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In Vitro Activity of an Oral Iminomethoxy Aminothiazolyl Cephalosporin, R-3746

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The in vitro activity of R-3746, an iminomethoxy aminothiazolyl cephalosporin with a CH_2OCH_3 moiety at position 3, was compared with those of other antibiotics. R-3746 inhibited the majority of hemolytic streptococci (groups A, B, C, F, and G) and *Streptococcus pneumoniae* at <0.06 μg/ml, which was comparable to the activity of amoxicillin, 2- to 8-fold more active than cefixime, and 16- to 64-fold more active than cefaclor and cephalexin. Ninety percent of β-lactamase-producing *Haemophilus influenzae* and *Neisseria gonorrhoeae* were inhibited at a concentration 0.25 μg/ml, but it was less active against *Branhamella* spp. It did not inhibit (MIC, >16 μg/ml) enterococci, viridans group streptococci, or methicillin-resistant staphylococci. The MICs of R-3746 for 90% of strains tested for *Escherichia coli*; *Klebsiella pneumoniae*; *Citrobacter diversus*; *Proteus mirabilis*; and *Salmonella*, *Shigella*, and *Yersinia* spp. were ≤1 μg/ml. It was two- to eightfold less active than cefixime but was markedly superior to cefaclor, cephalexin, amoxicillin-clavulanate, and trimethoprim-sulfamethoxazole. R-3746 inhibited 50% of *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Morganella* spp., *Providencia* spp., *Proteus vulgaris*, and *Serratia marcescens* at ≤8 μg/ml. *Pseudomonas* spp. were resistant. Fifty percent of *Clostridium* spp. were inhibited by 0.5 μg/ml, but MICs for *Bacteroides* spp. were >128 μg/ml. R-3746 was not appreciably hydrolyzed by most chromosomal and plasmid-mediated β-lactamases.

There has been great progress in the development of parenteral cephalosporins that are stable to attack by β-lactamases and that are active against a wide spectrum of gram-positive and -negative bacteria, but only recently have oral cephalosporins with extended antibacterial activity been synthesized (5). The older oral agents such as cephalexin and cephradine have relatively poor activity against important respiratory pathogens such as *Haemophilus influenzae* and *Branhamella catarrhalis*; and many β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*, which are important causes of nosocomial urinary tract infections, are resistant. Although cefaclor has activity against *Haemophilus influenzae*, it is not β-lactamase stable. Recently, cefixime, ceftetrame, and cefetamet have been investigated and shown to have excellent in vitro activity and favorable pharmacokinetic properties (2, 6, 7). We wished to compare the activities of R-3746, 1-[isopropoxycarbonyloxy-ethyl (6*R*, 7*R*)-(2-amino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetamido]3-methoymethyl-8-oxo-5-thia-1-azabicyclo(4,2,0)-oct-2-ene-2-carboxylate, a new oral cephalosporin, with those of other oral antibiotics against a variety of bacteria for which an oral cephalosporin could be used as initial or follow-up therapy to a broad-spectrum parenteral agent.

MATERIALS AND METHODS

The gram-positive and -negative bacteria used in this study were clinical isolates collected at the Columbia-Presbyterian Medical Center in New York City.

Standard antimicrobial powders were provided as follows: R-3746, Sankyo Co., Ltd., Tokyo, Japan; trimethoprim-sulfamethoxazole (TMP-SMX), Hoffmann-La Roche Inc., Nutley, N.J.; cephalexin and cefaclor, Eli Lilly & Co., Indianapolis, Ind.; amoxicillin-clavulanate, Beecham Laboratories, Bristol, Tenn.; cefixime, Fujisawa Pharmaceuticals, Osaka, Japan; and cefuroxime, Glaxo Inc., Research Triangle Park, N.C. Solutions of antimicrobial agents were prepared on the day of use, as directed by the manufacturers.

Susceptibility testing. Susceptibility testing was performed by a standard agar dilution technique with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% defibrinated sheep blood for testing streptococci and with 5% chocolate blood for testing *Haemophilus, Branhamella, or Neisseria* spp. Brucella agar supplemented with hemin and vitamin K was used for anaerobic species. Overnight cultures of test organisms in Mueller-Hinton broth (BBL), Todd-Hewitt broth (BBL) for streptococci, Schaedler broth for *Haemophilus* and *Neisseria* spp., or chopped meat-glucose (Scott Laboratories, Inc., Providence, R.I.) for anaerobic species were diluted in Mueller-Hinton broth. Final inocula of approximately 10^4 CFU were applied to plates with a multipoint spot inoculator. Plates were examined after 18 h of incubation of 35°C. Anaerobic organisms were incubated in GasPak jars (BBL) for 48 h at 35°C. Susceptibilities to all agents were tested at the same time.

Susceptibilities of five isolates each of five bacterial species were determined by the broth dilution technique. Tubes (1 ml) containing serial twofold dilutions of the compounds in Mueller-Hinton broth were inoculated with log-phase organisms to yield a final inoculum of approximately 5×10^5 CFU/ml. Tubes were incubated for 18 h at 35°C and inspected for the lack of turbidity. Samples of 0.01 ml were removed to antibiotic-free plates, which were incubated for 24 h at 35°C. The MBC, which was defined as a 99.9% reduction of the initial inoculum, was determined by the method of Pearson et al. (8), assuming a 5% pipetting error. Organisms were considered resistant to ampicillin, cephalexin, and cefaclor if MICs were ≥16 μg/ml.

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TABLE 1. Activity of R-3746 against gram-negative bacteria compared with those of other agents

Organism (no. tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli (30)	R-3746	0.12–2	0.25	1
	Cefixime	0.015–4	0.12	2
	Cefaclor	1–16	4	16
	Cephalexin	4–32	8	16
	Amox-clav[a]	2–32	16	16
	TMP-SMX[b]	0.12–>64	0.12	16
Klebsiella pneumoniae (30)	R-3746	0.03–8	0.25	1
	Cefixime	0.015–1	0.06	0.25
	Cefaclor	2–>128	8	32
	Cephalexin	4–>64	8	32
	Amox-clav	0.25–64	16	32
	TMP-SMX	0.12–>64	4	>64
Klebsiella oxytoca (20)	R-3746	0.12–8	0.25	2
	Cefixime	0.03–0.5	0.12	0.25
	Cefaclor	1–>128	4	>128
	Cephalexin	4–>64	8	>64
	Amox-clav	1–>32	8	>32
	TMP-SMX	0.06–16	0.25	8
Hafnia alvei (10)	R-3746	2–64	8	64
Enterobacter aerogenes (30)	R-3746	0.25–128	1	32
	Cefixime	0.5–>128	0.5	>128
	Cefaclor	1–>128	64	>128
	Cephalexin	>128	>128	>128
	Amox-clav	2–64	64	64
	TMP-SMX	0.12–1	0.25	0.5
Enterobacter cloacae (30)	R-3746	0.06–>128	4	>128
	Cefixime	0.06–>128	0.12	>128
	Cefaclor	8–>128	>128	>128
	Cephalexin	>128	>128	>128
	Amox-clav	16–128	64	64
	TMP-SMX	0.12–>16	0.25	0.5
Citrobacter freundii (30)	R-3746	2–>128	8	>128
	Cefixime	0.03–>128	2	>128
	Cefaclor	1–>128	>128	>128
	Cephalexin	>128	>128	>128
	Amox-clav	16–64	64	64
	TMP-SMX	0.06–>16	1	2
Citrobacter diversus (20)	R-3746	0.06–1	0.25	1
	Cefixime	0.06–4	0.12	0.5
	Cefaclor	0.5–2	1	1
	Cephalexin	4–8	8	8
	Amox-clav	1–16	2	4
	TMP-SMX	0.06–0.25	0.12	0.25
Proteus mirabilis (30)	R-3746	0.03–0.5	0.06	0.12
	Cefixime	≤0.015–0.06	≤0.0015	≤0.015
	Cefaclor	1–>128	2	64
	Cephalexin	1–>128	2	64
	Amox-clav	0.5–8	1	8
	TMP-SMX	≤0.06–8	≤0.06	0.5
Morganella morganii (20)	R-3746	0.03–32	0.5	8
	Cefixime	≤0.015–32	2	32
	Cefaclor	32–128	>128	>128
	Cephalexin	32–>128	>128	>128
	Amox-clav	2–>32	8	>32
	TMP-SMX	0.06–>16	0.25	8

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TABLE 1—*Continued*

Organism (no. tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Proteus vulgaris (30)	R-3746	0.03–64	0.25	16
	Cefixime	≤0.015–8	≤0.015	2
	Cefaclor	8–>128	>128	>128
	Cephalexin	16–>128	128	>128
	Amox-clav	2–>32	8	>32
	TMP-SMX	0.06–>16	0.25	2
Providencia rettgeri (30)	R-3746	0.015–16	0.12	8
	Cefixime	≤0.015–15	0.015	1
	Cefaclor	2–>128	128	>128
	Cephalexin	>128	>128	>128
	Amox-clav	2–>32	8	>32
	TMP-SMX	0.06–>16	0.25	8
Providencia stuartii (20)	R-3746	0.015–16	0.12	8
	Cefixime	≤0.015–1	≤0.015	0.5
	Cefaclor	8–>128	64	>128
	Cephalexin	32–>128	128	>128
	Amox-clav	8–>32	>32	>32
	TMP-SMX	0.12–>16	0.25	16
Serratia marcescens (20)	R-3746	0.5–128	2	32
	Cefixime	0.03–>128	2	128
	Cefaclor	64–>128	>128	>128
	Cephalexin	>128	>128	>128
	Amox-clav	8–>32	32	>32
	TMP-SMX	0.25–>16	0.5	4
Pseudomonas aeruginosa (20)	R-3746	>128	>128	>128
Pseudomonas cepacia (15)	R-3746	0.5–>128	32	>128
Pseudomonas maltophilia (10)	R-3746	>128	>128	>128
Pseudomonas spp., others (20)	R-3746	8–>128	64	>128
Acinetobacter antitratus (30)	R-3746	0.5–>128	16	>128
	Cefixime	1–>128	64	>128
	Cefaclor	8–>128	64	>128
	Cephalexin	>128	>128	>128
	Amox-clav	4–64	16	32
	TMP-SMX	0.25–8	0.5	1
Salmonella spp., ampicillin resistant (15)	R-3746	0.12–2	0.25	1
	Cefixime	0.03–0.5	0.06	0.25
	Cefaclor	2–32	8	16
	Cephalexin	4–16	16	16
	Amox-clav	0.5–>32	4	8
	TMP-SMX	0.06–16	0.12	2
Shigella spp., ampicillin resistant (15)	R-3746	0.25–4	0.5	1
	Cefixime	0.12–1	0.25	0.5
	Cefaclor	2–32	4	16
	Cephalexin	8–32	8	16
	Amox-clav	1–>32	8	8
	TMP-SMX	0.06–16	0.12	0.5
Aeromonas hydrophila (10)	R-3746	0.06–64	2	16
	Cefixime	1–>128	64	64
	Cefaclor	8–>128	64	>128
	Cephalexin	>128	>128	>128
	Amox-clav	4–>32	16	>32
	TMP-SMX	0.25–0.5	0.5	0.5

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TABLE 1—*Continued*

Organism (no. tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Yersinia enterocolitica (20)	R-3746	0.03–2	0.25	1
	Cefixime	0.03–8	1	4
	Cefaclor	1–>128	4	>128
	Cephalexin	4–>128	32	>128
	Amox-clav	0.5–>16	4	16
	TMP-SMX	0.06–0.5	0.12	0.25
Haemophilus influenzae (12)	R-3746	≤0.008–1	0.12	0.25
	Cefixime	0.12–0.25	0.12	0.25
	Cefaclor	0.12–16	2	8
	Cephalexin	0.12–32	4	16
	Amox-clav	0.25–1	0.5	0.5
	TMP-SMX	0.25–0.5	0.25	0.5
Branhamella catarrhalis (10)	R-3746	0.12–1	0.12	0.5
	Cefixime	≤0.015–0.12	≤0.015	0.12
	Cefaclor	0.12–64	0.12	8
	Cephalexin	0.12–128	0.25	32
	Amox-clav	0.12–1	0.12	1
Neisseria gonorrhoeae (17)	R-3746	≤0.008–0.25	0.015	0.25
	Cefixime	≤0.015–0.12	0.015	0.06
	Cefaclor	0.12–16	0.25	16
	Cephalexin	0.12–32	1	32

[a] Amoxicillin and clavulanate (Amox-clav) were combined at a ratio of 2:1. The concentration noted refers to that of amoxicillin.
[b] Trimethoprim and suflamethoxazole (TMP-SMX) were combined at a ratio of 1:5. The concentration noted refers to that of trimethoprim.

Time kill studies. Exponentially growing *Escherichia coli* and *Klebsiella pneumoniae*, at an inoculum of approximately 10^6 CFU, were exposed to R-3746 at concentrations of two-, four-, and eight-fold above the MIC, as determined in broth. Samples were obtained every 2 h for 8 h, and medium was removed by filtration. Bacteria were suspended in fresh medium, and dilutions were plated onto antibiotic-free medium. Organisms which were not exposed to drug were processed in a similar manner.

β-Lactamase assays. The presence of β-lactamases in clinical isolates was determined by the nitrocefin assay. β-Lactamases used for the determination of the stability of the compounds were either purified enzymes or partially purified enzymes, as previously described (4). The stabilities of the compounds to β-lactamase were determined by a spectrophotometric assay by using the change in absorption at the absorption maximum. The absorption used for R-3746 was 265 nm. Cephaloridine was used as a reference compound. Inhibition assays with nitrocefin as the substrate at a 10^{-4} M concentration, were performed in a final volume of 3 ml. Enzyme and inhibitor were incubated at various concentrations at 35°C for 10 min, and subsequently nitrocefin was added. The change in the A_{482} of nitrocefin was monitored for 10 min in a temperature-controlled recording spectrophotometer. As a control, the change in nitrocefin plus enzyme was monitored.

RESULTS

The susceptibilities of gram-negative bacteria to R-3746 and the other agents are given in Table 1. R-3746 inhibited 50% of the members of the family *Enterobacteriaceae* at ≤1 μg/ml, with the exception of *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* for which the MICs for 50% of strains tested (MIC_{50}s) were 4, 8, and 2 μg/ml, respectively. The MICs for 90% of strains tested (MIC_{90}s) for the *Enterobacteriaceae*, with the exception of the three aforementioned species, ranged from 1 to 8 μg/ml. The MIC_{90}s for *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* were ≥32 μg/ml. R-3746 was markedly more active than cephalexin and cefaclor against all aerobic, gram-negative species. It was generally somewhat less active than cefixime against most gram-negative species, except for *Enterobacter* spp. and *Morganella morganii*. R-3746 inhibited *Klebsiella* spp. that were resistant to amoxicillin-clavulanate and trimethoprim. R-3746 did not inhibit the *Pseudomonas* spp., which included *P. aeruginosa*, *P. cepacia*, *P. maltophilia*, *P. stutzeri*, and *P. fluorescens*; and most *Acinetobacter* spp. were resistant, with MICs of ≥16 μg/ml.

R-3746 was significantly more active than cefaclor and cephalexin against *Haemophilus influenzae*, *Branhamella catarrhalis*, and *Neisseria gonrrohoeae*, which included β-lactamase-producing isolates. It was, however, slightly less active than cefixime.

With respect to gram-positive bacteria (Table 2), R-3746 had activity similar to those of cefaclor and cephalexin and superior to that of cefixime against staphylococci. A total of 50% of methicillin-susceptible *Staphylococcus aureus* were inhibited by 4 μg/ml, and 50% of *Staphylococcus epidermidis* were inhibited by 8 μg/ml. None of the oral cephalosporins inhibited methicillin-resistant staphylococci. The majority of hemolytic streptococci (groups A, B, C, F, and G) were inhibited by 0.25 μg/ml, and *Streptococcus pneumoniae* was inhibited by 0.03 μg/ml. None of the isolates were penicillin resistant. R-3746 was slightly more active than cefixime and was markedly more active than cefaclor and cephalexin against streptococcal species. *Enterococcus faecalis* were resistant to R-3746, as was 20% of the viridans group streptococci. *Listeria monocytogenes* and *Corynebacterium* sp. strain JK were resistant to R-3746, as was *Bacteroides fragilis*. Most *Clostridium perfringens* were

TABLE 2. Activity of R-3746 against gram-positive and anaerobic organisms compared with those of other agents

Organism (no. tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (25)	R-3746	1–16	4	8
	Cefixime	4–64	16	16
	Cefaclor	1–16	2	8
	Cephalexin	0.5–16	2	8
Staphylococcus aureus, methicillin resistant (20)	R-3746	16–>64	32	>64
	Cefixime	16–>64	64	>64
	Cefaclor	16–>64	32	>64
	Cephalexin	16–>64	32	>64
Coagulase-negative staphylococci, methicillin susceptible (25)	R-3746	8–>32	8	16
	Cefixime	4–>64	16	64
	Cefaclor	0.5–8	2	8
	Cephalexin	0.5–8	2	8
Coagulase-negative staphylococci, methicillin resistant (20)	R-3746	4–>128	16	>128
	Cefixime	4–>64	16	64
	Cefaclor	>64	>64	>64
	Cephalexin	>64	>64	>64
Streptococcus pyogenes (20)	R-3746	0.008–0.06	0.03	0.06
	Cefixime	≤0.015–0.25	0.06	0.25
	Cefaclor	0.06–1	0.5	1
	Cephalexin	0.12–4	0.25	2
Streptococcus agalactiae (20)	R-3746	0.03–0.25	0.06	0.25
	Cefixime	0.03–0.25	0.12	0.25
	Cefaclor	1–16	1	4
	Cephalexin	1–8	4	4
Streptococcus spp., groups C, F, and G (17)	R-3746	≤0.06–1	≤0.06	0.25
	Cefixime	0.12–1	0.25	0.5
	Cefaclor	0.25–4	1	4
	Cephalexin	0.25–4	1	4
Streptococcus bovis (15)	R-3746	0.03–2	0.25	2
	Cefixime	0.03–2	0.5	2
	Cefaclor	0.25–4	0.5	4
	Cephalexin	0.25–4	0.5	4
Enterococcus faecalis (20)	R-3746	1–>128	>128	>128
Streptococcus pneumoniae (20)	R-3746	≤0.015–0.12	≤0.015	0.03
	Cefixime	≤0.015–0.5	0.06	0.25
	Cefaclor	0.25–1	0.5	1
	Cephalexin	0.25–4	0.5	4
Viridans group streptococci (20)	R-3746	0.015–64	0.25	32
	Cefixime	≤0.015–>128	0.5	16
	Cefaclor	0.25–4	0.5	4
	Cephalexin	0.25–4	0.5	4
Listeria monocytogenes (20)	R-3746	2–>32	32	>32
	Cefixime	4–>128	64	64
	Cefaclor	4–128	32	64
	Cephalexin	16–128	64	128
Corynebacterium sp. group JK (10)	R-3746	0.5–64	16	64
Bacteroides fragilis (20)	R-3746	32–>128	>128	>128
	Cefixime	8–>128	16	>128
	Cefaclor	>128	>128	>128
	Cephalexin	>128	>128	>128
Clostridium perfringens (10)	R-3746	0.5–4	0.5	4
Clostridium spp., others[a] (10)	R-3746	2–32	4	32

[a] Includes *C. difficile* (4 isolates), *C. novyi* (2 isolates), *C. septicum* (2 isolates), and *C. ramosum* (2 isolates).

TABLE 3. Comparison of MICs and MBCs of R-3746

Organism (no. tested)	Geometric mean concn (μg/ml)			
	MIC Range	MIC	MBC Range	MBC
Escherichia coli (5)	0.5–2	0.9	0.5–2	1
Klebsiella pneumoniae (5)	0.5–2	1	0.5–2	1.5
Enterobacter cloacae (5)	0.12–2	0.8	0.25–16	2
Proteus mirabilis (5)	0.06–0.12	0.1	0.25–1	0.5
Serratia marcescens (5)	0.5–4	1.7	8–32	14

inhibited by ≤4 μg/ml, but four *Clostridium difficile* tested were resistant.

Influence of growth conditions. MICs of R-3746 differed by no more than one doubling dilution against five isolates each of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, Proteus mirabilis, and *Serratia marcescens*, when tested on Mueller-Hinton agar, brain heart infusion agar, or nutrient agar. The MICs for 12 isolates each of *Escherichia coli* and *Staphylococcus aureus* were identical or within twofold, when determined under aerobic and anaerobic conditions. The MICs for *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* determined at pH 6.5 were within a twofold dilution of the MICs at pH 7.5.

The comparison of MICs and MBCs is shown in Table 3 for selected gram-negative species. All of the isolates produced β-lactamases. The MBCs were identical or within a doubling dilution of the MICs, with the exception of that for *Serratia marcescens*. The inoculum size had only a minor effect on the MICs for *Escherichia coli*; *Klebsiella pneumoniae*, and *Proteus mirabilis*; but at 10^7 CFU the MICs were markedly increased from geometric mean values of 0.06 to 16 μg/ml for *Enterobacter cloacae*, and for *Serratia marcescens* they were 1.4 to >128 μg/ml.

Stability to β-lactamases. The stability of R-3746 against hydrolysis by a number of different β-lactamases was determined. The relative rates of hydrolysis are shown in Table 4. R-3746 was relatively stable against attack by the common plasmid β-lactamases of the Richmond-Sykes type III and also the chromosomally mediated β-lactamases of *Pseudomonas aeruginosa* and *Enterobacter cloacae* P99. The cefuroxime-type enzyme of *Proteus vulgaris*, Richmond-Sykes type Ic, partially hydrolyzed R-3746; but the *Klebsiella oxytoca* K1 enzyme only minimally attacked R-3746. R-3746 at 100 μM only modestly inhibited the β-lactamase activity of TEM-1, with a 32% inhibition compared with a 100% inhibition for clavulanate at the same concentration. Conversely, R-3746 had a K_i of 8.2 μM for the P99 β-lactamase of *Enterobacter cloacae* and a 100 μM produced 97% inhibition, which was comparable to that found with cefotaxime at an equivalent concentration.

Time kill studies and effect on porin-defective bacteria. The effect of concentrations of R-3746 at two-, four-, and eightfold above the MIC was determined for *Escherichia coli* and *Klebsiella pneumoniae*. At twice the MIC there was a 3-log-unit decline in CFU by 4 h and no increase at 24 h. At four- and eightfold above the MIC, there was a decline in CFU at 24 h. There was no regrowth after exposure of either *Escherichia coli* or *Klebsiella pneumoniae* to two-, four-, and eightfold above the MIC. In contrast, for the same organisms there was regrowth at 24 h with both cephalexin and cefaclor at twofold above the MIC, but not at eightfold above the MIC.

The MIC and MBC for *Escherichia coli* JK, which was obtained from H. Nakaido and which possesses outer membrane proteins (OMPs) F and C and a mutant lacking OMPF, were determined. The MIC and MBC for the parent $OMPF^+$ $OMPC^+$ was 1 μg/ml, while that for $OMPF^-$ $OMPC^+$ was 2 μg/ml and for $OMPF^+$ $OMPC^-$ it was 0.5 μg/ml.

DISCUSSION

The synthesis of the aminothiazolyl iminomethoxy cephalosporins provided parenteral cephalosporins with excellent activity against most members of the family *Enterobacteriaceae* and increased the activity of cephalosporins against streptococcal species, with only a modest decrease in antistaphylococcal activity (5). Only recently have orally administered cephalosporins with in vitro activities similar to those of cefotaxime, ceftizoxime, and ceftriaxone been synthesized. Cefixime, which possesses a vinyl group at position 3 of the dihydrothiazine component of the cephem nucleus, has been shown to have activity against many organisms that are resistant to the older cephalosporins and to be β-lactamase stable (3, 6). Cefixime has excellent pharmacokinetic properties (1) and has been used clinically with success to treat a variety of respiratory, skin structure, and urinary tract infections. Ceftetrame and cefetamet are two other orally administered cephalosporins that have been shown to be active against many ampicillin-, cephalexin-, and cefaclor-resistant bacteria (2, 7). R-3746, which has a CH_2OCH_3 moiety at position 3 of the cephem nucleus, has excellent in vitro activity against hemolytic streptococci, *Streptococcus pneumoniae*, and most *Enterobacteriaceae*, with the exception of *Enterobacter* spp., *Citrobacter freundii*, and *Serratia marcescens*. Overall, R-3746 had activity very similar to that of cefixime and to those which we and others have found previously for ceftetrame and cefetamet (2, 7). Like these other agents, R-3746 does not inhibit enterococci or *Pseudomonas* spp.; and its activity, although

TABLE 4. Stability of R-3746 against hydrolysis by β-lactamases

Richmond-Sykes classification	β-Lactamase	Organism source	Relative rate of hydrolysis of[a]:		
			R-3746	Cefaclor	Cefuroxime
III	TEM-1	*Escherichia coli*	0.4	19	0.6
III	SHV-1	*Klebsiella pneumoniae*	3.8	21	0.6
Ia	P99	*Enterobacter cloacae*	1.2	24	0.8
Ic		*Proteus vulgaris*	13.9	240	108
Id	Sabath-Abraham	*Pseudomonas aeruginosa*	2.5	ND[b]	ND
IV	K1	*Klebsiella oxytoca*	3.4	55	11
V	PSE-1	*Pseudomonas aeruginosa*	3.9	ND	ND
	PC	*Staphylococcus aureus*	<0.1	30	<0.1

[a] Based on cephaloridine concentration of 100 μg/ml.
[b] ND, Not determined.

superior to that of cefixime against staphylococci, was much less than those of cephalexin, cefaclor, or amoxicillin-clavulanate. R-3746 inhibited many of the organisms that were not inhibited by amoxicillin-clavulanate and a number of trimethoprim-resistant organisms. The β-lactamase stability of R-3746 was superior to that of cefaclor and similar to that which we have found previously for cefixime (6). Although R-3746 was stable against attack by *Enterobacter* β-lactamases at 10^{-4}M, it is probable that it is hydrolyzed at the low concentrations in the periplasmic space, as are broad-spectrum cephalosporins, albeit at a low rate, which accounts for the high MIC_{90}s and the effect of a 10^7 inoculum on the MICs.

Recently, Utsui et al. (9) have shown that R-3746 binds to a lower degree to penicillin-binding protein of *Staphylococcus aureus* than does cefaclor, which could explain its lower activity against staphylococci. Utsui et al. (9) found MICs similar to those found in this study and similar β-lactamase stabilities. The one difference was the lower MICs for *Staphylococcus aureus* of 1.6 μg/ml compared with an MIC_{50} of 4 μg/ml in our study. Nishino et al. (T. Nishino, H. Takenouchi, M. Otsuki, and T. Tanino, 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 661, 1987) found an MIC_{90} of 3.13 μg/ml for R-3764, and Arai et al. (K. Arai, E. Suzuki, and T. Yokota, 27th ICCAC, abstr. no. 660, 1987) found a similar MIC_{90} (3.13 μg/ml).

Sawae et al. (Y. Sawae, E. Eto, and S. Ueda, 27th ICAAC abstr. no. 666, 1987) showed that R-3746, in an ester derivative named CS-807, yielded mean peak concentrations in serum of 2.86 μg/ml, with a half-life of 2 h after a 200-mg dose. Based on the results of in vitro studies, mouse protection studies (9), and these pharmacokinetics, it seems that, in its ester form, R-3746 is an appropriate agent to be investigated as a therapy for respiratory, skin structure, and urinary tract infections.

LITERATURE CITED

1. **Brittain, D. C., B. E. Scully, T. Hirose, and H. C. Neu.** 1985. The pharmacokinetic and bactericidal characteristics of oral cefixime. Clin. Pharmacol. Ther. **38**:590–594.
2. **Fass, R. J., and V. L. Helsel.** 1986. In vitro activities of Ro 19-5247 and Ro 15-8074, new oral cephalosporins. Antimicrob. Agents Chemother. **30**:429–434.
3. **Kamimura, T., H. Kojo, Y. Matsumoto, Y. Mine, S. Goto, and S. Kawahara.** 1984. In vitro and in vivo antibacterial properties of FK027, a new orally active cephem antibiotic. Antimicrob. Agents Chemother. **25**:98–104.
4. **Neu, H. C.** 1986. Antibiotic inactivating enzymes and bacterial resistance, p. 757–789. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
5. **Neu, H. C.** 1987. Beta-lactam antibiotics: structural relationships affecting in vitro activity and pharmacologic properties. Rev. Infect. Dis. **8**(Suppl):237–259.
6. **Neu, H. C., N. X. Chin, and P. Labthavikul.** 1984. Comparative in vitro activity and beta-lactamase stability of FR 17027, a new orally active cephalosporin. Antimicrob. Agents Chemother. **26**: 174–180.
7. **Neu, H. C., N. X. Chin, and P. Labthavikul.** 1986. In vitro activity and beta-lactamase stability of two oral cephalosporins, ceftetrame (Ro 19-5247), and cefetamet (Ro 15-8074). Antimicrob. Agents Chemother. **30**:423–428.
8. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal concentrations. Antimicrob. Agents Chemother. **18**:699–708.
9. **Utsui, Y., M. Ingue, and S. Mitsuhashi.** 1987. In vitro and in vivo antibacterial activity of CS-807, a new oral cephalosporin. Antimicrob. Agents Chemother. **31**:1085–1092.