RU 29 246, THE ACTIVE COMPOUND OF THE CEPHALOSPORIN-PRODRUG-ESTER HR 916

I. ANTIBACTERIAL ACTIVITY *IN VITRO*

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The aminothiazolyl-cephalosporin RU 29 246 is the active metabolite of the prodrug-pivaloyl-oxyethyl-ester HR 916. RU 29 246 *in vitro* activity includes a wide range of clinically relevant bacterial pathogens. Against methicillin-sensitive Staphylococci RU 29 246 (MIC_{90} of 0.25~2 μg/ml) was clearly more active than cefaclor, cefuroxime, cefpodoxime, cefixime and ceftibuten, but slightly less active than cefdinir. RU 29 246 inhibited hemolytic Streptococci of the serogroups A, B, C and G as well as penicillin-sensitive *Streptococcus pneumoniae* at concentrations similar to cefdinir, cefpodoxime and cefuroxime ($MIC_{90} \leq 0.13$ μg/ml), but less than the other oral cephalosporins investigated (cefixime, cefaclor and ceftibuten). MIC_{90}s of RU 29 246 against *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Salmonella* spp., *Shigella* spp., *Proteus mirabilis* and *Haemophilus influenzae* were ≤0.5 μg/ml. Only RU 29 246 and cefdinir demonstrated moderate activity against *Acinetobacter baumannii* ($MIC_{90} \geq 4$ μg/ml). Most strains of *Pseudomonas* spp., *Serratia marcescens*, *Enterobacter* spp., *Hafnia alvei* and *Bacteroides* spp. were resistant to RU 29 246.

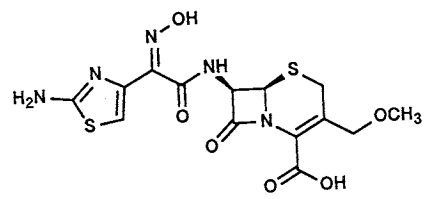
RU 29 246 killed *Escherichia coli* and *Staphylococcus aureus* at a rate of 99% to 99.9% at concentrations of two times MIC.

The pH value of the medium (range 5.5 to 8.5) and the inoculum size (range 10^5 to 10^7 cfu/ml) had no or only low influence on the antibacterial activity of RU 29 246.

RU 29 246 is a broad spectrum cephalosporin including in its activity both Gram-positive and Gram-negative pathogens and therefore—depending on the bioavailability of its prodrug—looks promising as to its therapeutic perspective.

RU 29 246 (7-β-[[2-(2-aminothiazol-4-yl)-2-*syn*-oximino]acetamido]ceph-3-em-3-methoxymethyl-4-carboxylic acid, Fig. 1) is a new cephem-antibiotic which has been esterified as the pivaloyl-oxyethyl-ester HR 916. RU 29 246 is characterized by a methoxymethyl-group at the 3-position of the cephem nucleus. Due to its low absorption in the gastrointestinal tract the compound is—like most of the structurally related cephalosporins of the third generation—not suitable for oral administration. Esterification of the carboxyl-group, a prodrug approach successful previously with penicillins and other cephalosporins, increases lipophilicity which is one of the factors involved in the absorption of drugs in the gastrointestinal tract[1~7].

Fig. 1. Chemical structure of RU 29 246.



In this study, the *in vitro* activity of RU 29 246

and representative other oral cephalosporins such as cefdinir (FK482, Fujisawa), cefpodoxime (CS-807, Sankyo), cefixime (FK027, Fujisawa), ceftibuten (7432S, Shionogi), cefuroxime (Glaxo) and cefaclor (Lilly) were compared by their MICs. In addition, the bactericidal activity of RU 29 246 was evaluated against representative strains. Furthermore, the influence of inoculum size and pH value of the medium on the MICs were investigated.

Materials and Methods

Strains

Test Strains: The MICs of 1,220 isolates of 84 species were determined. The isolates were cultured from specimens of blood, sputum and urine, and from swabs of wound sites, ears, noses and throats. Only one isolate was accepted from each patient.

Bactericidal kinetics, influence of inoculum size and pH value were determined with strains from laboratory stocks (Hoechst AG, Frankfurt/M., Germany): *Staphylococcus aureus* SG 511 and 503; *Escherichia coli* 1507 E, TEM-1, TEM-2, OXA-1, OXA-2, OXA-3, SHV-1; *Klebsiella pneumoniae* 1522 E and 1082 E; *Enterobacter cloacae* 1321 E; *Citrobacter diversus* 2046 E; *Salmonella typhimurium* MZ II.

Antibiotics

Amino-thiazol-cephalosporins: RU 29 246 (Hoechst-Roussel), cefpodoxime (Sankyo), cefixime (Fujisawa), cefdinir (Fujisawa), ceftibuten (Shionogi); glycyl-cephalosporin: cefaclor (Lilly); furanyl-methoximino-cephalosporin: cefuroxime (Glaxo).

RU 29 246 was provided by Hoechst AG. The other compounds were either commercially available or obtained from the manufacturer.

Susceptibility Testing

All isolates were tested by an agar dilution technique using Mueller-Hinton agar (Difco) or other acceptable media. For fastidious microorganisms, appropriate supplements were added to the Mueller-Hinton base. For *Gardnerella vaginalis*, Casman-Medium supplemented with 5% human blood was used. For anaerobes, Wilkins-Chalgren medium was supplemented with 5% sheep blood. An inoculum of 10^4 cfu/spot was delivered by a multipoint-inoculator to agar plates which contained 2-fold antibiotic dilutions. Most plates were incubated for 16 hours in ambient air at 35°C. Those containing methicillin-resistant Staphylococci were incubated for a 24-hour period at 32°C and supplemented by 2% NaCl. After incubation the MIC of each antibiotic was determined. The MIC was defined as the lowest concentration of antibiotic at which no visible growth or growth of ≤ 3 colonies was observed. *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains.

Influence of the Inoculum Size

The influence of the inoculum size on the activity of RU 29 246 was determined using a serial dilution test in Mueller-Hinton broth (Difco). Three simultaneously prepared geometric dilution series of the compound were inoculated with different suspensions of the test organisms. The initial numbers of viable bacteria were approximately 10^5, 10^6 and 10^7 cfu/ml. *Staphylococcus aureus* SG 511, *Escherichia coli* 1507 E, *Enterobacter cloacae* 1321 E and *Klebsiella pneumoniae* 1522 E served as representative pathogens. The effect of inoculum size was also investigated with 7 strains producing a plasmid-coded β-lactamase (*Staphylococcus aureus* 503, *Escherichia coli* TEM-1, TEM-2, OXA-1, OXA-2, OXA-3, SHV-1) and 2 strains producing a chromosomally coded β-lactamase (*Klebsiella pneumoniae* 1082 E, *Citrobacter diversus* 2046 E).

Influence of the pH Value of the Culture Medium

The effect of the pH value on the activity of RU 29 246 was determined in Brain Heart Infusion medium (Difco), which had been adjusted to different pH values between 5.5 and 8.5. The MICs of RU 29 246 for the test organisms *Staphylococcus aureus* SG 511, *Staphylococcus aureus* 503, *Escherichia coli*

1507 E and *Salmonella typhimurium* MZ II were determined by the serial dilution method.

Killing Curves

Mueller-Hinton broth was inoculated with approximately 10^5 cfu/ml of the test strains *Staphylococcus aureus* SG 511, *Staphylococcus aureus* 503, *Escherichia coli* 1507 E and *Escherichia coli* TEM-1 and then incubated with vigorous shaking at 35°C. After 2 hours, RU 29 246 was added to the cultures in concentrations corresponding to half the MIC, the MIC, two times the MIC and four times the MIC of the tested organism. One additional culture not exposed to RU 29 246 served as control. At intervals of 1, 2, 3, 4, 6 and 8 hours after inoculation, an aliquot was taken from each culture and, after adequate dilution, applied to the surface of Mueller-Hinton agar plates for viable bacterial count. After 18 hours of incubation at 35°C the colonies on these plates were counted and the numbers of cfu/ml in the original samples were calculated.

Results

Influence of the Inoculum Size on the MICs

Table 1 lists the MICs of RU 29 246 as determined for different sizes of inocula of various test organisms. With strains producing only low amounts of β-lactamase, there is no significant difference between the MICs for the various sizes of inoculum. The same can be said for the majority of the strains producing plasmid-coded β-lactamases, like *Staphylococcus aureus* 503, *Escherichia coli* SHV-1 and the *Escherichia coli*-strains producing OXA-enzymes. An increase in MIC due to higher inoculum size can be seen with *Escherichia coli* producing TEM-1 and TEM-2. The two strains producing a chromosomally mediated β-lactamase show high MICs of RU 29 246 (64 μg/ml) at an inoculum sizes of 10^5 cfu/ml, which increases to ≥256 μg/ml at an inoculum sizes of 10^7 cfu/ml.

Effect of the Initial pH of the Culture Medium on the MICs

The antibacterial activity of RU 29 246 against the Gram-negative test organisms *Escherichia coli* 1507 E and *Salmonella typhimurium* MZ II does vary by only one $\log_2$ within a pH-range of 5.5 to 8.5 of test medium (Table 2). The two *Staphylococcus aureus*-strains, however, show a slight decrease in sensitivity towards RU 29 246 with rising pH (three $\log_2$).

Comparative *In Vitro* Activities of RU 29 246 and Six Other Oral Cephalosporins

The activity of the oral cephalosporins against eight species of methicillin-susceptible Staphylococci (Table 3) was high for cefdinir (MIC_{90} 0.06~1 μg/ml), RU 29 246 (MIC_{90} 0.25~2 μg/ml), intermediate for cefuroxime (MIC_{90} 0.5~4 μg/ml), cefpodoxime (MIC_{90} 1~8 μg/ml), cefaclor (MIC_{90} 2~8 μg/ml), and therapeutically irrelevant for cefixime (MIC_{90} 4~>64 μg/ml) and ceftibuten (MIC_{90} 32~>64 μg/ml).

All oral cephalosporins included were inactive against methicillin-resistant Staphylococci and against Enterococci.

Cefuroxime, RU 29 246 and cefpodoxime had

Table 1. Dependence of the antibacterial activity of RU 29 246 on inoculum size.

Organism	MIC (μg/ml)		
	10^5 [a]	10^6	10^7
Staphylococcus aureus SG 511	0.25	0.25	0.25
Escherichia coli 1507 E	0.25	0.25	0.5
Enterobacter cloacae 1321 E	0.13	0.13	0.25
Klebsiella pneumoniae 1522 E	0.13	0.13	0.25
S. aureus 503	0.13	0.25	0.25
E. coli TEM-1	0.5	1	2
E. coli TEM-2	1	2	8
E. coli OXA-1	0.5	1	1
E. coli OXA-2	0.5	0.5	1
E. coli OXA-3	0.5	0.5	1
E. coli SHV-1	0.5	0.5	0.5
K. pneumoniae 1082 E	64	128	≥256
Citrobacter diversus 2046 E	64	128	≥256

[a] Inoculum size in colony forming units per ml.

Table 2. Antibacterial activity of RU 29 246 at various initial pH values of the culture medium.

Organism	MIC (μg/ml)						
	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0	pH 8.5
Staphylococcus aureus SG 511	0.16	0.16	0.31	0.63	0.63	1.25	0.63
S. aureus 503	0.16	0.31	0.31	0.63	1.25	0.63	1.25
Escherichia coli 1507 E	0.31	0.31	0.63	0.31	0.63	0.63	0.63
Salmonella typhimurium MZ II	0.63	0.63	0.63	0.63	0.63	0.31	0.31

Table 3. Comparative *in vitro* activity of RU 29 246 and six other oral cephalosporins.

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus MET-S (40)	RU 29 246	0.5 ~ 1	1	1
	Cefdinir	0.13 ~ 1	0.5	0.5
	Cefpodoxime	2 ~ 8	4	4
	Cefixime	8 ~ 64	32	32
	Ceftibuten	32 ~ >64	>64	>64
	Cefuroxime	1 ~ 4	2	2
	Cefaclor	2 ~ 16	2	8
S. aureus MET-R (20)	RU 29 246	8 ~ >64	>64	>64
	Cefdinir	4 ~ >64	>64	>64
	Cefpodoxime	16 ~ >64,	>64	>64
	Cefixime	>64	>64	>64
	Ceftibuten	>64	>64	>64
	Cefuroxime	8 ~ >64	>64	>64
	Cefaclor	>64	>64	>64
S. epidermidis MET-S (22)	RU 29 246	0.13 ~ 1	0.25	0.25
	Cefdinir	0.03 ~ 0.25	0.06	0.13
	Cefpodoxime	0.5 ~ 4	1	2
	Cefixime	1 ~ 16	2	4
	Ceftibuten	16 ~ >64	16	32
	Cefuroxime	0.25 ~ 2	0.5	1
	Cefaclor	1 ~ 8	1	2
S. epidermidis MET-R[a] (20)	RU 29 246	0.5 ~ 32	2	16
	Cefdinir	32 ~ >64	>64	>64
	Cefpodoxime	4 ~ >64	>64	>64
	Cefixime	32 ~ >64	64	>64
	Ceftibuten	>64	>64	>64
	Cefuroxime	0.5 ~ >64	4	>64
	Cefaclor	8 ~ >64	16	64
S. haemolyticus MET-S (19)	RU 29 246	0.25 ~ 8	0.5	2
	Cefdinir	0.03 ~ 1	0.13	0.5
	Cefpodoxime	1 ~ 4	2	4
	Cefixime	2 ~ 32	16	32
	Ceftibuten	4 ~ >64	64	>64
	Cefuroxime	0.5 ~ 2	1	2
	Cefaclor	0.25 ~ 4	1	2
S. haemolyticus MET-R (5)	RU 29 246	4 ~ >64	>64	>64
	Cefdinir	16 ~ >64	>64	>64
	Cefpodoxime	>64	>64	>64
	Cefixime	>64	>64	>64
	Ceftibuten	>64	>64	>64
	Cefuroxime	>64	>64	>64
	Cefaclor	64 ~ >64	>64	>64
S. simulans (14)	RU 29 246	0.13 ~ 0.5	0.25	0.5
	Cefdinir	0.03 ~ 0.13	0.06	0.13
	Cefpodoxime	0.5 ~ 2	1	2
	Cefixime	4 ~ 16	8	16
	Ceftibuten	8 ~ >64	64	>64
	Cefuroxime	0.13 ~ 1	0.5	1
	Cefaclor	0.5 ~ 2	1	2

Table 3. (Continued)

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
S. hominis (12)	RU 29 246	0.25 ~ 0.5	0.25	0.5
	Cefdinir	0.03 ~ 0.13	0.06	0.13
	Cefpodoxime	0.5 ~ 2	1	2
	Cefixime	2 ~ 8	4	8
	Ceftibuten	8 ~ >64	8	>64
	Cefuroxime	0.25 ~ 1	0.25	1
	Cefaclor	0.5 ~ 4	0.5	4
S. cohnii (10)	RU 29 246	0.5 ~ 2	2	2
	Cefdinir	0.25 ~ 1	0.25	1
	Cefpodoxime	2 ~ 8	8	8
	Cefixime	8 ~ >64	64	>64
	Ceftibuten	>64	>64	>64
	Cefuroxime	0.5 ~ 4	2	4
	Cefaclor	0.25 ~ 8	4	8
S. saprophyticus (9)	RU 29 246	0.5 ~ 1	1	1
	Cefdinir	0.13 ~ 0.25	0.25	0.25
	Cefpodoxime	2 ~ 8	4	8
	Cefixime	16 ~ 64	32	64
	Ceftibuten	64 ~ >64	>64	>64
	Cefuroxime	1 ~ 4	2	4
	Cefaclor	0.25 ~ 2	1	2
S. warneri (4)	RU 29 246	0.13 ~ 0.25	0.13	0.25
	Cefdinir	0.03 ~ 0.06	0.06	0.06
	Cefpodoxime	0.5 ~ 1	1	1
	Cefixime	2 ~ 8	2	8
	Ceftibuten	8 ~ 32	16	32
	Cefuroxime	0.25 ~ 0.5	0.25	0.5
	Cefaclor	1 ~ 4	2	4
Enterococcus faecalis (20)	RU 29 246	>64	32	64
	Cefdinir	4 ~ 32	8	16
	Cefpodoxime	>64	>64	>64
	Cefixime	>64	>64	>64
	Ceftibuten	>64	>64	>64
	Cefuroxime	>64	>64	>64
	Cefaclor	64	64	64
E. faecium (17)	RU 29 246	>64	>64	>64
	Cefdinir	8 ~ >64	>64	>64
	Cefpodoxime	>64	>64	>64
	Cefixime	>64	>64	>64
	Ceftibuten	>64	>64	>64
	Cefuroxime	>64	>64	>64
	Cefaclor	64 ~ >64	64	>64
E. liquefaciens (13)	RU 29 246	>64	>64	>64
	Cefdinir	2 ~ 8	4	8
	Cefpodoxime	>64	>64	>64
	Cefixime	64 ~ >64	>64	>64
	Ceftibuten	>64	>64	>64
	Cefuroxime	>64	>64	>64
	Cefaclor	16 ~ 64	32	64
Streptococcus pneumoniae PEN-S (20)	RU 29 246	0.03 ~ 0.25	0.03	0.06
	Cefdinir	0.008 ~ 0.25	0.06	0.13
	Cefpodoxime	0.016 ~ 0.13	0.03	0.06
	Cefixime	0.06 ~ 1	0.5	0.5
	Ceftibuten	4 ~ 8	4	4
	Cefuroxime	0.002 ~ 0.06	0.016	0.03
	Cefaclor	0.016 ~ 4	1	2

Table 3. (Continued)

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
S. pneumoniae PEN-R (MIC PEN 1~4 μg/ml) (15)	RU 29 246	0.5 ~ 4	2	4
	Cefdinir	0.25 ~ 8	2	4
	Cefpodoxime	0.5 ~ 4	2	4
	Cefixime	16 ~ 64	32	64
	Ceftibuten	16 ~ 64	32	64
	Cefuroxime	0.5 ~ 8	2	4
	Cefaclor	64 ~ >64	>64	>64
S. milleri group (17)	RU 29 246	0.008~ 0.25	0.03	0.25
	Cefdinir	0.016~ 0.25	0.06	0.25
	Cefpodoxime	0.016~ 0.25	0.03	0.25
	Cefixime	0.13 ~ 4	1	4
	Ceftibuten	4 ~ 32	16	32
	Cefuroxime	0.03 ~ 0.25	0.13	0.25
	Cefaclor	2 ~ 16	8	16
S. mitior (7)	RU 29 246	0.03 ~ 0.25	0.03	0.25
	Cefdinir	0.016~ 0.25	0.03	0.25
	Cefpodoxime	0.016~ 0.13	0.06	0.13
	Cefixime	0.13 ~ 4	0.5	4
	Ceftibuten	2 ~ 64	32	64
	Cefuroxime	0.03 ~ 0.13	0.03	0.13
	Cefaclor	2 ~ 16	4	16
Hemolytic Streptococci group A (19)	RU 29 246	0.016~ 0.06	0.016	0.06
	Cefdinir	0.008~ 0.06	0.016	0.03
	Cefpodoxime	0.016~ 0.06	0.03	0.06
	Cefixime	0.13 ~ 0.5	0.13	0.25
	Ceftibuten	0.25 ~ 4	1	1
	Cefuroxime	0.016~ 0.25	0.03	0.06
	Cefaclor	0.06 ~ 0.5	0.13	0.25
Hemolytic Streptococci group B (19)	RU 29 246	0.016~ 0.13	0.03	0.06
	Cefdinir	0.016~ 0.06	0.03	0.06
	Cefpodoxime	0.03 ~ 0.25	0.06	0.25
	Cefixime	0.25 ~ 1	0.5	0.5
	Ceftibuten	8 ~ 16	16	16
	Cefuroxime	0.03 ~ 0.13	0.06	0.13
	Cefaclor	0.5 ~ 4	2	4
Hemolytic Streptococci group C (5)	RU 29 246	0.016~ 0.03	0.016	0.03
	Cefdinir	0.016	0.016	0.03
	Cefpodoxime	0.03 ~ 0.06	0.03	0.06
	Cefixime	0.25	0.25	0.25
	Ceftibuten	1	1	1
	Cefuroxime	0.016	0.016	0.016
	Cefaclor	0.13	0.13	0.13
Hemolytic Streptococci group G (22)	RU 29 246	0.016~ 0.06	0.03	0.03
	Cefdinir	0.008~ 0.03	0.016	0.016
	Cefpodoxime	0.016~ 0.06	0.03	0.06
	Cefixime	0.13 ~ 0.5	0.13	0.25
	Ceftibuten	0.5 ~ 1	1	1
	Cefuroxime	0.016~ 0.13	0.03	0.06
	Cefaclor	0.06 ~ 0.13	0.13	0.13
Escherichia coli (24)	RU 29 246	0.13 ~ 0.5	0.25	0.5
	Cefdinir	0.13 ~ 0.25	0.25	0.25
	Cefpodoxime	0.13 ~ 1	0.25	0.5
	Cefixime	0.06 ~ 0.5	0.25	0.5
	Ceftibuten	0.03 ~ 0.5	0.13	0.25
	Cefuroxime	2 ~ 8	2	4
	Cefaclor	0.25 ~ 4	1	2

Table 3. (Continued)

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Klebsiella pneumoniae (16)	RU 29 246	0.13 ~ 1	0.25	0.5
	Cefdinir	0.06 ~ 0.5	0.13	0.5
	Cefpodoxime	0.03 ~ 4	0.13	0.5
	Cefixime	0.03 ~ 0.25	0.06	0.25
	Ceftibuten	0.03 ~ 0.25	0.06	0.13
	Cefuroxime	1 ~ 8	2	8
	Cefaclor	0.5 ~ 2	1	2
K. oxytoca (20)	RU 29 246	0.13 ~ 1	0.25	0.5
	Cefdinir	0.03 ~ 0.5	0.13	0.25
	Cefpodoxime	0.06 ~ 2	0.13	1
	Cefixime	0.016~ 2	0.03	0.5
	Ceftibuten	0.03 ~ 0.25	0.06	0.13
	Cefuroxime	1 ~ 8	2	4
	Cefaclor	0.25 ~ 1	0.5	1
Enterobacter cloacae (66)	RU 29 246	0.13 ~ >64	64	>64
	Cefdinir	0.13 ~ >64	16	>64
	Cefpodoxime	0.13 ~ >64	8	>64
	Cefixime	0.06 ~ >64	2	>64
	Ceftibuten	0.03 ~ >64	0.5	>64
	Cefuroxime	4 ~ >64	16	>64
	Cefaclor	2 ~ >64	>64	>64
E. aerogenes (13)	RU 29 246	0.25 ~ 64	2	64
	Cefdinir	0.5 ~ >64	1	>64
	Cefpodoxime	0.25 ~ >64	1	64
	Cefixime	0.25 ~ >64	1	>64
	Ceftibuten	0.25 ~ >64	0.5	64
	Cefuroxime	2 ~ >64	8	>64
	Cefaclor	8 ~ >64	>64	>64
E. sakazakii (11)	RU 29 246	0.13 ~ 2	0.13	0.5
	Cefdinir	0.13 ~ 1	0.13	0.25
	Cefpodoxime	0.13 ~ 1	0.25	0.5
	Cefixime	0.016~ 0.5	0.06	0.25
	Ceftibuten	0.016~ 0.13	0.03	0.06
	Cefuroxime	0.5 ~ 16	2	8
	Cefaclor	1 ~ 16	4	8
Hafnia alvei (15)	RU 29 246	0.5 ~ >64	8	>64
	Cefdinir	0.25 ~ 64	2	32
	Cefpodoxime	1 ~ >64	4	>64
	Cefixime	0.5 ~ >64	4	32
	Ceftibuten	0.13 ~ 16	0.5	8
	Cefuroxime	1 ~ >64	4	>64
	Cefaclor	4 ~ >64	16	>64
Citrobacter freundii (38)	RU 29 246	0.5 ~ >64	1	64
	Cefdinir	0.13 ~ >64	0.5	16
	Cefpodoxime	0.25 ~ 32	1	8
	Cefixime	0.13 ~ 64	1	8
	Ceftibuten	0.06 ~ 32	0.5	4
	Cefuroxime	2 ~ >64	4	64
	Cefaclor	4 ~ >64	32	>64
Serratia marcescens (38)	RU 29 246	2 ~ >64	32	>64
	Cefdinir	2 ~ >64	8	>64
	Cefpodoxime	0.5 ~ >64	4	64
	Cefixime	0.25 ~ >64	1	16
	Ceftibuten	0.06 ~ 8	0.25	2
	Cefuroxime	32 ~ >64	>64	>64
	Cefaclor	16 ~ >64	>64	>64

Table 3. (Continued)

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
S. liquefaciens (15)	RU 29 246	1 ~ >64	>64	>64
	Cefdinir	0.5 ~ >64	4	>64
	Cefpodoxime	0.25 ~ >64	2	>64
	Cefixime	0.25 ~ 32	2	32
	Ceftibuten	0.13 ~ 8	0.25	8
	Cefuroxime	16 ~ >64	64	>64
	Cefaclor	>64	>64	>64
Proteus mirabilis (20)	RU 29 246	0.25 ~ 0.5	0.25	0.5
	Cefdinir	0.06 ~ 0.13	0.06	0.13
	Cefpodoxime	0.06 ~ 0.13	0.06	0.13
	Cefixime	0.016~ 0.06	0.016	0.06
	Ceftibuten	0.03	0.03	0.03
	Cefuroxime	1 ~ 8	4	4
	Cefaclor	1 ~ 4	4	4
P. vulgaris (32)	RU 29 246	0.13 ~ 64	16	64
	Cefdinir	1 ~ 64	8	32
	Cefpodoxime	0.016~ 2	0.13	1
	Cefixime	0.004~ 0.06	0.016	0.03
	Ceftibuten	0.008~ 0.06	0.03	0.06
	Cefuroxime	16 ~ >64	>64	>64
	Cefaclor	16 ~ >64	>64	>64
P. penneri (3)	RU 29 246	>64	>64	>64
	Cefdinir	0.06 ~ 0.25	0.13	0.25
	Cefpodoxime	0.06 ~ 0.25	0.13	0.25
	Cefixime	0.016~ 0.03	0.03	0.03
	Ceftibuten	0.016~ 0.03	0.016	0.03
	Cefuroxime	16 ~ >64	32	>64
	Cefaclor	32 ~ >64	>64	>64
Providencia rettgeri (51)	RU 29 246	0.008~ 16	0.06	1
	Cefdinir	0.008~ 8	0.06	0.5
	Cefpodoxime	0.002~ 8	0.06	0.5
	Cefixime	0.002~ 4	0.016	0.13
	Ceftibuten	0.002~ 0.13	0.008	0.13
	Cefuroxime	0.03 ~ >64	2	8
	Cefaclor	1 ~ >64	>64	>64
P. stuartii (20)	RU 29 246	0.06 ~ 8	0.13	4
	Cefdinir	0.03 ~ 8	0.06	2
	Cefpodoxime	0.03 ~ 0.5	0.06	0.5
	Cefixime	0.008~ 0.25	0.016	0.13
	Ceftibuten	0.008~ 0.13	0.016	0.06
	Cefuroxime	1 ~ 16	2	4
	Cefaclor	4 ~ >64	64	>64
Morganella morganii (20)	RU 29 246	0.5 ~ >64	8	64
	Cefdinir	0.5 ~ 32	16	32
	Cefpodoxime	0.13 ~ 64	1	64
	Cefixime	0.25 ~ 64	2	64
	Ceftibuten	0.06 ~ 32	0.25	32
	Cefuroxime	16 ~ >64	64	>64
	Cefaclor	64 ~ >64	>64	>64
Salmonella spp. (12)	RU 29 246	0.13 ~ 1	0.25	0.5
(6 *S. typhimurium*,	Cefdinir	0.03 ~ 1	0.13	0.5
3 *S. enteritidis*,	Cefpodoxime	0.13 ~ 2	0.25	0.5
1 *S. brandenburg*,	Cefixime	0.03 ~ 0.13	0.03	0.13
1 *S. agona*,	Ceftibuten	0.016~ 0.03	0.03	0.06
1 *S. mendocina*)	Cefuroxime	0.5 ~ 16	8	16
	Cefaclor	0.13 ~ 32	2	8

Table 3. (Continued)

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Shigella spp. (22)	RU 29 246	0.13 ~ 0.5	0.25	0.5
(17 *S. flexneri*,	Cefdinir	0.06 ~ 0.25	0.13	0.25
3 *S. sonnei*,	Cefpodoxime	0.25 ~ 0.5	0.25	0.25
2 *S. boydii*)	Cefixime	1 ~ 4	2	2
	Ceftibuten	0.06 ~ 0.25	0.13	0.25
	Cefuroxime	2 ~ 4	2	4
	Cefaclor	1 ~ 2	1	1
Yersinia enterocolitica (15)	RU 29 246	1 ~ 2	2	2
	Cefdinir	0.5 ~ 1	0.5	1
	Cefpodoxime	0.5 ~ 2	1	2
	Cefixime	2 ~ 8	4	8
	Ceftibuten	0.06 ~ 0.5	0.13	0.25
	Cefuroxime	2 ~ 8	4	4
	Cefaclor	16	16	16
Acinetobacter baumannii (20)	RU 29 246	1 ~ 16	4	8
	Cefdinir	1 ~ 16	4	4
	Cefpodoxime	0.5 ~ 32	16	32
	Cefixime	8 ~ >64	16	32
	Ceftibuten	1 ~ >64	32	32
	Cefuroxime	2 ~ >64	32	64
	Cefaclor	>64	>64	>64
A. lwoffi (20)	RU 29 246	0.13 ~ 4	1	2
	Cefdinir	0.5 ~ 4	1	2
	Cefpodoxime	0.5 ~ 16	4	8
	Cefixime	2 ~ >64	8	16
	Ceftibuten	0.03 ~ 64	1	16
	Cefuroxime	4 ~ 16	4	8
	Cefaclor	16 ~ >64	32	64
A. calcoaceticus (12)	RU 29 246	0.5 ~ 2	1	2
	Cefdinir	2 ~ 8	4	8
	Cefpodoxime	4 ~ 32	16	32
	Cefixime	32 ~ 64	32	64
	Ceftibuten	8 ~ 32	16	32
	Cefuroxime	8 ~ 64	32	64
	Cefaclor	8 ~ >64	>64	>64
A. johnsonii (12)	RU 29 246	0.13 ~ 4	0.5	2
	Cefdinir	0.13 ~ 8	0.5	4
	Cefpodoxime	0.5 ~ 32	4	16
	Cefixime	2 ~ 64	8	32
	Ceftibuten	0.5 ~ 16	2	8
	Cefuroxime	1 ~ 64	4	32
	Cefaclor	0.5 ~ 32	4	16
A. genospecies 12 (6)	RU 29 246	0.25 ~ 2	1	2
	Cefdinir	0.25 ~ 2	0.5	2
	Cefpodoxime	2 ~ 4	2	4
	Cefixime	2 ~ 8	8	8
	Ceftibuten	2 ~ 8	4	8
	Cefuroxime	2 ~ 8	4	8
	Cefaclor	2 ~ 4	4	4
A. genospecies 3 (9)	RU 29 246	0.13 ~ 16	2	16
	Cefdinir	0.25 ~ 16	2	16
	Cefpodoxime	0.5 ~ 64	8	64
	Cefixime	2 ~ >64	32	>64
	Ceftibuten	32 ~ >64	>64	>64
	Cefuroxime	2 ~ >64	32	>64
	Cefaclor	32 ~ >64	>64	>64

Table 3. (Continued)

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Acinetobacter strain 84 (7)	RU 29 246	0.25 ~ 64	4	64
	Cefdinir	0.5 ~ 16	1	16
	Cefpodoxime	2 ~ >64	2	>64
	Cefixime	8 ~ >64	8	>64
	Ceftibuten	4 ~ >64	64	>64
	Cefuroxime	4 ~ >64	4	>64
	Cefaclor	4 ~ >64	4	>64
Pseudomonas aeruginosa (30)	RU 29 246	>64	>64	>64
	Cefdinir	>64	>64	>64
	Cefpodoxime	>64	>64	>64
	Cefixime	>64	>64	>64
	Ceftibuten	16 ~ >64	>64	>64
	Cefuroxime	>64	>64	>64
	Cefaclor	>64	>64	>64
P. fluorescens (20)	RU 29 246	>64	>64	>64
	Cefdinir	>64	>64	>64
	Cefpodoxime	>64	>64	>64
	Cefixime	8 ~ >64	>64	>64
	Ceftibuten	32 ~ >64	>64	>64
	Cefuroxime	>64	>64	>64
	Cefaclor	>64	>64	>64
P. putida (20)	RU 29 246	>64	>64	>64
	Cefdinir	>64	>64	>64
	Cefpodoxime	>64	>64	>64
	Cefixime	32 ~ >64	>64	>64
	Ceftibuten	32 ~ >64	>64	>64
	Cefuroxime	>64	>64	>64
	Cefaclor	>64	>64	>64
P. stutzeri (8)	RU 29 246	4 ~ >64	>64	>64
	Cefdinir	4 ~ >64	>64	>64
	Cefpodoxime	8 ~ >64	32	64
	Cefixime	16 ~ >64	64	>64
	Ceftibuten	8 ~ 32	16	32
	Cefuroxime	8 ~ >64	64	>64
	Cefaclor	>64	>64	>64
P. cepacia (24)	RU 29 246	2 ~ >64	>64	>64
	Cefdinir	1 ~ >64	8	>64
	Cefpodoxime	2 ~ >64	8	64
	Cefixime	8 ~ >64	>64	>64
	Ceftibuten	0.25 ~ >64	2	64
	Cefuroxime	4 ~ >64	16	>64
	Cefaclor	>64	>64	>64
P. maltophilia (20)	RU 29 246	>64	>64	>64
	Cefdinir	>64	>64	>64
	Cefpodoxime	>64	>64	>64
	Cefixime	>64	>64	>64
	Ceftibuten	16 ~ >64	>64	>64
	Cefuroxime	>64	>64	>64
	Cefaclor	>64	>64	>64
Haemophilus influenzae AMP-S (35)	RU 29 246	0.06 ~ 0.5	0.25	0.5
	Cefdinir	0.13 ~ 0.5	0.25	0.5
	Cefpodoxime	0.03 ~ 0.13	0.06	0.13
	Cefixime	0.03 ~ 0.5	0.13	0.25
	Ceftibuten	0.03 ~ 0.13	0.03	0.06
	Cefuroxime	0.25 ~ 1	1	1
	Cefaclor	4 ~ 16	8	16

Table 3. (Continued)

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
H. influenzae AMP-R	RU 29 246	0.25 ~ 1	0.5	1
(MIC AMP 16~64 μg/ml) (21)	Cefdinir	0.13 ~ 1	0.25	0.5
	Cefpodoxime	0.03 ~ 0.25	0.06	0.13
	Cefixime	0.016~ 0.25	0.03	0.13
	Ceftibuten	0.03 ~ 0.25	0.06	0.25
	Cefuroxime	0.5 ~ 2	1	1
	Cefaclor	4 ~ 32	8	32
H. parainfluenzae (10)	RU 29 246	0.03 ~ 0.25	0.13	0.25
	Cefdinir	0.03 ~ 0.5	0.13	0.25
	Cefpodoxime	0.06 ~ 0.13	0.06	0.13
	Cefixime	0.03 ~ 0.06	0.03	0.06
	Ceftibuten	0.03 ~ 0.06	0.06	0.06
	Cefuroxime	0.13 ~ 1	0.5	1
	Cefaclor	4 ~ 16	8	16
Moraxella catarrhalis (13)	RU 29 246	0.03 ~ 1	0.25	0.5
	Cefdinir	0.13 ~ 0.5	0.25	0.5
	Cefpodoxime	0.06 ~ 0.5	0.25	0.5
	Cefixime	0.06 ~ 0.5	0.13	0.5
	Ceftibuten	0.5 ~ 4	1	4
	Cefuroxime	0.25 ~ 2	0.5	2
	Cefaclor	0.5 ~ 4	4	4
Neisseria gonorrhoeae PEN-S (24)	RU 29 246	0.004~ 0.06	0.008	0.03
	Cefdinir	0.002~ 0.03	0.008	0.03
	Cefpodoxime	0.004~ 0.06	0.008	0.03
	Cefixime	0.002~ 0.016	0.008	0.016
	Ceftibuten	0.008~ 0.25	0.06	0.25
	Cefuroxime	0.016~ 0.13	0.06	0.13
	Cefaclor	0.25 ~ 64	8	32
N. gonorrhoeae PEN-R	RU 29 246	0.008~ 0.25	0.016	0.06
(MIC PEN 16~64 μg/ml) (20)	Cefdinir	0.004~ 0.06	0.016	0.06
	Cefpodoxime	0.004~ 0.13	0.03	0.06
	Cefixime	0.002~ 0.06	0.016	0.03
	Ceftibuten	0.03 ~ 0.5	0.13	0.5
	Cefuroxime	0.03 ~ 1	0.25	1
	Cefaclor	2 ~ 64	16	64
Brucella spp. (5)	RU 29 246	0.25	0.25	0.25
(3 *B. melitensis*,	Cefdinir	0.13 ~ 0.25	0.25	0.25
1 *B. abortus*,	Cefpodoxime	0.25	0.25	0.25
1 *B. suis*)	Cefixime	0.06 ~ 2	0.5	2
	Ceftibuten	8	8	8
	Cefuroxime	4	4	4
	Cefaclor	4	4	4
Listeria spp. (13)	RU 29 246	4 ~ 32	8	32
(8 *L. monocytogenes*,	Cefdinir	2 ~ 8	4	8
2 *L. inocua*,	Cefpodoxime	2 ~ >64	32	>64
1 *L. seeligeri*,	Cefixime	>64	>64	>64
1 *L. welshemeri*,	Ceftibuten	64 ~ >64	>64	>64
1 *L. ivanovii*)	Cefuroxime	1 ~ >64	32	>64
	Cefaclor	16 ~ >64	32	>64
Bordetella pertussis (7)	RU 29 246	16 ~ >64	64	>64
	Cefdinir	4 ~ >64	32	>64
	Cefpodoxime	8 ~ >64	32	>64
	Cefixime	2 ~ >64	32	>64
	Ceftibuten	64 ~ >64	64	>64
	Cefuroxime	8 ~ >64	>64	>64
	Cefaclor	64 ~ >64	>64	>64

Table 3. (Continued)

Species (No. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Gardnerella vaginalis (20)	RU 29 246	0.06 ~ 0.5	0.25	0.5
	Cefdinir	0.06 ~ 0.5	0.25	0.5
	Cefpodoxime	0.25 ~ 2	0.5	1
	Cefixime	2 ~ 32	4	16
	Ceftibuten	16 ~ >64	>64	>64
	Cefuroxime	0.5 ~ 8	1	2
	Cefaclor	1 ~ 32	16	32
Helicobacter pylori (12)	RU 29 246	0.5 ~ 2	1	2
	Cefdinir	0.5 ~ 2	0.5	2
	Cefpodoxime	0.5 ~ 4	2	4
	Cefixime	0.5 ~ 8	1	4
	Ceftibuten	2 ~ 8	4	8
	Cefuroxime	0.25 ~ 8	1	4
	Cefaclor	0.13 ~ 2	1	2
Clostridium difficile (12)	RU 29 246	0.13 ~ 8	0.13	8
	Cefdinir	64 ~ >64	64	>64
	Cefpodoxime	64 ~ >64	>64	>64
	Cefixime	>64	>64	>64
	Ceftibuten	>64	>64	>64
	Cefuroxime	64 ~ >64	>64	>64
	Cefaclor	32 ~ >64	64	>64
Clostridium spp. (5)	Ru 29 246	0.13 ~ 4	4	4
(1 *C. sordelli*,	Cefdinir	0.5 ~ 4	2	4
1 *C. sporogenes*,	Cefpodoxime	0.25 ~ 16	8	16
1 *C. histolyticum*,	Cefixime	1 ~ 64	1	64
1 *C. perfringens*,	Ceftibuten	1 ~ >64	4	>64
1 *C. ramosum*)	Cefuroxime	0.5 ~ 4	2	4
	Cefaclor	8 ~ 32	8	32
Peptococcus magnus (4)	RU 29 246	1 ~ 2	1	2
	Cefdinir	4 ~ 16	8	16
	Cefpodoxime	8 ~ 32	8	32
	Cefixime	>64	>64	>64
	Ceftibuten	64 ~ >64	>64	>64
	Cefuroxime	1 ~ 4	2	4
	Cefaclor	8	8	8
Bacteroides spp. (11)	RU 29 246	0.25 ~ >64	2	64
(4 *B. fragilis*,	Cefdinir	0.5 ~ 16	1	16
2 *B. thetaiotaomicron*,	Cefpodoxime	0.25 ~ >64	0.5	>64
1 *B. distasonis*,	Cefixime	1 ~ 16	1	16
1 *B. vulgatus*,	Ceftibuten	64 ~ >64	>64	>64
1 *B. disiens*,	Cefuroxime	0.5 ~ >64	16	64
1 *B. bivius*,	Cefaclor	8	8	8
1 *B. ovatus*)				

[a] 16 hours, 32°C, 2% NaCl.

highest activity against *Streptococcus pneumoniae* followed by cefdinir and cefixime, while cefaclor and ceftibuten were the least active of the cephalosporins included. Penicillin-resistant *Streptococcus pneumoniae* were much less susceptible to all compounds in comparison with the penicillin-susceptible strains (between 8 and 256 times). Strains of the *Streptococcus milleri* group and of *Streptococcus mitior* were about equally susceptible to RU 29 246, cefdinir, cefpodoxime and cefuroxime (MIC_{90} 0.13 to 0.25 μg/ml), but had MIC_{90}s of 4 μg/ml or above for cefixime, ceftibuten and cefaclor.

The MIC_{90} for hemolytic Streptococci of groups A, C and G were within a narrow range for RU 29 246, cefdinir and cefuroxime (0.016~0.06 μg/ml). Cefaclor (MIC_{90} 0.13~0.25 μg/ml), cefixime

(MIC_{90} 0.25 μg/ml) and particularly ceftibuten (MIC_{90} 1 μg/ml) were less active. Among the hemolytic Streptococci those of group B were less susceptible in comparison with the strains of groups A, C and G against all of the oral cephalosporins investigated except for RU 29 246 which demonstrated an equal MIC_{90} (0.06 μg/ml) both for group A and group B hemolytic Streptococci. The lower susceptibility of group B Streptococci in comparison with Streptococci of groups A, C and G was most pronounced for cefaclor and ceftibuten.

Among Enterobacteriaceae the acivity of the oral cephalosporins was highest against a group of species composed of *Escherichia coli*, *Klebsiella* spp., *Enterobacter sakazakii*, *Ptoteus mirabilis*, *Salmonella* spp. and *Shigella* spp. These species were highly susceptible (MIC_{90} ≤ 0.5 μg/ml) to ceftibuten, cefixime, cefpodoxime, cefdinir and RU 29 246, but less susceptible to cefaclor (MIC_{90} 0.5～8 μg/ml) and cefuroxime (MIC_{90} 4～8 μg/ml). *Proteus vulgaris* was less susceptible than *Proteus mirabilis* (cefpodoxime three $\log_2$, RU 29 246 seven $\log_2$, cefdinir eight $\log_2$, cefuroxime and cefaclor more than five $\log_2$) except for cefixime and ceftibuten, which were equally active against both species. These two compounds also inhibited both *Providencia* spp. at low concentrations, while the activities of cefpodoxime, cefdinir and RU 29 246 were moderate and cefaclor was inactive against both species. Cefuroxime activity was intermediate against *Providencia rettgeri* and *Providencia stuartii*. *Morganella morganii* showed high MIC_{90} for all compounds (32 μg/ml or above). However, a remarkable difference was observed at the MIC_{50} level for ceftibuten (0.25 μg/ml), cefpodoxime (1 μg/ml), cefixime (2 μg/ml) and RU 29 246 (8 μg/ml). Strains of *Serratia* spp. were highly susceptible to ceftibuten, moderately susceptible to cefdinir, cefpodoxime and cefixime, while RU 29 246, cefuroxime and cefaclor were mostly inactive. *Citrobacter freundii* was about equally susceptible to cefdinir, cefpodoxime, cefixime, ceftibuten; RU 29 246 demonstrated intermediate activity, while cefuroxime and cefaclor were inactive. *Yersinia enterocolitica* was about equally susceptible to all compounds, ceftibuten, however, was the most active agent. *Hafnia alvei* was resistant to all coumpounds (MIC_{90} equal to or above 32 μg/ml) except for ceftibuten (MIC_{90} 8 μg/ml). For *Enterobacter* spp. MIC_{90} of all oral cephalosporins were equal to or above 64 μg/ml, ceftibuten being still the most active compound (MIC_{50} of 0.5 μg/ml for *Enterobacter cloacae* and *Enterobacter aerogenes*).

Against *Acinetobacter baumannii* only RU 29 246 (MIC_{90} 8 μg/ml) and cefdinir (MIC_{90} 4 μg/ml) demonstrated marginal activities. *Acinetobacter lwoffi* was in addition moderately susceptible to all the other compounds except cefixime and cefaclor. All the oral cephalosporins included were inactive against the six species of *Pseudomonas* investigated.

MICs for each of the seven compounds investigated were mostly identical for *Haemophilus influenzae* and *Haemophilus parainfluenzae*. The activity of the oral cephalosporins included against ampicillin-resistant *Haemophilus influenzae* was equal to or only 2 to 4 times below that against ampicillin-susceptible isolates. Cefpodoxime, cefixime and ceftibuten were the most active compounds against *Haemophilus* spp. (MIC_{90} 0.06～0.25 μg/ml), followed by RU 29 246, cefdinir and cefuroxime (MIC_{90} 0.25～1 μg/ml) and cefaclor (MIC_{90} 16～32 μg/ml). *Moraxella catarrhalis* was equally susceptible to RU 29 246, cefdinir, cefpodoxime and cefixime, while ceftibuten, cefuroxime and cefaclor were 4 to 8 times less active.

Neisseria gonorrhoeae were about equally susceptible to RU 29 246, cefdinir and cefpodoxime (MIC_{90} 0.03～0.06 μg/ml), while cefixime was about twice as active (MIC_{90} 0.016～0.03 μg/ml). Cefuroxime and ceftibuten were less active. *Neisseria gonorrhoeae* resistant to benzylpenicillin had MIC_{90} only double as high as the penicillin-susceptible isolates except for cefuroxime (8 times higher). Cefixime was the most active oral cephalosporin against *Neisseria gonorrhoeae*, RU 29 246, cefdinir and cefpodoxime being half

as active as cefixime. Ceftibuten and cefuroxime were 16 to 32 times less active than cefixime. Cefaclor was the only one of the oral cephalosporins included without therapeutically relevant activity against the majority of Gonococci.

RU 29 246, cefdinir and cefpodoxime were superior to the other compounds in their activity against *Brucella* spp. *Listeria* spp. and *Bordetella pertussis* were resistant to all compounds investigated.

Against *Helicobacter pylori* the *in vitro* activity of all cephalosporins was about equal, cefdinir, RU 29 246 and cefaclor being the most active compounds (MIC_{90} 2 μg/ml).

Among anaerobic pathogens (*Clostridium difficile*, *Clostridium* spp., *Peptococcus magnus*) RU 29 246 was the most active of the oral cephalosporins followed by cefuroxime and cefdinir, which were, however, totally inactive against *Clostridium difficile*. Activity against *Bacteroides* spp. was only moderate for all compounds (MIC_{90} ≥16 μg/ml).

Bactericidal Activity

The kinetics of killing of two *Staphylococcus aureus*-strains and two *Escherichia coli*-strains are shown in Figs. 2 to 5.

Against *Staphylococcus aureus* SG 511 (Fig. 2), RU 29 246 exhibited a bactericidal effect (killing of about 99% within 8 hours) at concentrations 2 times and 4 times the MIC, which was stronger with *Staphylococcus aureus* 503 (Fig. 3, killing of between 99% and 99.9% within 8 hours). Exposure of both strains to a concentration equal to their MIC was bacteriostatic for *Staphylococcus aureus* SG 511 and weakly bactericidal for *Staphylococcus aureus* 503 (killing of about 90% within 8 hours).

RU 29 246 had only weak bactericidal activity at once the MIC against *Escherichia coli* 1507 E (Fig. 4), concentrations of 2 times or 4 times the MIC resulted in killing of more than 99% of the bacterial cells during the 8 hours test period. Against *Escherichia coli* TEM-1 (Fig. 5) the bacterial count was rapidly diminished at once, 2 times and 4 times the MIC. Four hours after introduction of RU 29 246, however, at once the MIC regrowth began, whereas killing proceeded at 2 times and 4 times the MIC. This regrowth

Fig. 2. Bactericidal activity of RU 29 246 against *Staphylococcus aureus* SG 511.

S. aureus SG 511, MIC=0.313 μg/ml.
● Control, △ 0.5×MIC, ▽ 1×MIC, □ 2×MIC, ○ 4×MIC.

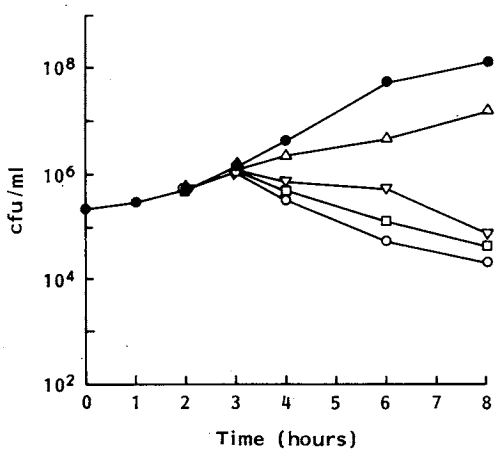


Fig. 3. Bactericidal activity of RU 29 246 against *Staphylococcus aureus* 503.

S. aureus 503, MIC=0.156 μg/ml.
● Control, △ 0.5×MIC, ▽ 1×MIC, □ 2×MIC, ○ 4×MIC.

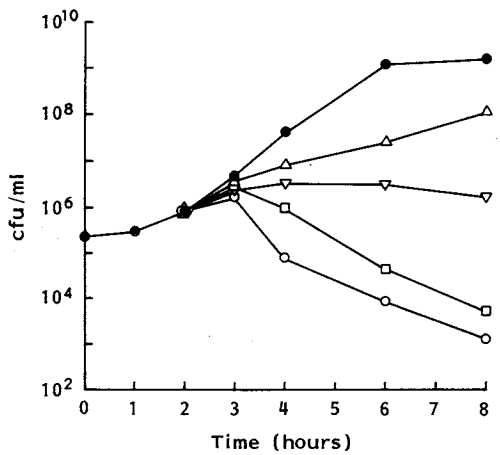


Fig. 4. Bactericidal activity of RU 29 246 against *Escherichia coli* 1507 E.

E. coli 1507 E, MIC=0.313 μg/ml.
● Control, △ 0.5×MIC, ▽ 1×MIC, □ 2×MIC, ○ 4×MIC.

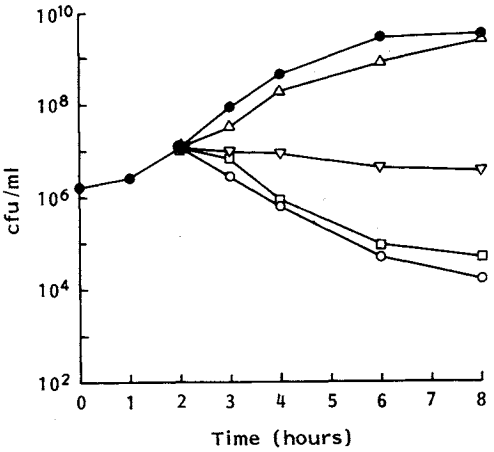
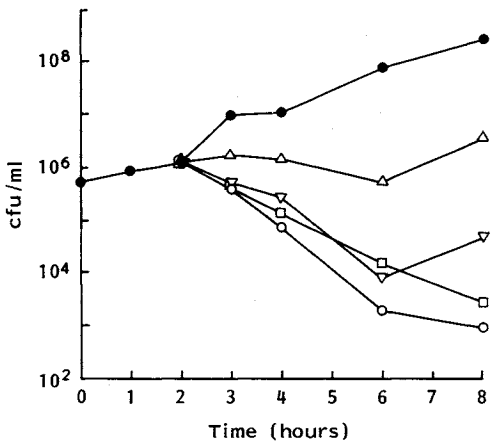


Fig. 5. Bactericidal activity of RU 29 246 against *Escherichia coli* TEM-1.

E. coli TEM-1, MIC=0.625 μg/ml.
● Control, △ 0.5×MIC, ▽ 1×MIC, □ 2×MIC, ○ 4×MIC.



was probably due to the degradation of RU 29 246 by the TEM-1 β-lactamase, as the MIC of this strain remained unchanged until the end of the assay period.

Discussion

A broad variety of resorbable cephalosporins or cephalosporins made resorbable by esterification are now available for oral therapy[8~10]. Microbiological progress made by the newer compounds in comparison with *e.g.* cefuroxime and cefaclor includes both intrinsic activity (*e.g. Haemophilus* spp., Streptococci) and their antibacterial spectrum (mostly within Enterobacteriaceae). Enhanced activity against Enterobacteriaceae for some of the new oral cephalosporins was achieved at the expense of low or no activity against Staphylococci (cefixime, cefetamet, ceftibuten). In this respect, these new compounds are inferior to established oral cephalosporins (*e.g.* cefuroxime-axetil). So, efforts are made to synthesize oral cephalosporins with improved activity against Staphylococci, *e.g.* cefprozil or Bay 3522[10,11]. Both compounds, however, are only moderately active against Enterobacteriaceae. A step forward to broad spectrum oral cephalosporins was achieved with cefdinir, cefpodoxime and RU 29 246.

RU 29 246 *in vitro* activity is highly stable for a wide range of pH and inoculum size. RU 29 246 possesses high activity against methicillin-susceptible Staphylococci, hemolytic Streptococci groups A, B, C and G and penicillin-susceptible *Streptococcus pneumoniae* similar to the activity of cefdinir, but superior to cefpodoxime, cefuroxime, ceftibuten, cefixime and also cefaclor. For most of the Gram-negative pathogens, MICs of RU 29 246 were higher than those of cefixime, cefpodoxime and ceftibuten. Nevertheless, MICs vs. the majority of strains of *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp. and *Proteus* spp. (MIC_{90} ≤0.5 μg/ml) should allow successful therapy of infections caused by these organisms. The majority of *Bacteroides* spp. are resistant to RU 29 246, whereas Gram-positive obligate anaerobes, even *Clostridium difficile*, are inhibited by relatively low concentrations (MIC_{90} ≤8 μg/ml). The activity of RU 29 246 against these species is clearly superior to that of the reference compounds. Enterococci, *Listeria* spp. and *Pseudomonas* spp. are insensitive to all oral cephalosporins included. RU 29 246 is bactericidal at concentrations of 2 times and 4 times the MIC killing *Escherichia coli* and *Staphylococcus aureus* between 2 and 3 logs within 8 hours. *Staphylococcus aureus* SG 511 is killed at a lower rate (between 1 and 2 logs).

So, RU 29 246 demonstrates broad spectrum *in vitro* activity including both Staphylococci and major Enterobacteriaceae. The bioavailability of its prodrug-ester HR 916 will be decisive for the relevance of this new compounds in therapy.

References

1) FOLTZ, E. L.; J. W. WEST, I. H. BRESLOW & H. WALLICK: Clinical pharmacology of pivampicillin. Antimicrob. Agents Chemother. -1970: 442～454, 1971
2) ROHOLT, K.: Pharmacokinetic studies with mecillinam and pivmecillinam. J. Antimicrob. Chemother. 3 (Suppl. B): 71～81, 1977
3) HARDING, S. M.; P. E. O. WILLIAMS & J. AYRTON: Pharmacology of cefuroxime as the 1-acetoxyethyl ester in volunteers. Antimicrob. Agents Chemother. 25: 78～82, 1984
4) SHIMIDA, K.; A. SHISHIDO & M. TSUNOO: Phase I study of cefdinir. Chemotherapy (Tokyo) 37 (Suppl. 2): 208～245, 1989
5) HAMASHIMA, Y.; T. KUBOTA, K. MINAMI, K. ISHIKURA, T. KONOIKE, M. YOSHIOKA, T. YOSHIDA, H. NAKASHIMIZU & K. MOTOKAWA: Synthesis and biological properties of 7β-(*Z*)-2-(2-amino-4-thiazolyl)-4-carboxy-2-butenoyl-amino]-3-cephem-4-carboxylic acid (7432-S), a new cephem antibiotic. J. Antibiotics 40: 1468～1470, 1987
6) UTSUI, U.; M. INOUE & S. MITSUHASHI: In vitro and in vivo antibacterial activities of CS-807, a new oral cephalosporin. Antimicrob. Agents Chemother. 31: 1085～1092, 1987
7) JONES, R. N.: Antimicrobial activity, spectrum and pharmacokinetics of old and new oral administerd cephems. Antimicrob. News Letters 5: 1～7, 1988
8) SEIBERT, G.; M. LIMBERT, I. WINKLER & T. DICK: The antibacterial activity *in vitro* and β-lactamase stability of the new oral cephalosporin HR 810 in comparison with five other cephalosporins and two aminoglycosides. Infection 11: 275～279, 1983
9) BAUERNFEIND, A.: Comparative antimicrobial spectrum and activity of ceftibuten against clinical isolates from West Germany. Diagn. Microbiol. Infect. Dis. 14: 63～74, 1991
10) BAUERNFEIND, A.; R. JUNGWIRTH, S. SCHWEIGHART & M. THEOPOLD: Antibakterielle Aktivität und β-Laktamase-Stabilität von elf Oralcephalosporinen. Infection 18 (Suppl. 3): 155～167, 1990
11) BAUERNFEIND, A. & R. JUNGWIRTH: In vitro evaluation of cefpodoxime, a new oral cephalosporin of the third generation. Infection 19 (Suppl. 5): 353～362, 1991