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Comparison of In Vitro Activity of GR 20263, a Novel Cephalosporin Derivative, with Activities of Other Beta-Lactam Compounds

R. WISE,* J. M. ANDREWS, AND K. A. BEDFORD

Department of Medical Microbiology, Dudley Road Hospital, Birmingham B18 7QH, United Kingdom

The in vitro activity of GR 20263, a new cephalosporin, was compared primarily with the activities of moxalactam (LY 127935), cefotaxime, cefoxitin, cefuroxime, and cefazolin against 293 clinical isolates of a variety of gram-positive and -negative bacteria. The minimal inhibitory concentrations of GR 20263 for 90% of group isolates were between 0.06 and 0.5 μg/ml for the *Enterobacteriaceae*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and Lancefield group A β-hemolytic streptococci; 2 μg/ml for *Pseudomonas aeruginosa*; 16 μg/ml for *Staphylococcus aureus*; and in excess of 128 μg/ml for *Bacteroides fragilis* and Lancefield group D streptococci. In comparison with the other agents, GR 20263 was markedly more active against the *Enterobacteriaceae* than cefuroxime, cefoxitin, and cefazolin, but marginally less active than moxalactam or cefotaxime. Against *S. aureus*, cefazolin was 16-fold and cefotaxime was 4-fold more active than GR 20263 and moxalactam. GR 20263 was eight-fold more active than cefotaxime and moxalactam against *P. aeruginosa*.

GR 20263 is a new parenteral aminothiazolyl cephalosporin from Glaxo Group Research which combines stability to a wide range of β-lactamases, a broad antibacterial spectrum, and low toxicity (3). In this study a wide range of recent clinical isolates were tested gainst GR 20263 and other relevant β-lactams, in particular cefotaxime (1, 7) and moxalactam, the novel oxa-β-lactam previously designated LY 127935 (2,6).

MATERIALS AND METHODS

Organisms and antimicrobial agents. A total of 293 strains were examined in this study, of which 284 are listed in Table 1. Of the total, 290 were recent clinical isolates; of the remaining 3, 1 was a β-lactamase-producing *Neisseria gonorrhoeae* strain from W.A. Ashford (Travis Air Force Base, Fairfield, Calif.), and 2 were Tem^+ strains of *Pseudomonas aeruginosa* from E. Lowbury (Birmingham, U.K.). All strains were identified by the API (API Laboratory Products Ltd., Farnborough, U.K.) method. β-Lactamase production by selected strains was verified by the use of nitrocefin (4).

Antibiotics of known potency were obtained from the following sources: GR 20263 and cefuroxime, Glaxo Research, Greenford, Middlesex, U.K.; cefotaxime, Roussel Laboratories, Wembley, U.K.; moxalactam and cefazolin, Lilly Research, Windlesham, U.K.; cefoxitin, Merck Sharp & Dohme, Hoddesdon, U.K.; ampicillin, penicillin, and carbenicillin, Beecham Research Laboratories, Brentford, U.K.; and azlocillin, Bayer Pharmaceuticals, Haywards Heath, U.K.

Methods. With the exceptions noted below, the activities of the β-lactams were measured by an agar plate dilution method using Isosensitest agar (pH 7.2; Oxoid, Basingstoke, U.K.). This medium was supplemented with 5% lysed human blood (in studies with *Bacteroides fragilis*), with a Levinthals preparation (in studies with *Haemophilus influenzae*), and with chocolate agar (in studies with *N. gonorrhoeae*).

With the exceptions of *B. fragilis*, *N. gonorrhoeae*, *H. influenzae*, and streptococci, inocula were prepared from overnight cultures in nutrient broth, yielding viable counts of about 10^9 colony-forming units (CFU) per ml. Inocula were prepared from 18-h cultures in thioglycolate broth for *B. fragilis*, in Levinthal broth for *H. influenzae*, and in Todd-Hewitt broth for streptococci, yielding about 10^9 CFU/ml. Inocula for *N. gonorrhoeae* were prepared by suspending overnight surface growth from chocolate agar in peptone water just before use, the viable count being about 10^8 CFU/ml. With the exception of *N. gonorrhoeae*, 10^3- and 10^6-CFU inocula were employed in testing all strains. This was accomplished by delivering 1 μl each of undiluted and a 1:1,000 dilution of the overnight culture to the antibiotic-containing agar plates using a multipoint inoculator (Denley Tech Ltd., Billingshurst, U.K.). Tests on *N. gonorrhoeae* were carried out with undiluted culture and a 1:10 dilution yielding final inocula of 10^5 and 10^4 CFU, respectively. Plates were incubated overnight at 37°C in air, except for the tests on *B. fragilis*, when a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) was used, and those on *H. influenzae* and *N. gonorrhoeae*, when 10% CO_2 was used.

The effects of serum on the activity of GR 20263 were studied on two strains each of *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *Staphylococcus aureus* and on one strain each of *Proteus mirabilis* and *Proteus morganii*.

The minimum inhibitory concentration (MIC) was

TABLE 1. *MICs inhibiting cumulative percentage of isolates at inocula of 10^3 and 10^6 CFU*

Organism (no. of strains)	Antibiotic	MIC (μg/ml of medium) at inoculum:					
		10^3 CFU			10^6 CFU		
		Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
E. coli (34)	GR 20263	0.06–2	0.25	0.5	0.12–4	0.25	2.0
	Cefotaxime	0.03–0.5	0.06	0.12	0.03–1.0	0.06	0.5
	Moxalactam	0.06–0.25	0.12	0.25	0.06–0.5	0.12	0.25
	Cefoxitin	1.0–16.0	4.0	8.0	2.0–32.0	4.0	16.0
	Cefuroxime	1.0–16.0	4.0	8.0	2.0–16.0	4.0	16.0
	Cefazolin	1.0–16.0	1.0	8.0	1.0–64.0	2.0	32.0
K. pneumoniae (25)	GR 20263	0.06–1.0	0.12	0.25	0.06–1.0	0.25	0.25
	Cefotaxime	0.03–0.12	0.03	0.12	0.03–0.25	0.06	0.12
	Moxalactam	0.06–0.25	0.12	0.12	0.06–0.25	0.12	0.25
	Cefoxitin	1.0–8.0	2.0	2.0	1.0–8.0	2.0	4.0
	Cefuroxime	1.0–16.0	2.0	4.0	1.0–>128	4.0	8.0
	Cefazolin	1.0–128	2.0	4.0	1.0–>128	2.0	32.0
P. mirabilis (20)	GR 20263	0.03–0.25	0.06	0.06	0.06–0.25	0.06	0.12
	Cefotaxime	0.06–0.5	0.06	0.12	0.12–0.5	0.12	0.25
	Moxalactam	≤0.008–0.25	0.015	0.03	0.015–0.5	0.03	0.12
	Cefoxitin	2.0–32.0	4.0	8.0	2.0–32.0	4.0	32.0
	Cefuroxime	2.0–32.0	2.0	4.0	2.0–32.0	2.0	16.0
	Cefazolin	2.0–64.0	4.0	32.0	4.0–>128	8.0	128
Indole-positive *Proteus* spp. (10)	GR 20263	0.03–0.25	0.06	0.25	0.03–1.0	0.06	0.5
	Cefotaxime	0.03–0.25	0.12	0.12	0.06–0.5	0.12	0.25
	Moxalactam	≤0.008–0.5	0.03	0.12	≤0.008–1.0	0.12	0.5
	Cefoxitin	0.5–16.0	2.0	8.0	1.0–16.0	4.0	16.0
	Cefuroxime	1.0–>128	16.0	128	2.0–>128	64.0	>128
	Cefazolin	16.0–>128	128	>128	64.0–>128	>128	>128
Enterobacter spp. (10)	GR 20263	0.12–0.25	0.12	0.25	0.25–0.5	0.25	0.25
	Cefotaxime	0.06–0.12	0.12	0.12	0.12–0.25	0.12	0.12
	Moxalactam	0.06–0.12	0.12	0.12	0.12–0.25	0.12	0.12
	Cefoxitin	64.0–>128	>128	>128	64.0–>128	>128	>128
	Cefuroxime	2.0–8.0	4.0	8.0	4.0–16.0	4.0	8.0
	Cefazolin	8.0–>128	64.0	>128	64.0–>128	>128	>128

TABLE 1—*Continued*

Organism (no. of strains)	Antibiotic	MIC (μg/ml of medium) at inoculum:					
		10^3 CFU			10^6 CFU		
		Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Providencia stuartii (10)	GR 20263	0.06–1.0	0.25	0.5	0.25–1.0	0.25	1.0
	Cefotaxime	0.06–0.25	0.12	0.25	0.06–0.5	0.25	0.25
	Moxalactam	0.06–0.12	0.06	0.12	0.06–0.25	0.12	0.12
	Cefoxitin	2.0–16.0	2.0	16.0	2.0–16.0	8.0	16.0
	Cefuroxime	2.0–16.0	2.0	16.0	2.0–64.0	4.0	16.0
	Cefazolin	2.0–128	16.0	32.0	4.0–>128	32.0	128
Serratia marcescens (10)	GR 20263	0.12–0.25	0.25	0.25	0.25–0.25	0.25	0.25
	Cefotaxime	0.12–0.25	0.12	0.25	0.12–0.5	0.25	0.5
	Moxalactam	0.12–0.25	0.25	0.25	0.12–0.5	0.25	0.25
	Cefoxitin	8.0–16.0	16.0	16.0	8.0–32.0	16.0	16.0
	Cefuroxime	16.0–64.0	32.0	64.0	16.0–128	64.0	128
	Cefazolin	>128	>128	>128	>128	>128	>128
P. aeruginosa (40)	GR 20263	0.5–4.0	1.0	2.0	0.5–4.0	1.0	2.0
	Cefotaxime	4.0–64.0	8.0	16.0	4.0–64.0	8.0	16.0
	Moxalactam	4.0–64.0	8.0	16.0	4.0–64.0	16.0	32.0
	Carbenicillin	8.0–>1,024	32.0	128	8.0–>1024	32.0	128
	Azlocillin	2.0–>128	4.0	8.0	2.0–>128	4.0	16.0
H. influenzae (29) (including 9 β-lactamase-positive)	GR 20263	0.03–0.12	0.06	0.12	0.03–0.12	0.06	0.12
	Cefoxatime	0.008–0.03	0.008	0.015	0.008–0.03	0.008	0.015
	Moxalactam	0.03–0.25	0.06	0.12	0.03–0.25	0.06	0.12
	Ampicillin	0.12–4.0	0.25	4.0	0.12–128	0.25	64.0
	Cefoxitin	0.5–4.0	2.0	2.0	0.05–4.0	2.0	2.0
	Cefuroxime	0.25–1.0	0.5	0.5	0.25–1.0	0.5	0.5
	Cefazolin	0.5–8.0	2.0	2.0	1.0–16.0	2.0	8.0
B. fragilis subsp. *fragilis* (31)	GR 20263	2.0–>128	16.0	>128	2.0–>128	16.0	>128
	Cefotaxime	0.5–128	2.0	32.0	0.5–>128	4.0	64.0
	Moxalactam	0.25–8.0	0.5	4.0	0.5–8.0	1.0	4.0
	Penicillin	1.0–>128	8.0	16.0	1.0–>128	8.0	16.0
	Cefoxitin	4.0–8.0	8.0	8.0	4.0–8.0	8.0	8.0
	Cefuroxime	0.25–>128	4.0	128	1.0–>128	8.0	128
	Cefazolin	2.0–>128	16.0	32.0	4.0–>128	16.0	64.0

N. gonorrhoeae (30) (including 1 β-lactamase-positive)	GR 20263	≤0.004–2.0	0.015	0.12	≤0.004–2.0	0.015	0.12
	Cefotaxime	≤0.004–0.06	≤0.004	0.015	≤0.004–0.06	≤0.004	0.015
	Moxalactam	≤0.004–0.12	0.03	0.12	≤0.004–0.12	0.03	0.12
	Penicillin	≤0.004–4.0	0.03	0.25	≤0.004–8.0	0.03	0.25
	Cefoxitin	0.12–0.5	0.12	0.5	0.12–0.5	0.12	0.5
	Cefuroxime	0.008–0.12	0.015	0.06	0.008–0.12	0.015	0.06
	Cefazolin	0.12–1.0	0.5	1.0	0.12–2.0	0.5	1.0
S. aureus (22) (including methicillin-resistant strains)	GR 20063	4.0–32.0	8.0	16.0	4.0–32.0	8.0	16.0
	Cefotaxime	1.0–16.0	2.0	4.0	1.0–32.0	2.0	4.0
	Moxalactam	4.0–16.0	8.0	16.0	4.0–32.0	8.0	16.0
	Cefoxitin	2.0–16.0	2.0	4.0	2.0–16.0	2.0	4.0
	Cefuroxime	0.25–8.0	1.0	4.0	0.25–128	2.0	4.0
	Azlocillin	0.12–4.0	0.25	1.0	0.12–64.0	0.25	1.0
Group A streptococci (8)	GR 20263	0.06–0.12	0.12	0.12	0.06–0.12	0.12	0.12
	Cefotaxime	0.015–0.03	0.03	0.03	0.015–0.03	0.03	0.03
	Moxalactam	0.5–1.0	1.0	1.0	0.5–1.0	1.0	1.0
	Cefoxitin	0.5–1.0	1.0	1.0	0.5–1.0	1.0	1.0
	Cefuroxime	≤0.008–0.015	0.015	0.015	≤0.008–0.015	0.015	0.015
	Cefazolin	0.12	0.12	0.12	0.12	0.12	0.12
Group D streptococci (5)	GR 20263	>128	>128	>128	>128	>128	>128
	Cefotaxime	>128	>128	>128	>128	>128	>128
	Moxalactam	>128	>128	>128	>128	>128	>128
	Cefoxitin	>128	>128	>128	>128	>128	>128
	Cefuroxime	>128	>128	>128	>128	>128	>128
	Cefazolin	32	32	32	32	32	32

defined as the concentration of antibiotic (micrograms per milliliter of media) at which no visible growth, or a minimal haze in the case of the heavy inocula, was apparent after 24 h of incubation. The tubes were subcultured onto agar at this time. The minimum bactericidal concentration (MBC) was the concentration of antibiotic per milliliter of original broth at which cultures failed to yield visible growth after a further 24 h of incubation. Measurements of MICs and MBCs were carried out in Isosensitest broth (pH 7.2) without serum and with 25% or 75% pooled human serum and inoculated with 10^3 to 10^4 CFU/ml of medium.

RESULTS

Table 1 summarizes the results obtained from 284 isolates tested at both inocula. GR 20263 has a high degree of activity against the *Enterobacteriaceae*; 90% of the strains tested at the lower inoculum were susceptible to between 0.06 and 0.5 μg/ml. Cefotaxime and moxalactam were about twice as active as GR 20263 against these strains. Ninety percent of the strains of *P. aeruginosa* were susceptible to 2.0 μg of GR 20263 per ml, which was fourfold more active than azocillin and eightfold more active than cefotaxime and moxalactam. Two Tem$^+$ strains were included; these were susceptible to 0.5 and 1 μg of GR 20263 per ml, yet were highly resistant to carbenicillin and azlocillin.

GR 20263 and moxalactam had similar high degrees of activity against *H. influenzae* (the MIC effective against 90% of the strains tested [MIC_{90}] at the lower inoculum was 0.12 μg/ml) in comparison with ampicillin (MIC_{90}, 4.0 μg/ml). The β-lactamase-producing strains were as susceptible to GR 20263 as the nonproducers.

B. fragilis were less susceptible to GR 20263 than to any other agent tested. The 30 strains of *N. gonorrhoeae* and 4 strains of *N. meningitidis* (not shown in Table 1) were somewhat more susceptible to GR 20263 than to benzylpenicillin; the β-lactamase-producing strain of the former species was inhibited by 0.015 μg of GR 20263 per ml.

GR 20263 and moxalactam were less active against *S. aureus* than the other agents tested, with MIC_{90}s of 16 μg/ml. Methicillin-resistant strains showed decreased susceptibility to GR 20263. The activity of this compound against Lancefield group A streptococci was similar to that of cefazolin, but none of the agents studied had any useful activity against Lancefield group D streptococci. Also included in the study, but not shown in Table 1, were three strains of *Streptococcus pneumoniae* and two strains of *Salmonella typhi*, which were highly susceptible to GR 20263 with MICs of 0.06 to 0.12 μg/ml.

An increase in inoculum from 10^3 to 10^6 CFU had little effect on the MIC_{90}, even in tests on Tem$^+$ strains of *P. aeruginosa*, *H. influenzae*, and *N. gonorrhoeae*.

The effect of serum on the MIC and MBC of GR 20263 is shown in Table 2. There was little or no difference between the MIC and MBC in the presence or absence of 25 or 75% serum.

DISCUSSION

This study shows that GR 20263 is a highly active broad-spectrum cephalosporin which shares many properties with cefotaxime (1, 7) and moxalactam (2, 6). All three compounds show high activity against the *Enterobacteriaceae* (including known β-lactamase producers); GR 20263 is marginally less active than the other two. This compound had a marked advantage over the other cephalosporins with respect to activity against *P. aeruginosa*, being 16- to 32-fold more active than cefotaxime or moxalactam and comparable to the narrow-spectrum cephalosporin, cefsulodin (CGP 7174/E) (5).

GR 20263 has comparatively low activity against *B. fragilis* and will probably be ineffective in treating infections caused by this organism. The mechanism of this poor performance

TABLE 2. *Effect of serum on the MIC and MBC (μg/ml) of GR 20263*

Strain tested		Broth		25% serum		75% serum	
		MIC	MBC	MIC	MBC	MIC	MBC
E. coli	I16	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
E. coli	I4	0.12	0.12	0.06	0.06	0.12	0.12
K. pneumoniae	H19	0.25	0.25	0.25	0.25	0.25	1.0
K. pneumoniae	H21	0.12	0.25	0.06	0.12	0.06	0.06
P. aeruginosa	G45	1.0	2.0	0.5	0.5	0.5	0.5
P. aeruginosa	G181	2.0	4.0	1.0	1.0	1.0	2.0
P. mirabilis	J15	0.06	0.25	0.06	0.12	0.25	1.0
P. morganii	J76	0.25	1.0	0.5	0.5	1.0	4.0
S. aureus	F34	8.0	8.0	8.0	8.0	8.0	8.0
S. aureus	F11	16.0	32.0	8.0	16.0	16.0	16.0

is difficult to deduce from this study. Some penicillin-resistant, high-penicillinase-producing strains were highly resistant to GR 20263, but others with similar qualities were susceptible to this compound.

The activity of GR 20263 against *H. influenzae* and *N. gonorrhoeae* was similar to that of moxalactam; both agents showed reduced activity against β-lactamase-producing strains. GR 20263, like moxalactam, had limited activity against *S. aureus*.

Others (4) have shown that GR-20263 is resistant to the commonly occurring β-lactamases of aerobes. The small decrease in susceptibility associated in the present study with an increase in inoculum supports this finding.

Studies by Acred et al. (P. Acred, D. M. Ryan, and P. W. Muggleton, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 560) indicate that GR 20263 has considerable activity against experimentally induced infections, especially those with *P. aeruginosa*. Clinical studies are awaited with interest.

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