ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1992, p. 2360–2364
0066-4804/92/112360-05$02.00/0

Vol. 36, No. 11

In Vitro Activity of RU 29246, the Active Compound of the Cephalosporin Prodrug Ester HR 916

G. RIESS,[1] J. ANDREWS,[2] D. THORNBER, AND R. WISE[2]*

Department of Microbiology, Dudley Road Hospital, Birmingham, United Kingdom,[2] and Hoechst AG, 6230 Frankfurt am Main 80, Germany[1]

Received 23 April 1992/Accepted 13 August 1992

The in vitro activity of RU 29246 was compared with those of other agents against 536 recent clinical isolates. The MICs of RU 29246 for 90% of members of the family *Enterobacteriaceae* tested (MIC_{90}s) were less than 2 μg/ml except those for *Morganella* spp. (16 μg/ml) and *Proteus* spp. (8 μg/ml). RU 29246 was active against *Staphylococcus aureus* (MIC_{90}, ≤8 μg/ml) and against *Staphylococcus saprophyticus* and coagulase-negative staphylococci (MIC_{90}s, ≤2 μg/ml). Streptococci and *Neisseria gonorrhoeae* were highly susceptible to RU 29246, and the activity of the agent against isolates of *Streptococcus pneumoniae* (MIC_{90}, ≤0.5 μg/ml), *Haemophilus influenzae* (MIC_{90}, ≤2 μg/ml), and *Moraxella catarrhalis* (MIC_{90}, ≤2 μg/ml) was comparable to those of the other cephalosporins tested. RU 29246 was insusceptible to hydrolysis by the common plasmid-mediated β-lactamases (TEM-1 and SHV-1). However, hydrolysis by the new extended-spectrum β-lactamases (TEM-3, TEM-5, and TEM-9) was detected. Results of the study suggested that RU 29246 should be investigated clinically for use in the treatment of a wide range of infections.

The aminothiazolyl cephalosporin RU 29246 is the active metabolite of the prodrug pivaloyl oxyethyl ester HR 916. Bioconversion occurs during drug absorption. For stability reasons, this compound must be used in vivo as a toluenesulfonic salt, which is called HR 916B. Preliminary studies suggest that the in vitro activity of RU 29246 includes a wide range of clinically relevant pathogens, among them staphylococci, streptococci, and members of the family *Enterobacteriaceae* (1, 5). RU 29246 is characterized by a methoximino group at the 3 position of the cephem nucleus (1). Because of its low level of absorption from the gastrointestinal tract, the compound is not suitable for oral administration. A prodrug approach (esterification of the carboxyl group) provides the necessary increase in lipophilicity for absorption from the gastrointestinal tract. In the study described here, we investigated the in vitro activity of the active metabolite RU 29246 against a wide range of pathogenic bacteria. As comparative agents, cephalosporins which also exhibited a broad spectrum of antibacterial activity were chosen.

MATERIALS AND METHODS

A total of 560 strains were tested, of which 536 were recent clinical isolates from different patients. The remainder were well-characterized resistant strains. The antibiotics and their sources were as follows: HR 916 (RU 29246 as the active metabolite; Hoechst AG, Frankfurt, Germany), cefuroxime (Glaxo, Greenford, United Kingdom), cefixime (Lederle, Farnham, United Kingdom), cefpodoxime (Roussel, Uxbridge, United Kingdom), cefaclor (Lilly, Windlesham, United Kingdom), and cephaloridine (Glaxo).

Susceptibility testing. The susceptibilities of the strains were studied by using a routine agar plate dilution method. The inocula were prepared as follows. For all strains except streptococci (including *Streptococcus pneumoniae*), enterococci, *Neisseria* spp., *Haemophilus influenzae*, and anaerobes, the organisms were grown overnight in digest broth (Southern Group, Hither Green Hospital, Lewisham, United Kingdom) to yield a viable count of about 10^9 CFU/ml. Streptococci, enterococci, *Haemophilus influenzae*, and *Neisseria* spp. were grown in Todd-Hewitt broth (CM189; Oxoid, Basingstoke, United Kingdom) plus 20 μg of NAD (Sigma Chemicals, Poole, United Kingdom) per ml. *Bacteroides fragilis* was grown in Wilkins-Chalgren anaerobe broth (CM643; Oxoid) supplemented with 25% sodium succinate (BDH Chemicals, Poole, United Kingdom), and *Clostridium* spp. were grown in Wilkins-Chalgren anaerobe broth supplemented with 1% Tween 80 (BDH). The viable counts in each broth were comparable.

One microliter of a 1:100 dilution of an overnight culture or, for a few selected strains for which increased inoculum sizes were to be studied, an undiluted inoculum was transferred to the surface of the antibiotic-containing agar by using a multipoint inoculator (Denley-Tech, Billingshurst, England). The final sizes of the inocula on the plates were therefore 10^4 and 10^6 CFU, respectively. The medium used for the agar dilution procedure was Iso-Sensitest agar (pH 7.2; CM885B; Oxoid) supplemented with 5% lysed horse blood plus 20 mg of NAD per liter to support growth of streptococci, *Haemophilus influenzae*, and *Neisseria* spp.; for anaerobes, Wilkins-Chalgren agar plus 5% horse blood was used.

All plates were incubated in air at 35 to 37°C for 24 h, except that the anaerobes were grown in an anaerobic cabinet (Don Whitely, Skipton, United Kingdom) in an atmosphere of 10% hydrogen–10% carbon dioxide–80% nitrogen. *Haemophilus influenzae* and *Neisseria* spp. were incubated in air enriched with 4 to 6% carbon dioxide. The MIC of the antibiotic was defined as that concentration at which no more than three colonies were detected. With the larger inoculum size, a slight haze of growth was ignored.

The effects of 0, 20, and 70% human serum (Bradsure Biologicals, Market Harborough, United Kingdom) on the MICs and MBCs of RU 29246 were studied with two strains each of *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Escherichia coli* and six strains of *Staphylococcus aureus* by a method based on that of Pearson et al. (8).

* Corresponding author.

TABLE 1. Activity of RU 29246 in comparison with those of other antimicrobial agents

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
Escherichia coli (49)	RU 29246	0.5	1	0.5–16
	Cefuroxime	2	4	1–64
	Cefixime	0.06	0.12	0.008–16
	Cefpodoxime	0.25	0.5	0.12–16
	Cefaclor	1	4	0.5–32
Klebsiella spp. (50)	RU 29246	0.5	2	0.25–4
	Cefuroxime	2	8	0.12–32
	Cefixime	0.015	0.25	0.004–0.5
	Cefpodoxime	0.06	0.5	0.03–2
	Cefaclor	0.25	4	0.25–8
Proteus mirabilis (50)	RU 29246	0.5	2	0.5–16
	Cefuroxime	1	4	0.5–>128
	Cefixime	0.008	0.008	0.004–4
	Cefpodoxime	0.06	0.5	0.03–8
	Cefaclor	1	4	0.5–>128
Morganella morganii (19)	RU 29246	1	16	0.5–128
	Cefuroxime	16	64	2–>128
	Cefixime	0.12	0.5	0.03–>128
	Cefpodoxime	0.12	2	0.12–>128
	Cefaclor	>128	>128	16–>128
Proteus vulgaris (22)	RU 29246	4	8	0.5–32
	Cefuroxime	64	>128	4–>128
	Cefixime	0.008	0.015	0.004–0.06
	Cefpodoxime	0.25	1	0.06–4
	Cefaclor	128	>128	1–>128
Staphylococcus aureus (35) (including 4 methicillin-resistant strains)	RU 29246	2	8	1–>128
	Cefuroxime	1	8	0.5–>128
	Cefixime	8	32	4–>128
	Cefpodoxime	2	16	1–>128
	Cefaclor	2	32	0.5–>128
Staphylococcus saprophyticus (20)	RU 29246	1	2	1–4
	Cefuroxime	1	2	0.5–4
	Cefixime	32	64	8–64
	Cefpodoxime	4	8	1–8
	Cefaclor	2	4	1–8
Staphylococcus epidermidis (19)	RU 29246	0.5	2	0.25–4
	Cefuroxime	0.25	2	0.12–2
	Cefixime	8	16	1–64
	Cefpodoxime	0.5	4	0.25–8
	Cefaclor	0.5	2	0.25–8
Group A streptococci (8)	HR916			0.03–0.06
	Cefuroxime			0.008–0.03
	Cefixime			0.06–0.12
	Cefpodoxime			0.015–0.03
	Cefaclor			0.06–0.12
Enterococcus faecalis (9)	RU 29246			2–>128
	Cefuroxime			2–>128
	Cefixime			2–>128
	Cefpodoxime			1–>128
	Cefaclor			32–>128
Streptococcus pneumoniae (22)	RU 29246	0.25	0.5	0.03–4
	Cefuroxime	0.015	0.25	0.004–2
	Cefixime	0.25	1	0.12–8
	Cefpodoxime	0.03	0.12	0.015–1
	Cefaclor	0.25	0.5	0.06–32

Continued on following page

TABLE 1—*Continued*

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
Moraxella catarrhalis (26)	RU 29246	1	2	0.5–2
	Cefuroxime	1	2	0.5–8
	Cefixime	0.12	0.5	0.06–0.5
	Cefpodoxime	0.5	1	0.12–1
	Cefaclor	0.5	1	0.5–4
Haemophilus influenzae (34) (including 13 β-lactamase producers)	RU 29246	1	2	0.5–4
	Cefuroxime	1	2	0.5–4
	Cefixime	0.06	0.25	0.03–0.5
	Cefpodoxime	0.12	0.5	0.03–0.5
	Cefaclor	2	8	1–32
Neisseria gonorrhoeae (21) (including 3 penicillinase producers)	RU 29246	0.015	0.06	0.004–0.12
	Cefuroxime	0.015	0.12	0.004–0.5
	Cefixime	0.004	0.008	0.004–0.008
	Cefpodoxime	0.004	0.015	0.004–0.03
	Cefaclor	0.12	0.5	0.06–0.5
Bacteroides fragilis (24)	RU 29246	16	>128	8–>128
	Cefuroxime	8	>128	4–>128
	Cefixime	16	>128	4–>128
	Cefpodoxime	32	>128	8–>128
	Cefaclor	>128	>128	32–>128
Peptostreptococci (15)	RU 29246	0.5	1	0.015–8
	Cefuroxime	0.25	1	0.12–8
	Cefixime	1	2	0.5–8
	Cefpodoxime	0.25	0.5	0.12–8
	Cefaclor	0.5	16	0.12–32
Clostridium spp. (9)[b]	RU 29246			0.12–2
	Cefuroxime			0.25–16
	Cefixime			1–32
	Cefpodoxime			0.12–4
	Cefaclor			0.12–1

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] Seven *C. perfringens*, one *C. sporogenes*, and one *C. tertium*.

Increasing concentrations of RU 29246 were prepared in Iso-Sensitest broth (CM473; Oxoid) and broth containing human serum. Overnight broth cultures were diluted to inoculate the broth to give a final inoculum of 10^5 CFU/ml. After overnight incubation at 35 to 37°C, the MIC was defined as that concentration which gave no visible growth. Lethality of 99.9% was determined by subculture of 0.1 ml into antibiotic-free medium.

Protein binding. Protein binding of RU 29246 was determined by using a micropartition system (Centrifree System; Amicon, Beverly, Mass.). Concentrations of 5, 10, and 20 μg/ml were prepared in pooled human serum (Bradsure Biologicals). After centrifugation, protein-free extracts were assayed against standards prepared in phosphate buffer (pH 6.6) by using Antibiotic Medium No. 1 (CM327; Oxoid) and *Escherichia coli* NCTC 10418.

β-Lactamase stability. The enzymes were studied by using cell-free sonic extracts from the strains containing the single characterized enzymes and were prepared from midexponential-phase cultures in tryptone soy broth as described previously (2, 6). BRO-1 was released from sonicated cell debris by suspension in 4% Triton X-100. Preparative isoelectric focusing was carried out on ampholine Ultrodex gel with an LKB Multiphor II electrophoretic unit (Pharmacia,

TABLE 2. Susceptibility of RU 29246 to various β- lactamases

Antibiotic	TEM-1			TEM-3			TEM-5			TEM-9			SHV-1			K-1			BRO-1		
	V_{max}[a]	K_m (μM)	Rel V/K[b]	V_{max}	K_m (μM)	Rel V/K	V_{max}	K_m (μM)	Rel V/K	V_{max}	K_m (μM)	Rel V/K	V_{max}	K_m (μM)	Rel V/K	V_{max}	K_m (μM)	Rel V/K	V_{max}	K_m (μM)	Rel V/K
Cephaloridine	100	ND[c]	100	100	38	100	100	173	100	100	122	100	100	ND	100	100	39	100	100	6	100
Cefuroxime	<0.1	ND	<0.1	46	86	20	6.6	356	3.2	29	581	6.0	<0.1	ND	<0.1	27	70	15	62	143	2.6
Cefpodoxime	<0.1	ND	<0.1	75	38	77	1.3	26	8.4	33	48	85	<0.1	ND	<0.1	1.9	41	1.8	85	270	1.9
Cefixime	<0.1	ND	<0.1	6.5	28	9.0	0.7	11	11	20	357	6.8	<0.1	ND	<0.1	0.15	62	0.1	7.3	55	0.8
RU 29246	<0.1	ND	<0.1	75	71	40	2.5	91	4.7	13	64	24.2	<0.1	ND	<0.1	3.9	69	3.1	67	76	5.3

[a] V_{max} values are relative to cephaloridine (100%).
[b] Rel V/K, relative V_{max}/K_m.
[c] ND, not determined (rates too slow to be accurately determined).

Milton Keynes, United Kingdom) with a constant 2.5-kV power supply. Analytical isoelectric focusing was carried out on LKB ampholine polyacrylamide gel plates by using the electrophoretic unit described above. Nitrocefin was used to detect β-lactamase activity.

Hydrolysis studies were carried out on cephaloridine, cefixime, cefuroxime, cefpodoxime, and RU 29240 with a temperature-controlled UV spectrophotometer (Lambda 2; Perkin-Elmer, Beaconsfield, England) at 37°C. All antibiotic solutions were prepared immediately before use in 50 mM phosphate buffer (pH 7), and the reactions were monitored at 260 nm, but the reaction for cefixime was recorded at 290 nm. The reported kinetic constants were derived by half-time analysis of single reaction progress curves (7). The relative V_{max}/K_m values were compared with that of cephaloridine, which was taken to be 100%. The value calculated for V_{max}/K_m can be taken as a measure of the efficiency of hydrolysis as defined by Pollock (9).

RESULTS

The activity of RU 29246 is given in Table 1 and is compared with those of the other cephalosporins. A higher inoculum size (10^6 instead of 10^4 CFU per spot) did not produce a significant influence on the MICs, with the exception of those for three strains of *Proteus vulgaris*, in which the higher inoculum size produced a 32-fold increase in the MICs for the strains.

Against members of the family *Enterobacteriaceae*, RU 29246 displayed activities similar to those of the other cephalosporins. Against *Escherichia coli*, RU 29246 was more active than cefuroxime and cefaclor but it was less active than cefixime and cefpodoxime. The same applied to the results obtained against *Klebsiella* spp., *Proteus mirabilis*, *Proteus vulgaris*, and *Morganella morganii*.

Against methicillin-susceptible strains of *Staphylococcus aureus*, RU 29246 showed activity comparable to that of cefuroxime, while it was two to four times more active than cefixime, cefpodoxime, and cefaclor. The four methicillin-resistant strains were less susceptible to RU 29246 (MIC, ≥8 μg/ml) and the other agents. Against *Staphylococcus epidermidis*, the MIC of RU 29246 for 90% of strains tested was comparable to those of cefuroxime and cefaclor, but the new agent was twofold more active than cefpodoxime and at least eightfold more active than cefixime. A similar result was obtained for *Staphylococcus saprophyticus*.

All *Neisseria* spp. were susceptible to the cephalosporins tested, with cefixime and cefpodoxime being the most active compounds, while RU 29246 was as active as cefuroxime but more active than cefaclor. Those strains of *Neisseria gonorrhoeae* which produced β-lactamase were as susceptible to RU 29246 as the β-lactamase nonproducers were.

Among the respiratory pathogens tested, *Streptococcus pneumoniae* was susceptible to all the cephalosporins tested. Interestingly, three strains that had reduced susceptibilities to penicillin also showed reductions in their susceptibilities to the cephalosporins (0.5 to 8 μg/ml). The strains of *Haemophilus influenzae* and *Moraxella catarrhalis* were susceptible to all the agents studied. The β-lactamase producers of both genera were as susceptible as the β-lactamase nonproducers were.

Lancefield group A and B streptococci were susceptible to RU 29246 and the other compounds tested. *Enterococcus faecalis* was insusceptible to RU 29246, as it was to the other cephalosporins. Against strains of *Streptococcus milleri* and *Streptococcus sanguis* (data not shown), RU 29246 (mode MIC, 2 μg/ml) was more active than cefixime (mode MIC, 8 μg/ml) but was not as active as cefpodoxime and cefuroxime (mode MICs, 0.25 μg/ml). Again, strains that showed a reduced susceptibility to penicillin were also less susceptible to the cephalosporins.

None of the cephalosporins had significant activity against *Bacteroides fragilis*. They showed activity against the anaerobic streptococci and *Clostridium* spp. tested.

In the absence of serum there was no difference or, at most, there was a two- to fourfold difference between the MICs and MBCs except for those for one strain of *Staphylococcus aureus*. The addition of up to 70% human serum to the medium had no significant effect. The protein bindings of RU 29246 at concentrations of 5, 10, and 20 μg/ml were 69.4, 59.6, and 59%, respectively.

In Table 2 the relative efficiency of hydrolysis of RU 29246 to seven β-lactamase preparations is shown. RU 29246 showed marked insusceptibility to hydrolysis by TEM-1 and SHV-1, but relative to cefixime, RU 29246 showed susceptibility to TEM-3 and TEM-9 enzymes. The chromosomal enzyme K-1 showed hydrolytic activity against RU 29246; cefixime was the least susceptible stable substrate to β-lactamases. RU 29246 was the least susceptible of the compounds to the BRO-1 enzyme.

DISCUSSION

A variety of cephalosporins are now available for oral therapy, some being absorbed intact and others, such as HR 916, being administered as a prodrug (3, 4). Unfortunately, the enhanced activity against members of the family *Enterobacteriaceae* has, in most cases, been achieved at the expense of less activity against staphylococci.

RU 29246 has many in vitro similarities to the other cephalosporins tested in the study described here, with the major exception that it exhibits activity against methicillin-susceptible staphylococci, hemolytic streptococcal groups A and B, and penicillin-susceptible *Streptococcus pneumoniae*.

In common with other cephalosporins, RU 29246 is a bactericidal compound, with little difference between the MICs and MBCs. The presence of serum generally altered the MIC only by two- to fourfold, which is reflected in the low protein binding of the agent.

Although RU 29246 was insusceptible to hydrolysis by the common TEM-1 and SHV-1 enzymes, it was hydrolyzed by the extended-spectrum plasmid-mediated enzymes, TEM-3, TEM-5, and TEM-9. The susceptibility to the BRO-1 enzyme of *Moraxella catarrhalis* accords well with the higher observed MIC of RU 29246 for 90% of strains tested (MIC_{90}) (2 μg/ml) compared with that of cefixime (MIC_{90}, 0.5 μg/ml), which was less susceptible to hydrolysis.

RU 29246 demonstrated broad-spectrum in vitro activity against both staphylococci and most members of the family *Enterobacteriaceae*. The relevance of the new compound for antibacterial therapy will depend on the bioavailability of its prodrug ester HR 916.

ACKNOWLEDGMENT

We thank Hoechst AG for financial support for the study.

REFERENCES

1. **Adam, F., J. Blumbach, W. Durckheimer, B. Mencke, and T. Wollmann.** 1991. HR916B, a new oral cephalosporin. I. Synthesis and structure-activity relations. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 835.

2. **Ashby, J. P., B. Kirkpatrick, L. J. V. Piddock, and R. Wise.** 1987. The effect of imipenem on strains of Enterobacteriaceae. J. Antimicrob. Chemother. **20:**15–22.
3. **Bauernfeind, A.** 1991. Comparative antimicrobial spectrum and activity of ceftibuten against clinical isolates from West Germany. Diagn. Microbiol. Infect. Dis. **14:**63–74.
4. **Bauernfeind, A., and R. Jungwirth.** 1991. In vitro evaluation of cefpodoxime, a new oral cephalosporin of the third generation. Infection **19**(Suppl. 5):353–362.
5. **Bauernfeind, A., and R. Jungwirth.** 1991. HR 916B, a new oral cephalosporin. II. Antibacterial activity in vitro. Prog. Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 836.
6. **Diver, J. M., D. Thornber, and R. Wise.** 1989. Protection of piperacillin and ticarcillin from β-lactamase hydrolysis by tazobactam (YTR 830) and clavulanic acid. J. Antimicrob. Chemother. **24:**23–28.
7. **Nichols, W. W., and R. G. Hewinson.** 1987. Rapid and automated measurement of Km and Vmax values of β-lactamases in bacterial extracts. J. Antimicrob. Chemother. **19:**285–289.
8. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemother. **18:**699–708.
9. **Pollock, M. R.** 1965. Purification and properties of penicillinase from two strains of Bacillus licheniformis: a chemical, physiochemical and physiological comparison. Biochem. J. **94:**666–675.