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Bacteriological Comparison of the Activities of Ceftriaxone, a New Long-acting Cephalosporin, with Those of Other New Cephalosporins

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In vitro antibacterial activities of a new cephalosporin, ceftriaxone, were bacteriologically characterized in comparison with those of cefotaxime and ceftizoxime. Minimal inhibitory concentrations (MIC) of cefriaxone determined on 680 fresh clinical isolates in Japan showed extraordinarily high activity against all gram-negative bacteria. Especially notable was its high activity against *Proteeae* species and *Haemophilus influenzae*; in this respect it was greatly superior to both cefotaxime and ceftizoxime. It also showed high activity against *Pseudomonas aeruginosa* and some anaerobic pathogens. Against other strains, in general ceftriaxone exhibited activity comparable to those of 2 structurally related cephalosporins, except for *Klebsiella* sp. and *Pseudomonas maltophilia*, against which it showed lower activity.

Its activity is bactericidal and, in contrast to cefotaxime and ceftizoxime, its minimal bactericidal concentration (MBC) value was less than 3 times the MIC except for *Ps. aeruginosa*. Its mode of action was morphologically assessed. Ceftriaxone showed an unusually high stability to most bacterial β-lactamases except to so-called cefuroximases from *Bacteroides fragilis*, *Pseudomonas cepacia* and *Proteus vulgaris*. In addition, ceftriaxone was found to be a very potent inhibitor of cephaloridine hydrolysis by various β-lactamases.

Keywords——ceftriaxone; aminothiazolyl-oxyiminoacetamidocephalosporin; bacteriological property; β-lactamase inhibition; morphological effect

Introduction

Recent developmental efforts have led to a variety of semisynthetic cephalosporins with extraordinary potency and expanded antibacterial spectra. Notable among them is a group of aminothiazolyl-oxyimino acetamido cephalosporins (ATOICs: see Fig. 1) including cefotaxime,[1] ceftizoxime,[2] and cefmenoxime.[3] Ceftriaxone, synthesized by Reiner *et al.*,[4,5] also belongs to the ATOIC category, but is unique with respect to its extremely long plasma half-life in man[6] as compared with other ATOICs. This property was tentatively ascribed to the presence of an enolate anion at the dihydrotriazinone moiety in position 3 of the cephalosporin nucleus, which should also be responsible for differences of other *in vitro* bacteriological parameters from those of other ATOICs, if any.

Although its antibacterial properties were reported from several laboratories (ref. 4, 7—9) as well as ours (M. Arisawa *et al.*, 20th Interscience Congress on Antimicrobial Agents and Chemother., Sept. 21, 1980, in New Orleans, La, U.S.A.), very few workers made a close side-by-side comparison among ATOICs. Therefore, we have undertaken a comparative study on several key parameters using clinical strains freshly isolated in Japan to evaluate the *in vitro* efficacy of ceftriaxone. The results[10] are herewith communicated.

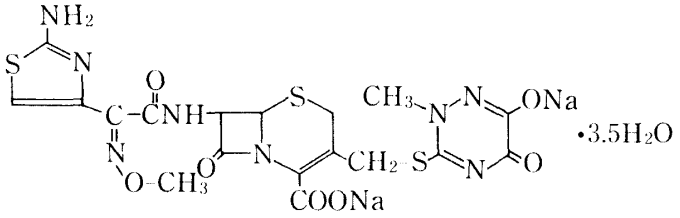
Experimental

Chemical——Ceftriaxone (Ro 13-9904), cefotaxime and ceftizoxime were synthesized and supplied for this study by F. Hoffmann-La Roche AG, Switzerland (Fig. 1).

Microorganisms——Clinical isolates (680 strains) were collected from various clinical materials in 18

(6*R*, 7*R*)-7-[2-(2-Amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-[[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-as-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid disodium salt (*Z* isomer)

ceftriaxone
(R_0 13-9904)



ceftizoxime(FK-749) $\diagdown$H

cefotaxime(HR-756) $\diagdown CH_2OCOCH_3$

Fig. 1

hospitals in the Kanto area of Japan from August 1978 to February 1979, and identified by the previously reported method.[11]

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal concentration (MBC) Determination——MIC was determined by the agar dilution method according to the Japanese standard procedure[12] using heart infusion (HI) agar; inoculation was done with a loopful of 10^6 cells (CFU)/ml throughout. *Pseudo-monas* species except *Ps. aeruginosa* were all cultured at 30°C. For the culture of anaerobes, GAM Bouillon (Nissui) medium for preculture and GAM Agar (Nissui) for the MIC determination[13] were employed in a Gas Pak system. The 24 h preculture (without shaking) was inoculated after appropriate dilution with 0.05% yeast extract and MIC was determined after a further 24 h incubation at 37°C.

For MBC determination, MIC was first determined by the broth dilution method (10^5 cells/ml) on a microtiter plate. Portions (50 μl) taken from the wells showing macroscopically no turbidity were inoculated on Tryptosoya agar plates. After 18 h incubation at 37°C, a well that gave less than 5 colonies per plate was judged as negative (99.9% killing) and the minimum concentration in a growth-negative well was taken as MBC.

Morphological Examination——Loopfuls of 10^6 cells/ml were inoculated onto HI agar plates containing 2-fold serially diluted series of ceftriaxone, cefotaxime or cefazolin. At 0, 1, 2, 4, 7 and 22 h at 37°C after the inoculation, cell morphology on the plates was directly observed by means of a microscope (Nikon, type S-ke) at a magnification of 400 under phase contrast. The effects of drugs on cell morphology were examined after photography. MIC was determined after 22 h incubation on the same plates as were used for morphology study.

***β*-Lactamase Preparations and Assays**——Partially purified β-lactamases were prepared by a published method[14] using cephaloridine as the substrate. A modification of O'Callaghan's spectrophotometric assay was used to follow the reaction.[15]

Results

Comparative Antibacterial Activities

The comparative inhibitory activities of ceftriaxone, ceftizoxime and cefotaxime against 26 species of aerobic and anaerobic bacteria isolated from various clinical specimens are shown in Table I. Ceftriaxone exhibited very low MICs against a wide variety of species except for several non-fermentative gram-negative bacilli other than *Pseudomonas aeruginosa*, *Acinetobacter anitratus*, and *Flavobacterium* sp. Against staphylococci, ceftriaxone had no advantage over cefazolin, like two other ATOICs.

TABLE I. *In Vitro* Antibacterial Activity of Ceftriaxone compared with Other Cephalosporins

Microorganism (No. of strains)	Compound	MIC (μg/ml) Range	50% inhibition	90% inhibition
Staphylococcus aureus (34)	Ceftriaxone	3.13—≧200	6.25	12.5
	Ceftizoxime	1.56—≧200	3.13	12.5
	Cefotaxime	0.78—≧200	3.13	6.25
	Cefazolin	0.2—≧200	0.78	1.56
Staphylococcus epidermidis (22)	Ceftriaxone	0.78—≧200	6.25	50
	Ceftizoxime	0.2—≧200	6.25	≧200
	Cefotaxime	0.39—≧200	3.13	25
	Cefazolin	0.1—≧200	0.78	6.25
Escherichia coli (47)	Ceftriaxone	0.025—0.78	0.05	0.2
	Ceftizoxime	≦0.006—12.5	0.05	0.39
	Cefotaxime	≦0.006—1.56	0.05	0.39
	Cefazolin	0.78—≧200	3.13	50
Klebsiella pneumoniae (56)	Ceftriaxone	0.025—0.2	0.1	0.2
	Ceftizoxime	≦0.006—0.1	0.025	0.05
	Cefotaxime	≦0.006—0.39	0.05	0.1
	Cefazolin	0.78—25	1.56	6.25
Citrobacter freundii (21)	Ceftriaxone	0.1—≧200	0.39	100
	Ceftizoxime	0.1—100	0.39	100
	Cefotaxime	0.1—100	0.39	50
	Cefazolin	12.5—≧200	≧200	≧200
Proteus mirabilis (40)	Ceftriaxone	≦0.006—0.025	≦0.006	0.012
	Ceftizoxime	≦0.006—0.025	0.012	0.012
	Cefotaxime	0.012—0.1	0.025	0.05
	Cefazolin	3.13—≧200	6.25	6.25
Proteus vulgaris (29)	Ceftriaxone	≦0.006—50	0.2	12.5
	Ceftizoxime	≦0.006—0.78	0.025	0.2
	Cefotaxime	0.012—12.5	0.39	6.25
	Cefazolin	≦200	≧200	≧200
Proteus morganii (40)	Ceftriaxone	0.006—6.25	0.025	0.39
	Ceftizoxime	0.012—100	0.39	6.25
	Cefotaxime	0.025—50	0.2	3.13
	Cefazolin	50—≧200	≧200	≧200
Proteus rettgeri (12)	Ceftriaxone	≦0.006—0.39	0.012	0.1
	Ceftizoxime	≦0.006—0.05	≦0.006	0.025
	Cefotaxime	≦0.006—0.39	0.012	0.2
	Cefazolin	3.13—≧200	12.5	≧200
Serratia marcescens (45)	Ceftriaxone	0.2—≧200	3.13	50
	Ceftizoxime	0.1—50	0.78	12.5
	Cefotaxime	0.2—≧200	3.13	50
	Cefazolin	100—≧200	≧200	≧200
Enterobacter cloacae (40)	Ceftriaxone	0.1—≧200	0.39	12.5
	Ceftizoxime	0.025—≧200	0.39	6.25
	Cefotaxime	0.1—≧200	0.39	12.5
	Cefazolin	1.56—≧200	≧200	≧200
Enterobacter aerogenes (17)	Ceftriaxone	0.05—50	0.2	12.5
	Ceftizoxime	0.012—50	0.05	12.5
	Cefotaxime	0.05—50	0.1	6.25
	Cefazolin	1.56—≧200	50	≧200
Haemophilus influenzae (16)	Ceftriaxone	≦0.006—0.05	≦0.006	0.012
	Ceftizoxime	≦0.006—0.2	0.012	0.025
	Cefotaxime	≦0.006—0.2	0.012	0.025
	Cefazolin	6.25—≧200	25	100
Pseudomonas aeruginosa (64)	Ceftriaxone	3.13—≧200	12.5	50
	Ceftizoxime	6.25—≧200	25	100
	Cefotaxime	3.13—≧200	12.5	100
	Gentamicin	0.39—≧200	1.56	100

Microorganism (No. of strains)	Compound	MIC (μg/ml) Range	50% inhibition	90% inhibition
Pseudomonas putida	Ceftriaxone	12.5—100	50	100
(9)	Ceftizoxime	12.5—100	50	100
	Cefotaxime	25—100	50	100
	Gentamicin	1.56—≧200	6.25	≧200
Pseudomonas maltophilia	Ceftriaxone	3.13—100	25	100
(9)	Ceftizoxime	6.25—≧200	12.5	100
	Cefotaxime	1.56—25	6.25	25
	Gentamicin	≧200	≧200	≧200
Pseudomonas cepacia	Ceftriaxone	6.25—50	25	50
(7)	Ceftizoxime	3.13—12.5	6.25	12.5
	Cefotaxime	6.25—25	12.5	25
	Gentamicin	6.25—≧200	100	≧200
Pseudomonas fluorescens	Ceftriaxone	50—≧200	100	≧200
(8)	Ceftizoxime	≧200	≧200	≧200
	Cefotaxime	50—≧200	100	≧200
	Gentamicin	0.39—12.5	0.78	12.5
Acinetobacter anitratus	Ceftriaxone	6.25—50	25	25
(28)	Ceftizoxime	3.13—25	6.25	12.5
	Cefotaxime	3.13—25	12.5	25
	Cefazolin	100—≧200	≧200	≧200
Acinetobacter lwoffi	Ceftriaxone	3.13—≧200	100	≧200
(10)	Ceftizoxime	0.39—≧200	50	≧200
	Cefotaxime	0.78—100	25	100
	Cefazolin	25—≧200	≧200	≧200
Bacteroides fragilis	Ceftriaxone	6.25—≧200	12.5	>100
(31)	Ceftizoxime	3.13—≧200	25	>100
	Cefotaxime	6.25—≧200	25	>100
	Cefoxitin	6.25—100	12.5	50
Bacteroides sp.	Ceftriaxone	0.39—≧200	12.5	50
(other than *B. fragilis*)	Ceftizoxime	0.39—≧200	12.5	50
(25)	Cefotaxime	0.39—100	6.25	50
	Cefoxitin	3.13—100	25	50
Peptococcus sp.	Ceftriaxone	0.1—6.25	0.39	6.25
(15)	Ceftizoxime	0.05—50	3.13	25
	Cefotaxime	0.05—6.25	0.39	6.25
	Cefoxitin	0.05—6.25	0.39	0.78
Peptostreptococcus sp.	Ceftriaxone	0.05—1.56	0.39	1.56
(8)	Ceftizoxime	0.05—0.78	0.2	0.78
	Cefotaxime	0.05—0.78	0.2	0.78
	Cefoxitin	0.2—12.5	0.78	12.5
Fusobacterium sp.	Ceftriaxone	0.39—>100	50	>100
(8)	Ceftizoxime	0.2—>100	>100	>100
	Cefotaxime	0.39—>100	100	>100
	Cefoxitin	6.25—>100	6.25	>100
Clostridium sp.	Ceftriaxone	0.1—25	0.39	25
(10)	Ceftizoxime	0.05—>100	1.56	25
	Cefotaxime	0.1—50	0.39	12.5
	Cefoxitin	0.39—25	3.13	6.25

The activity of ceftriaxone against *Enterobacteriaceae* was very high, and either MIC_{50} or MIC_{90} values of all the species tested were unusually low, the highest MIC_{50} value being 3.13 μg/ml on *Serratia marcescens*. Against *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteeae* other than *Proteus vulgaris*, ceftriaxone showed high activity; the MICs were particularly low and no strain was resistant to the antibiotic. Ceftriaxone was clearly superior to cefotaxime and ceftizoxime against *Proteus mirabilis* and *Proteus morganii*. The superiority of ceftriaxone to other ATOICs against *P. inconstans* was also noted (data not shown).

TABLE II. Effect of Medium, pH and Serum on the MIC of Ceftriaxone

Organism	MIC (μg/ml)[a] with given medium:											
	Heart infusion agar							BHI	MH	TSA	mMH	NA
	pH 7.4	pH 9	pH 8	pH 7	pH 6	pH 7.4 + 10% HB	pH 7.4 + 25% HB	pH 7.2	pH 7.4	pH 7.3	pH 7.4	pH 7.0
Staphylococcus aureus 209P JC-1	3.13	6.25	3.13	3.13	0.78	3.13	3.13	3.13	3.13	3.13	1.56	3.13
Escherichia coli NIHJ JC-2	0.1	0.05	0.05	0.1	0.1	0.1	0.05	0.2	0.1	0.1	0.1	0.1
Klebsiella pneumoniae ATCC 27736	0.05	0.025	0.05	0.05	0.05	0.05	0.05	0.025	0·025	0.025	≦0.012	0.025
Citrobacter freundii IFO 12681	0.78	1.56	0.39	0.2	0.2	0.78	0.2	0.2	0.2	0.1	0.2	0.2
Proteus vulgaris ATCC 6380	≦0.012	≦0.012	≦0.012	≦0.012	≦0.012	≦0.025	≦0.025	≦0.025	≦0.012	≦0.012	0.025	≦0.012
Proteus vulgaris ATCC 6898	≦0.012	≦0.012	≦0.012	≦0.012	≦0.012	≦0.012	≦0.012	≦0.012	≦0.025	≦0.012	0.025	≦0.012
Serratia marcescens IFO 12648	0.39	0.78	1.56	0.78	0.2	0.78	0.78	0.1	0.78	0.2	0.1	0.39
Enterobacter cloacae ATCC 13047	12.5	0.78	1.56	12.5	25	25	12.5	12.5	25	6.25	12.5	25
Pseudomonas aeruginosa IFO 12689	12.5	12.5	12.5	12.5	12.5	25	12.5	25	50	25	25	25
Pseudomonas aeruginosa ATCC 9721	3.13	3.13	3.13	3.13	6.25	3.13	3.13	6.25	3.13	6.25	6.25	6.25

a) Inoculum, 10^6 μg/ml

BHI: brain heart infusion agar "Eiken,"
MH; Mueller Hinton Medium "Eiken,"
TSA: trypto-soy Agar "Eiken",
mMH: modified Mueller Hinton medium "Eiken,"
NA: nutrient agar "Eiken."

Against *Haemophilus influenzae*, ceftriaxone was far more active than any other cephalosporin tested. Eighty-eight percent of the isolates was sensitive to 0.006 μg/ml or less of ceftriaxone, while the inhibitions at the same concentration of ceftizoxime and cefotaxime were 19 and 13%, respectively. The same situation was observed with *H. parainfluenzae* (4 strains; not shown).

The activity of ceftriaxone against *Ps. aeruginosa* was moderate; superior to that of ceftizoxime and equivalent to that of cefotaxime. More than half of the strains were inhibited at 12.5 μg/ml or less of ceftriaxone, while only 25% of strains were sensitive to the same concentration of ceftizoxime. Among non-fermentative species other than *Ps. aeruginosa*, *Acinetobacter anitratus* and *Flavobacterium* sp. (not shown) were the only species susceptible to ceftriaxone. The ATOICs tested here were in general hardly active against other non-fermentative gram-negative bacilli. Thus, *Pseudomonas putida* and *Pseudomonas fluorescens*, both sensitive to gentamicin, were totally resistant to ceftriaxone ($MIC_{50} \geqq 50$ μg/ml), while *Pseudomonas maltophilia* and *Pseudomonas cepacia* were only slightly sensitive to ceftriaxone. All ATOICs were also equally inactive against *Alcaligenes odorans* (2 strains) and *Achromobacter xylosoxidans* (3 strains), though the data are not shown.

As regards anaerobic isolates, ceftriaxone was moderately active against *Bacteroides fragilis*, being as effective as cefoxitin, but it was superior to ceftizoxime, in accord with previous data on type strains.[13] Naturally resistant strains (MIC>25 μg/ml) amounted to 48% with ceftriaxone and 94% with ceftizoxime. Ceftriaxone was also fairly active against *Peptococcus* sp., *Peptostreptococcus* sp., *Clostridium* sp., *Veillonella paravula* (not shown) and *Gaffkya anaerobia* (not shown). Among ATOICs, ceftriaxone was similar to cefotaxime, and superior to ceftizoxime in terms of activity against *Peptococcus* sp. Against *Fusobacterium* sp., ceftriaxone was practically inactive with 60% of strains being resistant to ceftriaxone; cefotaxime was equally ineffective, and ceftizoxime was even more so.

Effects of Media, pH and Serum on the Activity of Ceftriaxone

The effects of the medium, pH and the inoculum size for testing varied among the antibiotics. These factors were examined for ceftriaxone in comparison with other cephalosporins. Table II illustrates the effect of different media on MICs. No distinct difference due to the test medium was found. When the pH was changed on HI agar under conditions allowing cell growth in the controls, almost the same (at largest, a 2-fold difference) MIC values were obtained except for *Enterobacter*, for which higher pH resulted in greater susceptibility to the cephalosporins. The addition of horse blood did not cause inactivation.

The inoculum size in the test had a significant effect on the MICs of ceftriaxone (Table III). Little change in MIC was seen in the range of inoculum concentration from 10^4 to 10^7 cells/ml, while at 10^8 cells/ml, the MIC of ceftriaxone was 2- to 8-fold greater than at 10^6 cells/ml, except

Table III. Effect of the Inoculum Size on the MIC of Ceftriaxone

Strain	MIC (μg/ml) in HI agar at inoculum size (cells/ml):				
	10^4	10^5	10^6	10^7	10^8
Staphylococcus aureus 209P JC-1	1.56	3.13	3.13	3.13	6.25
Escherichia coli NIHJ JC-2	0.1	0.1	0.1	0.1	0.1
Klebsiella pneumoniae ATCC27736	0.025	0.025	0.05	0.05	0.39
Citrobacter freundii IFO12681	0.2	0.2	0.78	1.56	3.13
Proteus vulgaris ATCC6380	≦0.012	≦0.012	≦0.012	0.025	25
Proteus vulgaris ATCC6898	≦0.012	≦0.012	≦0.012	≦0.012	12.5
Serratia marcescens IFO12648	0.2	0.2	0.39	0.78	50
Enterobacter cloacae ATCC13047	3.13	6.25	12.5	50	50
Pseudomonas aeruginosa IFO12689	12.5	25	12.5	50	>100
Pseudomonas aeruginosa ATCC9721	3.13	3.13	3.13	6.25	12.5

with *Proteus* and *Serratia*. MICs of ceftriaxone against *Proteus*, which is quite sensitive at 10^6 cells/ml inoculation, showed a large increase, by a factor of 1024—2048. It should be noted, however, that even at this MIC, ceftriaxone is the most potent anti-*Proteus* agent so far reported.

Minimal Bactericidal Concentration (MBC)

When MBC was determined with randomly selected strains of *E. coli*, *K. pneumoniae*, *Citrobacter freundii*, *P. mirabilis*, *P. morganii*, *S. marcescens*, *Enterobacter cloacae*, and *Ps. aeruginosa*, it was found that ceftriaxone gave an MBC value identical to or at most two-fold higher than its MIC with most of the test strains except *Ps. aeruginosa*, suggesting a potent bactericidal action (Table IV). With *Ps. aeruginosa*, the majority (50%) of the isolates tested exhibited 8-fold greater value of MBC than MIC. When compared with ceftizoxime and cefotaxime, a stronger bactericidal activity of ceftriaxone than ceftizoxime was evident against *P. morganii*, *P. mirabilis*, and *S. marcescens*, while the reverse was seen with *Ps. aeruginosa*.

Table IV. Relation of MBC to MIC

Organism (No. of strains)	Antibiotic	MBC/MIC (fold) 1	2	4	8	≧16	Mean
Escherichia coli (8)	Ceftriaxone	5	3				1.38
	Ceftizoxime	6	2				1.25
	Cefotaxime	8					1.00
	Cefazolin	7	1				1.13
Klebsiella pneumoniae (8)	Ceftriaxone	7	1				1.13
	Ceftizoxime	5	2		1		2.13
	Cefotaxime	6	2				1.25
	Cefazolin	6		1	1		2.25
Citrobacter freundii (6)	Ceftriaxone	2	3	1			2.00
	Ceftizoxime	4	1			1	6.33
	Cefotaxime	4	2				1.33
Proteus mirabilis (8)	Ceftriaxone	3	2	2	1		2.88
	Ceftizoxime	3		2	1	2	8.38
	Cefotaxime	1	4			3	13.13
	Cefazolin	1	4	3			2.71
Proteus morganii (5)	Ceftriaxone	5					1.00
	Ceftizoxime	2	2	1			2.00
	Cefotaxime	4	1				1.20
Serratia marcescens (8)	Ceftriaxone	7	1				1.13
	Ceftizoxime	3	2	2	1		2.88
	Cefotaxime	3	4		1		2.38
Enterobacter cloacae (8)	Ceftriaxone	2	4	1	1		2.75
	Ceftizoxime	6		2			1.75
	Cefotaxime	1	3	2	2		3.8
Pseudomonas aeruginosa (8)	Ceftriaxone		3	1	4		5.25
	Ceftizoxime	2	4		2		3.25
	Cefotaxime	2	5		1		2.50

Morphological Effect of Ceftriaxone on Selected Species

Figure 2 shows the morphological change after 4 h incubation at 37°C as a function of antibiotic concentration. With three strains sensitive to cefazoline, ceftriaxone had a different morphological effect from that of cefazolin: The cell shape became filamentous over a wide range of concentration, with little induction of spheroplasts. On the other hand, cefazolin formed shorter filaments over a much narrower concentration range, above which spheroplasting and cell lysis ensued. Cefotaxime was found to cause a very similar effect to ceftriaxone. Thus, both ATOICs began forming filaments from *E. coli* NIHJ, for example, at 0.025—3.13

μg/ml, while spheroplasts were scarcely observed. In contrast, cefazolin induced filamentous form only at a concentration from 0.78 to 1.56 μg/ml, followed by rapid lysis so that the filamentous form was detected as a minor morphological population only in a much narrower concentration range with cefazolin-treated cells. The difference was more clearly demonstrated on two other cefazolin-sensitive strains, *Klebsiella pneumoniae* 1R535 and *Proteus mirabilis* IV31. On the other hand, with five strains intrinsically resistant to cefazolin, ceftriaxone and cefotaxime showed the same features as with cefazolin-sensitive strains regardless of the presence or absence of β-lactamase able to hydrolyze ceftriaxone, as revealed in *P. vulgaris* 5D63-1 which produces a β-lactamase active on ceftriaxone, and in *P. vulgaris* strain 1X113 having a β-lactamase unable to hydrolyze ceftriaxone. In general, ceftriaxone resembled cefotaxime in terms of induced morphological change in spite of the lower MICs of ceftriaxone than of cefotaxime on *Ps. aeruginosa* 6F120-1 and *P. morganii* 1AB669.

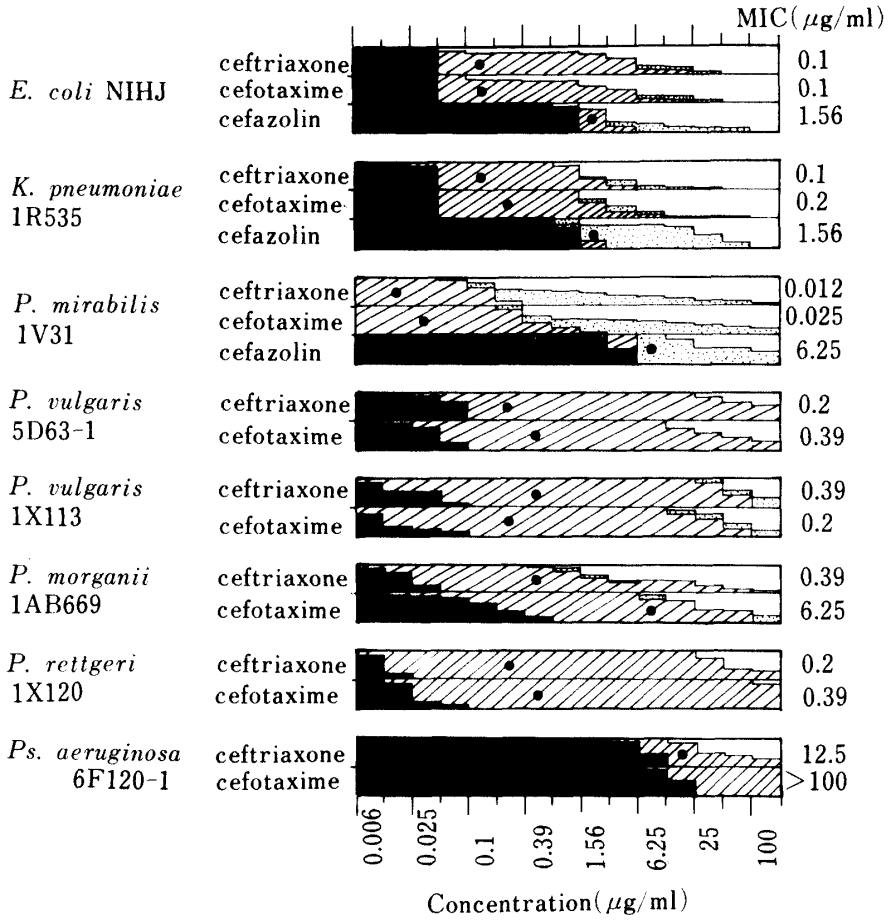


Fig. 2. Morphological Change as a Function of Antibiotic Concentration

Cell morphology was examined by taking photographs directly on theplates at 4 h after inoculation. The height of the bar indicates the relative population in the field (100% at 0 h). Solid circles indicate MIC. Symbols; solid bars, rod shape; striped bars, filamentous shape; dotted bars, spheroplasts.

When the relationship of individual MIC to changes in 4-h morphology was examined, a difference between ATOICs and cefazolin was again noticed (Fig. 2). Thus, the MICs of cefazolin coincided with the concentration at which rod cells disappeared but spheroplasts and filaments appeared, whereas concentrations of ATOICs required to make rod shapes disappear and to lyse the majority of cells were 4—16-fold lower and 2—512-fold higher than their MICs, respectively. In fact, the MIC of cefotaxime on *P. rettgeri* 1X120 was low (0.39

TABLE V. Comparative Stability to and Inhibitory Activity against Various β-Lactamases

Enzyme source	Type	Antibiotic	Relative rate	K_i (μM)
Penicillinase from				
Escherichia coli W3630	Type I	Ceftriaxone	<1[a]	108
		Cefotaxime	<1	—
Escherichia coli W3630	Type II	Ceftriaxone	<1	2.5
		Cefotaxime	<1	—
Escherichia coli W3630	Type III	Ceftriaxone	<1	13.8
		Cefotaxime	<1	—
Cephalosporinase from				
Escherichia coli GN5482		Ceftriaxone	<1[b]	0.09
		Cefotaxime	<1	0.13
Citrobacter freundii GN7391		Ceftriaxone	<1	0.05
		Cefotaxime	<1	—
Enterobacter cloacae GN7471		Ceftriaxone	<1	0.06
		Cefotaxime	<1	0.05
Serratia marcescens GN10857		Ceftriaxone	<1	2.2
		Cefotaxime	<1	3.4
Proteus morganii GN5407		Ceftriaxone	<1	0.18
		Cefotaxime	<1	0.07
Proteus rettgeri GN4430		Ceftriaxone	<1	145
		Cefotaxime	<1	2.1
Pseudomonas aeruginosa GN10362		Ceftriaxone	<1	0.10
		Cefotaxime	<1	0.27
Cefuroximase from				
Proteus vulgaris GN7919		Ceftriaxone	112[c]	111[d]
		Cefotaxime	84	—
		Ceftizoxime	39	135
Pseudomonas cepacia GN11164		Ceftriaxone	171	105
		Cefotaxime	174	250
		Ceftizoxime	98	135
Bacteroides fragilis GN11147		Ceftriaxone	8	48
		Cefotaxime	7	77
		Ceftizoxime	3	19

a) Relative hydrolysis rate with penicillin G=100.
b), *c*) Relative hydrolysis rate with cephaloridine=100.
d) K_m(μM).

μg/ml), but cell lysis was not induced even at 100 μg/ml within 4 h at 37°C. The situation was also clearly demonstrated in the lytic pattern analysis (not shown). This result seemed to be inconsistent with the bactericidal activity demonstrated by MBC study (Table IV). However, when the morphology of *E. coli* NIHJ was examined after 22 h incubation, which is usually used for MIC determination, MICs of ATOICs as well as cefazolin corresponded exactly with the concentration at which no cell was detected microscopically at all. This fact, in the light of results in the previous section, indicates that ATOICs at attainable concentrations in serum[6] easily induce the filament form, which has a prolonged lifetime. Thus, cell lysis occurs at the latest within 22 h, confirming that the drug action is ultimately bactericidal.

Stability to the Inhibitory Activity on β-Lactamases of Ceftriaxone

As shown in Table V, all cephalosporins tested here were in general very stable to various cephalosporinases (CSases) and penicillinases (PCases) derived from *E. coli*, *C. freundii*, *S. marcescens*, *E. cloacae*, *P. morganii*, *P. rettgeri*, and *Ps. aeruginosa*, as evidenced by an average hydrolysis rate more than 50 times lower than that of cephaloridine. However, ceftriaxone was hydrolyzed by what we call cefuroximases (CXases)[16] from *P. vulgaris* and *Ps. cepacia* at the same rate as and more rapidly than cephaloridine, respectively, and to a similar extent to cefotaxime. To *B. fragilis* CXase which is a well known contributor to resistance to β-lactam

antibiotics,[17] ceftriaxone was moderately stable, like cefotaxime and ceftizoxime. These facts are also reflected in their K_m values.

Ceftriaxone was found to possess inhibitory activity on the hydrolysis of either penicillin G or cephaloridine by these enzymes except for the CXases. Table V (right column) shows K_i values of ceftriaxone, which are comparable to those of cefotaxime in general. The mode of inhibition was mostly competitive, but some CSases from *Citrobacter freundii* were non-competitively inhibited only by ceftriaxone, as will be described in detail elsewhere.

Discussion

Extraordinarily broad and highly potent *in vitro* activity of ceftriaxone was confirmed with clinical isolates in Japan. The susceptible genera/species to ceftriaxone can be summarized as follows, together with the results of another independent evaluation.[13] In view of the high efficacy on *Neisseria* as reported by Angehrn *et al.*,[4] *N. gonorrhoea* should also be placed in the first group.

Highly Susceptible Species ($MIC_{50}<1$ μg/ml): *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Citrobacter* sp., *Proteus* sp. (including *P. inconstans*), *Salmonella* sp., *Shigella* sp., *Enterobacter cloacae*, *Enterobacter aerogrenes*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Bacteroides praeacutus*, *Propionibacterium acnes*, *Veillonella parvula*, *Gaffkya anaerobia*, *Peptococcus* sp., *Peptostreptococcus* sp., *Clostridium befermentans*, *Bifidobacterium adolescens*.

Fairly Susceptible Species ($1 \text{ μg/ml} \leqq MIC_{50} < 10$ μg/ml): *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Alcaligenes faecalis*, *Bacteroides distasonis*, *Bacteroides vulgatus*.

Less Susceptible Species ($10 \text{ μg/ml} \leqq MIC_{50} < 25$ μg/ml): *Pseudomonas aeruginosa*, *Pseudomonas maltophilia*, *Pseudomonas cepacia*, *Acinetobacter anitratus*, *Flavobacterium* sp., *Bacteroides fragilis*, *Bacteroides ovatus*, *Clostridium perfringens*

Practically Insensitive Species (more than 50% of Population having $MIC>25$ μg/ml): *Streptococcus faecalis*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Acinetobacter lwoffi*, *Achromobacter xylosoxidans*, *Alcaligenes odorans*, *Bacteroides thetaiotaomicron*, *Eubacterium lentum*, *Fusobacterium* sp.

When compared with other ATOICs, ceftriaxone showed higher activity against all *Proteeae* species (except *P. vulgaris*), *H. influenzae*, *H. parainfluenzae*, *Ps. aeruginosa* and several anaerobic species. These findings in general confirm that was separately reported from various laboratories,[4,7-9] is also valid with Japanese isolates, suggesting that the sensitivity of the above species are greatly affected by the substitution at position 3 of the cephalosporin nucleus. Against other species, the activity of ceftriaxone was comparable to those of two other ATOICs, cefotaxime and ceftizoxime, including its generally lower efficacy on gram-positive strains and non-fermenter species other than *Ps. aeruginosa*.

Side-by-side comparison of other bacteriological parameters indicates that ceftriaxone is more or less comparable to cefotaxime and ceftizoxime in the effect of media, pH and inoculum size on MIC values, morphological effects and resistance to PCases and CSases, but is superior in bactericidal effect as judged by the ratio of MBC to MIC (Table IV). The susceptibility of ceftriaxone to CXases was comparable to that of cefotaxime but higher than that of ceftizoxime (Table V).

Morphological observation with time at various concentrations revealed that ceftriaxone, like cefotaxime, could easily induce filamentous cells, but it took a fairly long time for the agent to bring about cell lysis in comparison with cefazolin on a MIC basis. This common feature of ATOICs indicates that a sufficiently long contact of ATOICs with cells is prerequisite for them to exert *in vivo* the potent efficacy expected from their extraordinary *in vitro* activities. It is therefore expected that ceftriaxone, in view of its very long serum half-life (6—8 h in

man),[16] might show potent antibacterial activity *in vivo*. All in all, the variation in position 3 substitution among the 3 ATOICs tested here did cause a distinct difference in the antibacterial spectrum, but their fundamental antibacterial nature remained essentially the same. Thus, the clinical efficacy would be expected to be highly dependent on the pharmacokinetic behavior of these drugs in man.

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