographs. The binding affinity of the compounds was expressed as the concentration of the nonradiolabeled compound that inhibited radiolabeling with [14C]benzylpenicillin by 50% (IC_{50}) compared with that of a control sample.

**Pharmacokinetic study**

Male ICR mice were administered the test compounds subcutaneously at a dose of 25 mg/kg of body weight. Blood was obtained from one mouse at each time point of 5, 15, and 30 min, and 1 and 2 h after the administration. The collected blood was allowed to stand at 4°C for 2 h and was centrifuged at 3000 rpm for 10 min. To remove proteins from serum, an equivalent volume of methanol was added to the serum, and the mixture was centrifuged at 12000 rpm for 3 min. The supernatant was filtered, using a commercially available filtration device (porous size, 0.45 μm; Millipore Japan, Tokyo, Japan). The concentration of the test compound in the serum sample was measured by HPLC. Pharmacokinetic parameters, AUC and t_{1/2}, were calculated by the Gauss-Newton method.

Subsequently, three ICR male mice, which received the test compounds subcutaneously at the same dose as noted above, were kept in a metabolic cage (MM type; Sugiyamagen, Tokyo, Japan), and urine was collected during 4 h after the administration. The collected urine was filtered in the manner described above for serum. The concentration of the test compound in the urine was measured by HPLC, and the percent recovery was calculated.

**Results**

**Antibacterial activities**

The antibacterial activities of CP6679 against 19 reference strains are shown in Table 1. CP6679 showed a broad antibacterial spectrum against gram-positive bacteria, including MRSA and *Enterococcus hirae*, and against gram-negative bacteria, including *P. aeruginosa*. The antibacterial activities of CP6679 were similar to those of cefozopran, except that it was more active against high-level MRSA isolate and *E. hirae*. Against *P. aeruginosa*, CP6679 was more potent than cefpirome and cefoselis. However, the antibacterial activity of CP6679 against *Proteus vulgaris* GN76/C-1 was inferior to that of ceftazidime and cefoselis. In particular, among the β-lactam agents evaluated, CP6679 showed the strongest activity against both MRSA and *P. aeruginosa*. 

**Pulmonary infection**

Male ICR mice (n = 8) were administered 200 mg/kg i.p. of cyclophosphamide, an immunosuppressant, 4 days before infection. To facilitate the erosion caused by pathogens in the lungs, they were treated with 2% formaldehyde intranasally 1 day before infection. An MRSA isolate, *S. aureus* M-126 or MF681, suspended in saline, was introduced intranasally as a challenge. The mice were administered the test compounds subcutaneously twice a day, at a dose of 50 mg/kg, for 3 days after infection. The lungs were extracted and homogenized with saline buffer 1 day after the final dosing, and viable bacterial cells were counted.

**Infection models**

**Systemic infection**

The protective effects of CP6679 on systemic infection models were compared with those of reference agents. Five male ICR mice were challenged intraperitoneally with 0.5 ml of bacterial suspension containing 2.5% gastric mucin (Difco Laboratories, Detroit, MI, USA). The animals were administered the test compounds subcutaneously, once at 1 h, or twice at 1 and 3 h after the challenge. The 50% effective dose (ED_{50}) was calculated by probit analysis from the number of mice still alive 7 days after infection.