Mechanism of Therapeutic Effectiveness of Cefixime against Typhoid Fever

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β-Lactams have been considered ineffective against organisms growing inside mammalian cells because of their poor penetration into cells. However, cefixime has been shown to be clinically effective against typhoid fever. The probable mechanism of therapeutic effectiveness of cefixime against typhoid fever was investigated using *Salmonella enterica* serovar Typhimurium instead of *S. enterica* serovar Typhi both in a cellular and in a mouse infection model. Cefixime was able to inhibit the growth of serovar Typhimurium inhabiting monocyte-derived THP-1 cells. Elongation of serovar Typhimurium in THP-1 cells was observed microscopically. Apparent morphological changes of serovar Typhimurium in THP-1 cells were also observed by electron microscopy. The concentration of cefixime inside THP-1 cells was almost half (46 to 48%) of the concentration outside the cells when serovar Typhimurium coexisted in the solution. The length of time after oral dosing (8 mg/kg) that cefixime was present—calculated from levels in serum—at a concentration above the MIC at which 90% of the serovar Typhi organisms inside human cells were inhibited was presumed to be more than 12 h. Cefixime also showed excellent activity in the mouse systemic and oral infection models based on infections caused by serovar Typhimurium. It is concluded that a fair amount of cefixime can enter mammalian cells and inhibit the growth of bacteria inside cells when the bacteria are sensitive enough to cefixime, as are serovars Typhimurium and Typhi.

Typhoid fever continues to be one of the major public health problems in developing countries. Moreover, a transferable plasmid encoding resistance to chloramphenicol, co-trimoxazole, and ampicillin has spread to *Salmonella enterica* serovar Typhi in many countries (7, 14, 17, 19, 22). This changing trend in the antibiotic susceptibility of serovar Typhi has encouraged the use of new agents, such as oxymino-cephalosporins and quinolones, for treatment of typhoid fever caused by multi-drug-resistant strains. Although resistance to these agents has been observed in some cases, it is lower than that of serovar Typhi to the older agents (1–3, 7–9, 14, 15, 17, 18, 20, 22).

While the strong *in vitro* activity of newer cephalosporins against enteropathogenic bacteria is well known, these drugs have been considered ineffective for the treatment of enteric infectious diseases caused by organisms such as serovar Typhi grown in mammalian cells. Recent clinical studies have shown excellent efficacy of cefixime and ceftriaxone against typhoid fever (3, 9), which can be considered a systemic infection rather than a local intestinal infection like shigellosis. Cefixime (21), the first oral extended-spectrum cephalosporin, has strong activity against serovar Typhi (MIC at which 90% of the strains are inhibited [MIC₉₀] of 0.25 μg/ml) (13), and its clinical usefulness has also been proven in several studies (3, 7–9, 14, 17, 18). This activity is comparable to those of ceftriaxone (3, 9) and the new quinolones (1, 2, 15, 20, 22). The mechanism of its therapeutic effectiveness was investigated in an *in vitro*

model using *Salmonella* serovar Typhimurium instead of serovar Typhi, which does not cause morbidity in mice.

MATERIALS AND METHODS

Bacterial strains. Clinically isolated *Salmonella* serovar Typhimurium FP39, LT2 (virulent strain), and FP1671 (TEM-1-type β-lactamase producer) were used. Orally virulent serovar Typhimurium C5 (10) was kindly provided by Takeshi Sasahara of Kitasato University (Sagamihara, Japan).

Human cells. Monocyte-derived THP-1 cells from Dainippon Pharmaceutical Co. were used.

Antibiotics. Cefixime (Fujisawa), amoxicillin (Fujisawa), ciprofloxacin (Bayer), and chloramphenicol (Sankyo), used in *in vivo* studies, and ceftriaxone (Roche) were commercially obtained. The cefixime, ceftiozime, amoxicillin, and ciprofloxacin used in *in vitro* studies were synthesized in our laboratories.

Susceptibility testing. MICs were determined with serial dilutions of antibiotics in Mueller-Hinton agar (Difco), with inoculum sizes of 10⁴ CFU per spot. The MIC was defined as the lowest concentration of antibiotic that inhibited visible growth after 18 h of incubation at 35°C.

Protective effect against mouse systemic infection caused by serovar Typhimurium. Male ICR mice (4 weeks old) were used. Serovar Typhimurium strains were cultured overnight on Trypticase soy agar (BBL) at 35°C and suspended in saline. A 0.2-ml portion containing approximately one minimum lethal dose (MLD) (determined on day 21 after infection) was inoculated intravenously into each mouse. Eight mice were allocated to each group. Antibiotics were administered orally twice a day at doses of 160, 40, 10, 2.5, and 0.625 mg/kg of body weight, from 1 day after infection for 3 days.

Protective effect against mouse oral infection caused by serovar Typhimurium C5. Male 4-week-old BALB/c mice were used. Orally virulent serovar Typhimurium C5 (4.4 × 10⁷ CFU in 0.2 ml of saline/mouse, 1 MLD) was inoculated orally. Antibiotics were administered orally twice a day for 5 days, from day 3 to day 7 after infection.

Viability of THP-1 cells after infection with serovar Typhimurium FP39. Antibacterial activity of cefixime for serovar Typhimurium inside THP-1 cells was evaluated by determining the viability of THP-1 cells at 6 h after infection with serovar Typhimurium FP39. The cells were colored using a LIVE/DEAD reduced biohazard viability/cytotoxicity kit from Molecular Probes. Results were analyzed by FACScan and LysysII (Becton Dickinson).

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TABLE 1. MICs of antibiotics for serovar Typhimurium strains

Serovar Typhimurium strain	β -Lactamase production	MIC ^a (μ g/ml)			
		Cefixime	Amoxicillin	Chloramphenicol	Ciprofloxacin
FP39	—	≤ 0.0078	0.5	4	≤ 0.0078
FP1671	+	0.125	>128	>128	0.016
LT2	—	0.063	0.5	4	0.016
C5	—	0.031	0.5	4	0.016

^a MICs were determined by agar dilution using Mueller-Hinton medium (Difco).

Morphological changes of serovar Typhimurium inside THP-1 cells caused by the presence of cefixime. The morphological changes of serovar Typhimurium FP39 in THP-1 cells were studied by light microscopy and transmission electron microscopy. Infected THP-1 cells were treated for 4 h with 0.5 μ g of cefixime/ml.

Bactericidal activity of cefixime for serovar Typhimurium inside THP-1 cells. To exclude the effect of bactericidal activity of cefixime for serovar Typhimurium outside THP-1 cells, the viable cell counts of serovar Typhimurium inside THP-1 cells were monitored. After 1 h of incubation of THP-1 cells with serovar Typhimurium, the cells were treated with 50 μ g of gentamicin/ml for 30 min to eliminate the bacteria outside of the cells and were washed three times with the same medium before the addition of cefixime. Sample cell solutions were washed once with the medium and lysed by suspension in the same volume of sterile distilled water, and viable bacteria were counted with a conventional plating method.

Penetration of antibiotics into THP-1 cells. The penetration of antibiotics into THP-1 cells was determined by means of a velocity gradient centrifugation technique (11, 12). Antibiotics were added to THP-1 cell suspensions at concentrations of 10 or 50 μ g/ml. The cell-antibiotic mixtures were incubated at 37°C in 5% CO₂ for 30 min. Concentrations of antibiotics were determined by bioassay. We also evaluated the effect of the coexistence of cefixime with serovar Typhimurium. THP-1 cells were incubated with serovar Typhimurium FP39 at a ratio of 1:5 for 1 h at 37°C in 5% CO₂, followed by incubation with cefixime for 30 min under the same conditions.

RESULTS

Antibacterial activity of cefixime against *Salmonella* serovar Typhimurium. Table 1 shows the MICs of antibiotics for the serovar Typhimurium strains used. The sensitivity of these strains to tested antibiotics was similar to the sensitivity of *Salmonella* serovar Typhi strains (13).

Therapeutic effectiveness of cefixime in mouse systemic infection caused by serovar Typhimurium. To confirm the clinical effectiveness of cefixime against typhoid fever, the in vivo efficacy of cefixime was evaluated in a mouse systemic infection model based on infections caused by serovar Typhimurium FP39, FP1671, and LT2 (Table 2). Against FP39, 3 days of cefixime demonstrated potent therapeutic activity, with a 50% effective dose (ED₅₀) of 1.48 mg/kg at 14 days after infection, while the ED₅₀s of the other three antibiotics were more than 10 times higher.

Cefixime also showed therapeutic activity against the β -lactamase-producing amoxicillin-resistant strain FP1671 of serovar Typhimurium, although the ED₅₀ was higher than that for FP39. However, dosages of 320 mg each of amoxicillin and chloramphenicol per kg per day had almost no effect on this strain. The ED₅₀ of ciprofloxacin was similar to that of cefixime for this strain, although its MIC was lower than that of cefixime.

Against serovar Typhimurium LT2 (virulent strain), cefixime showed potent therapeutic activity, with ED₅₀s of 1.48 mg/kg after 6 days and 32.6 mg/kg after 14 days. The strong in vitro activity of cefixime correlated well with its in vivo therapeutic

activity. On the other hand, the ED₅₀ of ciprofloxacin was higher than that of cefixime, even though the MIC was the same.

Therapeutic effectiveness of cefixime in mouse oral infection caused by serovar Typhimurium C5. A 4-mg/kg/day dosage of cefixime demonstrated potent therapeutic activity in this model, and the dosage figures for amoxicillin, chloramphenicol, and ciprofloxacin were 20, 100, and 20 mg/kg/day, respectively (Fig. 1).

Viability of THP-1 cells after infection with serovar Typhimurium FP39. The viability of THP-1 cells at 6 h after infection with serovar Typhimurium FP39 was reduced to 65%, in comparison with 98% in the negative control. A 1- μ g/ml concentration of cefixime increased the viability to 94%, and 10 μ g of cefixime/ml increased the viability to 97%.

Morphological changes of serovar Typhimurium inside THP-1 cells induced by the presence of cefixime. After 4 h of treatment with 0.5 μ g of cefixime/ml, obvious elongation of serovar Typhimurium FP39 inside THP-1 cells was observed, although the bacteria were not as long as those outside THP-1 cells (Fig. 2). Figure 3 shows micrographs detailing the morphological changes of serovar Typhimurium FP39 in THP-1 cells, as revealed by transmission electron microscopy. Apparent morphological changes were also observed inside bacterial cells. Although the concentration of cefixime inside THP-1 cells seemed to be lower than that outside the cells, it was able to cause real damage to serovar Typhimurium.

Bactericidal activity of cefixime for serovar Typhimurium inside THP-1 cells. Cefixime was able to inhibit the growth of serovar Typhimurium FP39 inhabiting THP-1 cells at 1 μ g/ml and was able to decrease the number of CFU at 10 μ g/ml. Cefixime was also active against the β -lactamase-producing amoxicillin-resistant strain FP1671. High concentrations of cefixime were able to effectively kill serovar Typhimurium inside THP-1 cells.

TABLE 2. Therapeutic effect of cefixime on a mouse systemic infection model with serovar Typhimurium^a

Strain no. (MLD) and antibiotic	ED ₅₀ (mg/kg) at day postinfection		
	6	10	14
FP39 (1.8×10^8 CFU/mouse)			
Cefixime	≤ 0.625	0.86	1.48
Amoxicillin	7.2	11.2	20
Chloramphenicol	40	56	130
Ciprofloxacin	11.2	27.9	32.6
FP1671 (2.8×10^7 CFU/mouse)			
Cefixime	5.00	12.5	20
Amoxicillin	>160	>160	>160
Chloramphenicol	35.7	>160	>160
Ciprofloxacin	8.38	33	35.7
LT2 (9.8×10^6 CFU/mouse)			
Cefixime	1.48	11	32.6
Amoxicillin	20	>160	>160
Chloramphenicol	36	130	≥ 160
Ciprofloxacin	6.97	36	67.6

^a Male 4-week-old ICR mice were infected intravenously with 1 MLD of serovar Typhimurium in 0.2 ml of saline per mouse and were treated orally with antibiotics at 160, 40, 10, 2.5, and 0.625 mg/kg twice a day for 3 days.

FIG. 1. Therapeutic effect of cefixime on a mouse oral infection model. Male 4-week-old BALB/c mice (10/group) were infected orally with 4.4×10^7 CFU of serovar Typhimurium C5 per mouse and were treated orally with cefixime twice a day for 5 days, beginning 3 days after infection.

Penetration of cefixime into mammalian cells. The intracellular/extracellular concentration ratios of cefixime inside THP-1 cells were 34% at 50 μg of cefixime/ml and 28% at 10 μg /ml (Table 3). The penetration of cefixime increased to 48 and 46%, respectively, when the cells were incubated with serovar Typhimurium. The penetration values for ceftizoxime, ceftriaxone, and amoxicillin were 29, 31, and 35%, respectively. Under the same conditions, the penetration of ciprofloxacin was 600%, which seems to indicate active uptake (5).

DISCUSSION

Therapy with newer quinolones and β -lactams has been introduced to cope with multidrug-resistant *Salmonella* serovar Typhi. This was quickly followed by the emergence of quinolo-

ne-resistant serovar Typhi (16, 23). Wistrom and Norrby recommended restricting quinolones to the early empirical treatment of severely ill and vulnerable patients, although they are highly effective in the treatment of bacterial enteritis caused by various organisms, because of the high risk of the rapid development of resistance (24). Additionally, quinolones are not recommended for treatment of children, who often urgently require effective antimicrobial therapy.

Some extended-spectrum β -lactamases inactivate newer cephalosporins, including cefixime. Currently, however, the incidence of extended-spectrum- β -lactamase or cephalosporinase-producing serovar Typhi is very low.

The anti-serovar Typhi activity of cefixime was reported at the Third Asia-Pacific Symposium on Typhoid Fever and Other Salmonellosis in Bali in 1997, and the findings have been

FIG. 2. Cefixime-induced morphological changes of serovar Typhimurium FP39 inside THP-1 cells. (A) Control; (B) infected THP-1 cells after treatment for 4 h with 0.5 μg of cefixime/ml.

FIG. 3. Cefixime-induced morphological changes of serovar Typhimurium FP39 inside THP-1 cells (transmission electron microscopy). (A) Control; (B) infected THP-1 cells after treatment for 4 h with 0.5 µg of cefixime/ml. →, serovar Typhimurium inside THP-1 cells.

published (13). More than 70% of the strains were inhibited at less than 0.031 µg of cefixime/ml, and the MIC₉₀ of cefixime for 73 strains of serovar Typhi, including 18 chloramphenicol-resistant strains, was 0.25 µg/ml. This activity is comparable to those of ceftriaxone and the new quinolones ofloxacin and ciprofloxacin. The MICs of cefixime for *Salmonella* serovar Typhimurium strains were similar to those for serovar Typhi strains.

β-Lactam antibiotics have been considered ineffective against organisms which grow inside mammalian cells, such as serovars Typhi and Typhimurium, despite their excellent *in vitro* activity, because their concentrations inside mammalian cells are much lower than outside (11, 12). However, the efficacy of newer cephalosporins, including oral cefixime for typhoid fever, has been proven in several clinical studies (3, 7–9, 14, 17, 18).

We used serovar Typhimurium in this study since this pathogen, unlike serovar Typhi, causes systemic infection in mice. In addition, the susceptibility of serovar Typhimurium to the tested

antibiotics is similar to that of serovar Typhi, providing a useful model for investigation.

It was presumed that cefixime can inhibit the growth of bacteria in mammalian cells when the bacteria are highly sensitive to cefixime, as are serovars Typhimurium and Typhi. How great a concentration of cefixime inside human cells could be induced? We detected a 28 to 34% concentration outside cells in the absence of serovar Typhimurium. However, the concentration increased to 46 to 48% when the cells were incubated with serovar Typhimurium. This penetration of cefixime into THP-1 cells was comparable to that of other β-lactams, such as ceftizoxime, ceftriaxone, and amoxicillin. On the other hand, ciprofloxacin, a quinolone, was actively taken up.

The intracellular concentration was estimated from levels in serum after oral administration of 8 mg of cefixime/kg (6), and the concentration of cefixime inside cells was 34%. The duration of time above a MIC₉₀ of cefixime for serovar Typhi, obtained from the estimated intracellular concentration, was

FIG. 4. Bactericidal activity of cefixime for serovar Typhimurium inside THP-1 cells. —, control; —○—, 1 µg/ml; —□—, 10 µg/ml; —△—, 100 µg/ml.

TABLE 3. Penetration of cefixime into mammalian cells and effect of presence of serovar Typhimurium

Antibiotic (concn [µg/ml])	Presence of serovar Typhimurium ^a	Ratio ^b (mean %)
Cefixime (50)	—	34
	+	48
Cefixime (10)	—	28
	+	46
Ceftizoxime (50)	—	29
Ceftriaxone (50)	—	31
Amoxicillin (50)	—	35
Ciprofloxacin (50)	—	600

^a THP-1 cells were incubated with serovar Typhimurium FP39 at a ratio of 1:5 for 1 h at 37°C under 5% CO₂, followed by incubation with antibiotic for 30 min under the same conditions.

^b Ratio of intracellular/extracellular concentration.

more than 12 h. Typhoid fever is a relatively severe infection, which warrants a higher than normal dosage to ensure clinical efficacy. Previous studies conducted with cefixime for typhoid fever have used a dosage of 10 to 20 mg/kg/day (3, 7–9, 14, 17, 18). One preliminary report showed good clinical efficacy with a dose of 10 mg/kg/day (4). A dose of 5 to 7.5 mg of cefixime per kg twice a day should result in a sufficient time of intracellular concentration above the MIC₉₀ for clinical efficacy against pediatric typhoid fever.

In conclusion, the effectiveness of cefixime against typhoid fever was presumed to come from its strong activity against serovar Typhi and its reasonable penetration into monocytes, which was increased to about 47% in the presence of serovar Typhimurium. This was proven by the growth inhibition of serovar Typhimurium inside THP-1 cells differentiated to macrophages, the morphological changes of serovar Typhimurium inside THP-1 cells, and the increasing viability of THP-1 cells infected with serovar Typhimurium. A long half-life that leads to a long time above the MIC inside mammalian cells also supports the clinical effectiveness of cefixime. Furthermore, cefixime had good therapeutic effect in a mouse model based on infections caused by serovar Typhimurium.

The discrepancy between the weaker therapeutic activity and strong in vitro activity and high penetration into cells of ciprofloxacin is not fully understood. Its pharmacokinetics and the effects of intestinal flora are possible explanations.

The findings of the present investigation support the therapeutic effectiveness of oral cefixime for treatment of typhoid fever.

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