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Sch 29482, laboratory evaluation of a new penem antibiotic

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The antibacterial activity of a new penem antibiotic, Sch 29482 (SCH), was examined in comparison with appropriate cephalosporins and penicillins. The drug inhibited penicillinase-positive and negative staphylococci equally well, being 2–5 times more active than cephalothin or cefamandole and 10–20 times more active than methicillin. Staphylococci resistant to methicillin were susceptible to SCH in agar dilution tests. Staphylococci tolerant to methicillin were also tolerant to SCH. Streptococci and pneumococci were highly susceptible to the drug. The agent was of only moderate activity against enterococci, especially *Streptococcus faecium* strains. MICs of ampicillin and penicillin G against enterococci were 4–8 times lower than those of SCH. SCH was bactericidal. Neither the choice of the method used for susceptibility testing, nor the size of the inoculum nor various test media influenced the *in-vitro* activity of this drug against a representative collection of Gram-negative and Gram-positive bacteria.

Introduction

Sch 29482 (SCH) is a new penem antibiotic which is chemically related to the carbapenem antibiotic thienamycin. The present study was undertaken to compare the *in-vitro* efficacy of this drug with various other β-lactam antibiotics. Some 250 clinical isolates of Gram-positive cocci were included in the study to provide a good indication of the potential efficacy of SCH in clinical application for Gram-positive infections. With a representative number of Gram-negative and Gram-positive bacteria, the influence of various *in-vitro* parameters, such as method of susceptibility testing, test medium and inoculum size, on results obtained with SCH was examined. All our data emphasize the fact that this penem antibiotic possesses promising *in-vitro* characteristics and, therefore, warrants further laboratory and clinical evaluation.

Methods

Bacteria

The micro-organisms used were isolated from clinical material and identified according to standard procedures. About 250 Gram-positive organisms were examined. The collection of Gram-negative bacteria included: *Escherichia coli* (16 strains); *Klebsiella pneumoniae* (8 strains) ; *Enterobacter aerogenes* (4 strains); *Enterobacter cloacae* (4 strains); *Serratia marcescens* (5 strains); *Proteus mirabilis* (4 strains); *Proteus vulgaris* (4 strains); *Providentia rettgeri* (4 strains); *Morganella morganii* (4 strains) and *Acinetobacter calcoaceticus* (4 strains).

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Antibiotics

Stock solutions of SCH, methicillin, penicillin G, ampicillin, cephalothin, cefamandole, cefaclor, cephalexin, cefoperazone and *N*-formimidoyl thienamycin were prepared in sterile distilled water and either used immediately or stored at −80°C, if necessary. All drugs were kindly provided by the pharmaceutical companies.

Susceptibility tests

Minimal inhibitory concentrations (MICs) were determined by serial twofold dilutions of antibiotics, usually in Mueller–Hinton agar (BBL), or in other broth or agar media as indicated in the text. Pneumococci and haemolytic streptococci were tested on agar containing 1% (w/v) Isovitalex (BBL) and 1% (w/v) haemoglobin (BBL). The inoculum in agar dilution was about 10^4 per spot, and in micro-broth dilution 5×10^5 to 10^6 per ml MICs were read after incubation at 37°C for 18 to 24 h. The MIC techniques corresponded to the procedures proposed by the National Committee of Clinical Laboratory Standards (1980).

Minimal bactericidal concentrations (MBCs) were determined by subculturing approximately 5 μl of the medium in each well of an MIC plate on Mueller–Hinton agar. The MBC was the lowest concentration of antibiotic that caused a 99·9% reduction in the number of viable organisms.

Populations of methicillin-resistant staphylococci were analysed by disaggregation of overnight broth cultures (brief, controlled exposure to 20-kilocycle sound) and surface inoculation of appropriate dilutions on drug-containing Mueller–Hinton agar plates. Colony counts after 48 h of incubation at 30 or 37°C allowed calculation of the number of viable units among 10^8 colony forming units (cfu) resistant to each concentration of antimicrobial agent.

Tolerance in staphylococci was examined by plating 20 μl of each well of an MIC plate on Mueller–Hinton agar and counting the number of cfu. Kinetics of killing was investigated in 20 ml Mueller–Hinton broth, to which 100 times the MIC of drug was added. At appropriate time intervals, 0·4 ml samples were withdrawn, and incubated for 10 to 15 min at 20°C with 0·1 ml of a crude *Bacillus cereus* enzyme preparation (Sabath *et al.*, 1971). This served to destroy any drug and, thus to avoid carry-over of bacteriostatic activity. Destruction of drug in the broth was examined by a plate assay, using *Bacillus subtilis* ATCC 6633 as test organism (Bennet *et al.*, 1966).

Results

Activity against Gram-positive organisms

Table I summarizes MIC data of SCH against Gram-positive bacteria. As can be seen, the drug showed excellent activity against staphylococci and streptococci including the pneumococci. The drug showed activity against methicillin-resistant staphylococci also. Enterococci, especially *Str. faecium*, were not very susceptible to the agent. However, the incidence of *Str. faecium* among enterococci is only 1·8% in our area.

Table II compares the activity of various β-lactam antibiotics against *Staphylococcus aureus* ($N = 35$), *Staph. epidermidis* ($N = 48$) and methicillin-resistant *Staph. aureus* ($N = 40$). *Staph. epidermidis* includes coagulase-negative organisms. No further speciation was done. SCH proved to be highly active against penicillinase-positive

Table I. Activity of Sch 29482 against Gram-positive bacteria

Organism	No. of strains	Minimal inhibitory concentration (mg/l) Mean	Mode	MIC_{90}*
Staphylococcus				
Methicillin-resistant	40	0·26	0·25	0·46
Penicillinase-positive	51	0·08	0·125	0·14
Penicillinase-negative	32	0·08	0·06	0·1
Streptococcus faecalis	31	7·6	8·0	9·3
Streptococcus faecium	14	19·5	wide range	78
Streptococcus durans	3	5·0	8·0	6·5
Haemolytic streptococci				
Group A	25	0·03	0·03	0·04
Group B	9	0·12	0·12	0·13
Group C	7	0·14	0·12	0·71
Pneumococci	30	0·01	0·03	0·07

*Concentration required to inhibit 90% of the strains examined.

Table II. Comparative activity of β-lactam antibiotics against *Staph. aureus* ($N = 35$), *Staph. epidermidis* ($N = 48$), and methicillin-resistant *Staph. aureus* ($N = 40$)

Antibiotic	Penicillinase positive MIC (mean)	Penicillinase negative MIC (mean)	Methicillin-resistant MIC (mean)	MIC_{90}*
SCH	0·08	0·08	0·26	0·46
Methicillin	2·0	1·2	4·8	7·2
Penicillin G	—	0·02	—	—
Cephalothin	0·2	0·16	0·9	2·5
Cefamandole	0·34	0·24	1·9	4·8
Cefaclor	4·22	1·96	33·7	73
Cefoperazone	1·33	1·02	8·7	18·7
N-Formimidoyl thienamycin	0·03	0·03	0·08	0·1
SCH (30°C)	—	—	1·6	4

*Concentration required to inhibit 90% of the examined strains.
Incubation was carried out at 37°C, unless otherwise indicated, for 24 h.

and negative strains. Only the thienamycin compound had similar activity. SCH and thienamycin also showed activity against methicillin-resistant organisms, when tested in agar dilution with an inoculum of 10^4 per spot and incubated for 24 h at 37°C. These two drugs were more active than cefamandole or cephalothin, which were shown elsewhere to be the most active β-lactam drugs against methicillin-resistant staphylococci (Kayser, 1980). Incubation of plates at 30°C increased MICs of SCH approximately 10 times.

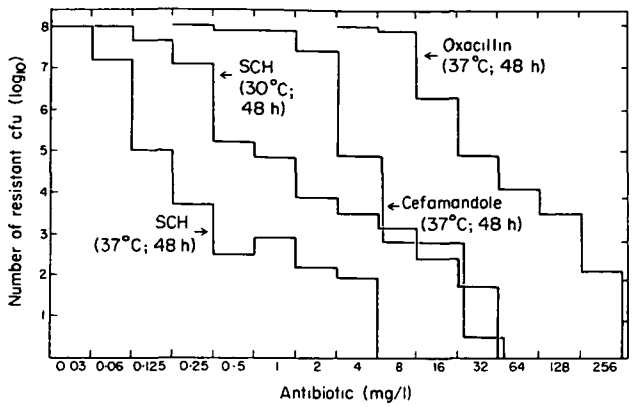

Figure 1. Composition of highly methicillin-resistant *Staph. aureus* strain EK 695 of cells with different levels of resistance to SCH, cefamandole and oxacillin

It is well known that populations of methicillin-resistant staphylococci are composed of cells differing widely in their resistance to β-lactam antibiotics. This heterogeneity was also observed in case of SCH (see Figure 1). However, the resistant minorities in populations are less resistant to SCH than to oxacillin or cefamandole.

Penicillin-tolerant staphylococci are known to be susceptible to penicillins in MIC tests, but highly resistant to the killing effect of these drugs (Sabath *et al.*, 1977). We examined the tolerance phenomenon in 32 *Staph. aureus* and *epidermidis* strains (16 of each species) against methicillin and SCH. Only one *Staph. aureus* culture was found to be tolerant to the two drugs. Figure 2 demonstrates the kinetics of killing of the tolerant

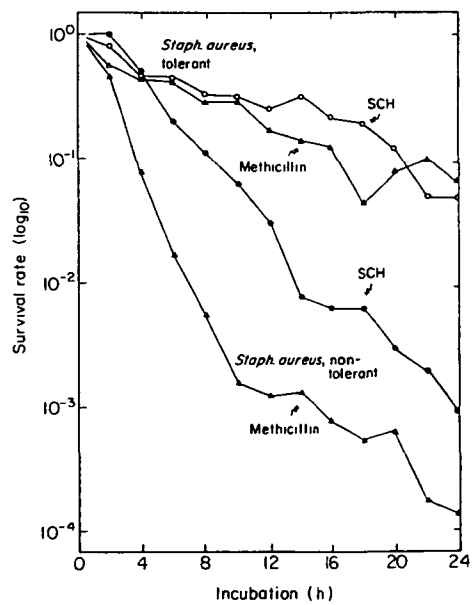

Figure 2. Rates of killing of *Staph. aureus* strain FK 484 and FK 485 by methicillin and SCH. Strain FK 484: ● methicillin 60 mg/l, ▲ SCH 6 mg/l; strain FK 485: ○ methicillin 60 mg/l, △ SCH 6 mg/l.

Table III. Comparative activity of β-lactam antibiotics against enterococci ($N = 48$)

Antibiotic	Minimal inhibitory concentration (mg/l) Mean	Mode	MIC_{90}*
SCH	9·8	8	30·0
Penicillin G	2·1	2	10·0
Ampicillin	0·7	1	2·9
Methicillin	34·9	32	157·0
Cephalothin	34·4	32	130·0
Cefamandole	29·8	32	100·0
Cefaclor	119·0	128	220·0
Cefoperazone	28·5	32	90·0
N-Formimidoyl thienamycin	4·3	2	8·0

*Concentration required to inhibit 90% of the examined strains.

and a non-tolerant strain by methicillin and SCH. Destruction of drugs during growth of the tolerant culture in comparison with the non-tolerant culture did not occur.

Table III summarizes MIC data of β-lactam antibiotics against enterococci. Although SCH was not as active as penicillin or ampicillin against enterococci, it showed some activity, which was about 4 times greater than the activity of cephalosporins. Thienamycin also proved to have a moderate activity against these problem bacteria.

All drugs were active against β-haemolytic streptococci and pneumococci (see Table IV). Penicillin G and thienamycin were most active, closely followed by SCH, ampicillin and cefamandole.

Examination of bactericidal activity of SCH gave MBC values which were identical to MICs against most of 80 Gram-positive strains examined. MBCs for methicillin-resistant staphylococci were 4–16 times, and for the tolerant *Staph. aureus* culture, more

Table IV. Comparative activity of β-lactam antibiotics against streptococci and pneumococci

Antibiotic	Mode MIC (mg/l) Group A ($N = 25$)	Group B ($N = 9$)	Group C ($N = 7$)	Pneumococci ($N = 30$)
SCH	0·03	0·13	0·13	0·03
Penicillin G	0·004	0·03	0·01	0·008
Ampicillin	0·01	0·1	0·03	0·01
Methicillin	0·13	0·5	0·25	0·06
Cephalothin	0·25	0·25	0·25	0·06
Cefaclor	0·25	8·0	2·0	0·25
Cefalexin	0·5	8·0	2·0	1·0
Cefamandole	0·03	0·13	0·06	0·06
Cefoperazone	0·13	0·25	0·25	0·06
N-Formimidoyl thienamycin	⩽ 0·03	⩽ 0·03	⩽ 0·03	⩽ 0·03

Table V. Effect of methods and media on *in-vitro* activity of Sch 29482

Method and medium	Minimal inhibitory concentration (mean) (mg/l) Enterobacteria ($N = 67$)	Staphylococci ($N = 32$)	Enterococci ($N = 8$)
Agar dilution procedure (NCCLS)			
Mueller-Hinton-Agar (Oxoid)	0·91	0·12	5·7
Isosensitest-Agar (Oxoid)	0·85	0·10	4·4
Diagnostic Sensitivity Test-Agar (Oxoid)	0·93	0·07	6·2
Micro-broth dilution procedure (NCCLS)			
Mueller–Hinton broth (Oxoid)	1·08	0·25	6·8
Mueller–Hinton broth plus cation supplement (see NCCLS)	1·37	0·16	9·8

than 100 times higher than MICs. Killing of *Staph. aureus* and enterococci was often less effective at higher drug concentrations.

In-vitro *factors and activity of SCH*

β-lactam antibiotics are known to be of varying stability to β-lactamases. Instability of a drug to a β-lactamase can result in wide differences in MIC results according to the method used. Instability also can result in a significant inoculum effect. Activity of antimicrobials *in vitro* can also be influenced by the composition of the test medium. We, therefore, examined the influence of some *in-vitro* parameters on results obtained with SCH. The enterobacteria used for these tests were a representative collection of organisms as described in the 'Methods' section. Table V demonstrates that neither the method nor various media influenced MIC results of SCH. Table VI shows that the size of the inoculum also had no effects on test results. The only exceptions were methicillin-resistant staphylococci. Because of the typical heterogeneity (see Figure 1), MICs for methicillin-resistant staphylococci were greater at higher inoculum sizes.

Discussion

The penem antibiotic SCH has been shown in this study to be an excellent antibiotic against most species of Gram-positive organisms. In particular it exhibited some activity against methicillin-resistant staphylococci, although the typical characteristics of these bacteria—heterogeneity and influence of incubation temperature on phenotypic expression of resistance—were also observed with SCH. The drug was as active as penicillin G against penicillinase-negative variants of methicillin-resistant strains (data were not shown here) and, thus can be considered as a possible choice in the treatment of infections caused by such bacteria. This might be important in the future, because

Table VI. Effect of inoculum size on activity of Sch 29482

Organism	Mean MIC (mg/l) in agar dilution 10^3/spot	10^4/spot	10^5/spot	10^6/spot
Enterobacteria ($N = 10$)	0·34	0·45	1·10	1·40
Staphylococci ($N = 10$)	0·03	0·13	0·16	0·25
Staph. aureus (methicillin-resistant) ($N = 2$)	0·50	0·70	16	32
Enterococci ($N = 5$)	3·5	4·6	6·1	7·0
	Mean MIC (mg/l) in broth dilution 10^4/ml	10^5/ml	10^6/ml	10^7/ml
Enterobacteria	0·28	0·23	0·45	0·37
Staphylococci	0·09	0·08	0·17	0·25
Staph. aureus (methicillin-resistant)	0·70	2·80	4·00	90·00
Enterococci	4·60	6·10	5·30	12·10

there is a world-wide increase of epidemic infections in hospitals caused by staphylococci, simultaneously resistant to β-lactam antibiotics, aminoglycosides and other antimicrobials (Shanson, Kensit & Duke, 1976; Crossley *et al.*, 1979; Graham *et al.*, 1980; Amirak *et al.*, 1981. Kayser, F. H., 1981 personal observation). SCH has also been shown to be highly active against streptococci and pneumococci. Enterococci, however, were not as susceptible to SCH as to ampicillin or penicillin G, although they were more sensitive to this agent than to cephalosporin antibiotics. The good activity of SCH against the small collection of Gram-negative bacteria suggests that this drug has a broad antibacterial spectrum of activity.

Neither the size of the inoculum, the method used for susceptibility testing nor various test media affected the *in-vitro* activity of SCH against Gram-negative and Gram-positive bacteria. Such parameters can influence susceptibility testing of bacteria against β-lactam antibiotics that have a reduced stability to β-lactamases. It was shown, for instance, that the *in-vitro* activity of cefoperazone, cefamandole and cephalothin against Gram-negative bacteria producing large amounts of TEM-β-lactamase, is highly dependent on the test method and the size of the inoculum used (Kayser, Huf & Homberger, 1981). Since such TEM-producers were among the strains examined and since neither the inoculum nor the method influenced the activity of SCH against these strains, it can be concluded that this drug is remarkably stable against the most frequently occurring β-lactamase of Gram-negative bacteria (Mathew, 1979).

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