IN VITRO ACTIVITY OF KITASAMYCIN AGAINST GRAM-POSITIVE COCCI

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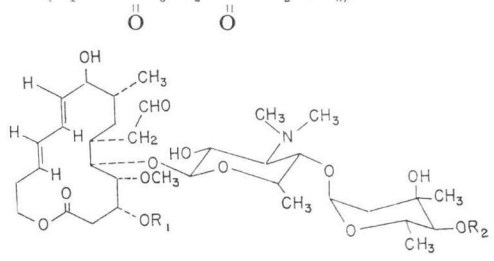
(Received for publication January 7, 1974)

Kitasamycin was tested *in vitro* against 214 clinical isolates of gram-positive cocci and its activity compared to minocycline, cephalothin, erythromycin, clindamycin and dicloxacillin. All isolates of *Streptococcus pyogenes* were inhibited by 0.39 μg/ml. At a concentration of 1.56 μg/ml, kitasamycin inhibited all isolates of *Diplococcus pneumoniae*, 98% of isolates of *Staphylococcus aureus* sensitive to penicillin G and 99% of isolates of *Staphylococcus aureus* resistant to penicillin G. Most of the other antibiotics were as active or more active than kitasamycin against gram-positive cocci.

In 1953, HATA, *et al.*[3] reported the isolation of a new antibiotic complex known as kitasamycin (leucomycin). The organism producing this antibiotic complex was obtained from soil samples and named *Streptomyces kitasatoensis* HATA. In 1967, eight components were separated and their chemical structures determined[10]. Fig. 1 shows the chemical structure of kitasamycin which corresponds to leucomycin A6. Kitasamycin was found to be active against gram-positive and some gram-negative cocci, rickettsiae and large viruses. The antibiotic has been used in Japan for the treatment of acute infections for several years. It has been administered orally, intravenously and topically. Peak serum concentrations of 5.7 μg/ml have been obtained following oral administration[9]. Peak serum levels of 11.5 μg/ml have been obtained following intravenous administration. Kitasamycin has low toxicity and is effective against erythromycin-resistant and penicillin-resistant strains of *Staphylococcus aureus*[2,6,7]. Although this antibiotic was evaluated many years ago, it was felt of interest to determine its activity against recent isolates of gram-positive cocci. Consequently, the *in vitro* activity of kitasamycin was determined against 214 gram-positive cocci isolated from clinical specimens.

Fig. 1. Chemical structure of kitasamycin A_6 (R_1=C-CH_3 R_2=C-CH_2-CH_3).



Materials and Methods

The activity of kitasamycin was determined against 214 clinical isolates of gram-positive

Supported in part by Grant CA 10042 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland 20014, and by Grant-in-Aid from Ayerst Research Laboratories, Montreal, Canada.

* Dr. BODEY is a Scholar of The Leukemia Society of America, Inc.

cocci, representing 3 genera and compared to the activity of 5 other antibiotics using a microdiluter[4] Isolates of *Diplococcus pneumoniae* and *Streptococcus pyogenes* were inoculated into Tryptose-phosphate broth containing 2% human blood. The isolates were incubated for 18 hours at 37°C. A 50 μl aliquot of a 10^{-3} dilution of this broth was added to wells containing 50 μl volumes of the antibiotics, resulting in a total of 100 μl in each well. The antibiotics used were clindamycin (The Upjohn Co., Kalamazoo, Michigan), dicloxacillin (Beecham-Massengill, Inc., Clifton, New Jersey), erythromycin (Abbott Laboratories, North Chicago, Illinois), minocycline (Lederle Laboratories, Pearl River, New York), cephalothin (Eli Lilly and Co., Indianapolis, Indiana) and kitasamycin tartrate (Ayerst Laboratories, New York, New York). They were dissolved in MUELLER-HINTON broth and two-fold serial dilutions were prepared, ranging in concentration from 25 μg/ml to 0.025 μg/ml. The minimum inhibitory concentration (MIC) was determined after incubation at 37°C for 18 hours. All studies were performed in triplicate. Disk sensitivity testing was performed according to the method of BAUER *et al*[1].

Seventeen isolates of *Dipl. pneumoniae*, 47 isolates of *Str. pyogenes*, and 150 isolates of *Staphylococcus aureus* were tested. One hundred isolates of *S. aureus* were resistant to penicillin G at a concentration of 50 μg/ml. The remaining 50 isolates were inhibited by less than 0.1 μg/ml penicillin G. Most of the organisms were isolated from cancer patients hospitalized at this institution between August 1971 and January 1973.

Results

Tables 1 and 2 compare the activity of kitasamycin using the microdilutor and disk sensitivity techniques. Fifty of the 150 isolates of *S. aureus* and 35 of the 47 isolates of *Str. pyogenes* were selected for testing. All of the isolates of *S. aureus* which had an MIC of no greater than 1.56 μg/ml produced zones of inhibition of at least 19 mm by the disk technique. One isolate resistant to 25 μg/ml produced a zone less than 19 mm diameter. All of the isolates of *Str. pyogenes* were inhibited by no greater than 1.56 μg/ml and all but one isolate produced zones of inhibition of at least 19 mm diameter. The one isolate producing a smaller zone of inhibition had an MIC of 0.20 μg/ml.

All of the isolates of *Dipl. pneumoniae* were inhibited by 0.39 μg/ml of kitasamycin. All of the isolates of *Str. pyogenes* and 98% of the isolates of penicillin G sensitive *S. aureus* were inhibited by 1.56 μg/ml of kitasamycin. The remaining 2% of penicillin G sensitive *S. aureus* were resistant to 25 μg/ml. Among the 100 isolates of penicillin G resistant *S. aureus*, 92% had an MIC of no greater than 1.56 μg/ml and an additional 7% had an MIC of no greater

Table 1. Correlation between zone of inhibition using disk sensitivity technique and minimum inhibitory concentration of kitasamycin using microdilutor for 50 isolates of *Staphylococcus aureus*

MIC (μg/ml)	Diameter of zone of inhibition (mm)		
	<19	19~21	>21
>25	1		
1.56		3	1
0.78		29	10
0.39		4	2

Table 2. Correlation between zone of inhibition using disk sensitivity technique and minimum inhibitory concentration of kitasamycin using microdilutor for 35 isolates of *Streptococcus pyogenes*

MIC (μg/ml)	Diameter of zone of inhibition (mm)		
	<19	19~21	>12
1.56			2
0.78		1	4
0.39		5	6
0.20	1	4	6
0.10		1	5

Fig. 2. *In vitro* activity of six antibiotics against *Diplococcus pneumoniae*.

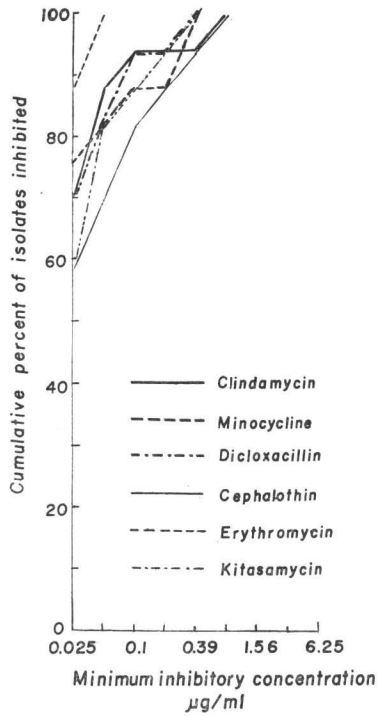


Fig. 3. *In vitro* activity of six antibiotics against *Streptococcus pyogenes*.

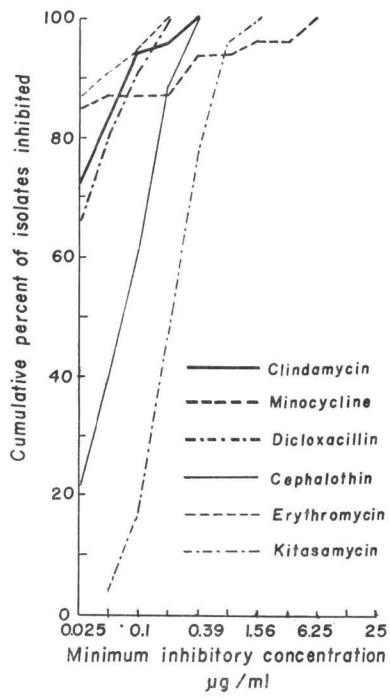


Fig. 4. *In vitro* activity of six antibiotics against penicillin G sensitive *Staphylococcus aureus*.

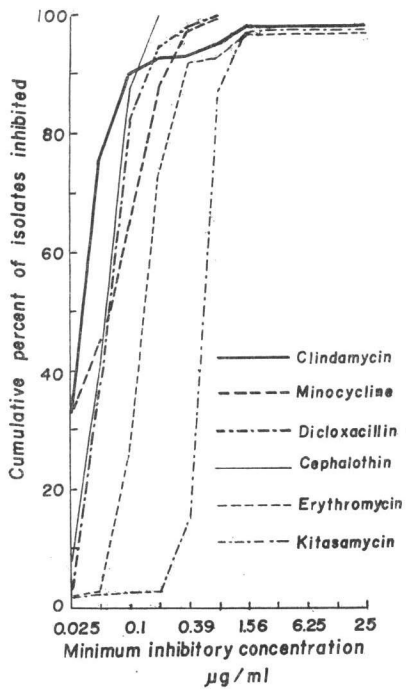
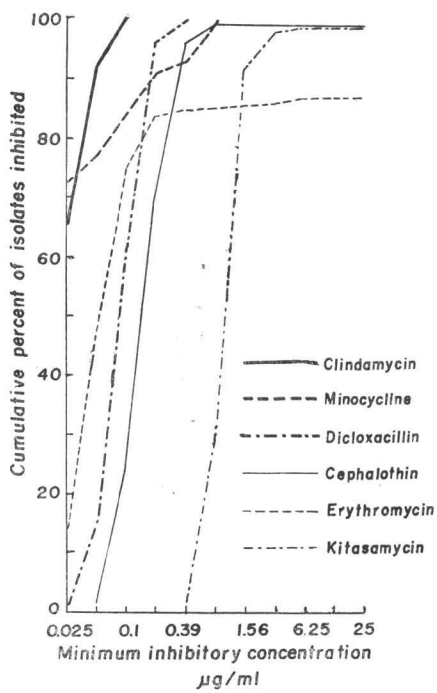


Fig. 5. *In vitro* activity of six antibiotics against penicillin G resistant *Staphylococcus aureus*.



than 6.25 μg/ml of kitasamycin. The remaining 1% were resistant to 25 μg/ml.

Erythromycin was the most active of the 6 antibiotics against *Dipl. pneumoniae* (Fig. 2). All of the isolates were inhibited by 0.05 μg/ml. Although the other 5 antibiotics were comparable in activity, dicloxacillin was the least active. All of the 17 isolates of *Dipl. pneumoniae* were inhibited by 1.56 μg/ml of all 5 antibiotics.

Erythromycin was the most active antibiotic against *Str. pyogenes* (Fig. 3). All of the isolates were inhibited by 0.2 μg/ml. Cephalothin and kitasamycin were the least active antibiotics against *Str. pyogenes*. At a concentration of 0.1 μg/ml, erythromycin inhibited 95% of isolates, clindamycin inhibited 94%, dicloxacillin inhibited 92%, cephalothin inhibited 61% and kitasamycin inhibited 17%.

Cephalothin was the most active antibiotic against the 50 isolates of *S. aureus* sensitive to penicillin G (Fig. 4). All of the isolates were inhibited by 0.2 μg/ml. Dicloxacillin and minocycline also inhibited all of the isolates, at a concentration of 0.78 μg/ml. One isolate was resistant to 25 μg/ml of the other 3 antibiotics. Kitasamycin was substantially less active than the other antibiotics.

Clindamycin was the most active antibiotic against the 100 isolates of *S. aureus* resistant to penicillin G (Fig. 5). All of the isolates were inhibited by 0.1 μg/ml. Minocycline and dicloxacillin also inhibited all of the isolates at higher concentrations. One isolate was resistant to kitasamycin and cephalothin and 13 isolates were resistant to erythromycin. Kitasamycin was the least active antibiotic against most of the isolates.

Discussion

Our results are comparable to those obtained by other investigators who tested kitasamycin against isolates of *Dipl. pneumoniae* and *Str. pyogenes*[5,8]. In our study there was no difference in the activity of kitasamycin against staphylococci, regardless of their sensitivity to penicillin G. Only 1% of isolates of *S. aureus* which were resistant to penicillin G were resistant to kitasamycin. All of these isolates were sensitive to clindamycin, minocycline and dicloxacillin, but 13% were resistant to erythromycin. Kitasamycin has activity against the vast majority of clinical isolates of *S. aureus*, *Str. pyogenes* and *Dipl. pneumoniae*, but *in vitro* studies do not suggest that it has any advantages over currently available antibiotics, although it would be expected to be effective in clinical situations in which erythromycin is indicated.

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