SPENOLIMYCIN, A NEW SPECTINOMYCIN-TYPE ANTIBIOTIC

III. BIOLOGICAL PROPERTIES

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Spenolimycin is a new spectinomycin-type antibiotic isolated from *Streptomyces gilvospiralis* sp. nov. *In vitro*, it was active against a wide variety of aerobic Gram-positive and Gram-negative bacteria and *Neisseria gonorrhoeae*. It was two to four-fold more active against *N. gonorrhoeae* than spectinomycin. Spenolimycin was effective in the standard mouse protection test against *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae*.

Spenolimycin is a new spectinomycin-like antibiotic isolated from *Streptomyces gilvospiralis* sp. nov. Its discovery, the taxonomy of the producing culture, fermentation, isolation and structure determination have been described in the two preceding papers[1,2]. The microbiological profile of this antibiotic is described in this paper. Spectinomycin was used as the reference compound for all studies.

Table 1. Potency of spenolimycin against a variety of aerobic bacteria.

Organism	MIC (μg/ml)	
	Spenolimycin	Spectinomycin
Staphylococcus aureus ATCC 6538P	50	50
S. aureus CMX 686B	100	50
S. aureus A5177	>100	>100
S. aureus 45	100	50
S. epidermidis 3519	50	50
Lactobacillus casei ATCC 7469	3.1	25
Streptococcus faecium ATCC 8043	25	100
S. bovis A5169	12.5	12.5
S. agalactiae CMX 508	12.5	50
S. pyogenes EES 61	12.5	25
S. pyogenes 930	6.2	25
Micrococcus luteus 9341	50	25
Escherichia coli Juhl	50	12.5
E. coli SS	6.2	6.2
E. coli DC-2	25	12.5
E. coli H560	6.2	6.2
E. coli KNK 437	25	12.5
Enterobacter aerogenes ATCC 13048	50	25
Klebsiella pneumoniae ATCC 8045	25	25
Providencia stuartii CMX 640	>100	>100
Pseudomonas aeruginosa BMH 10	6.2	25
P. aeruginosa 5007	>100	>100
P. aeruginosa K799/wt	>100	>100
P. aeruginosa K799/61	100	50
P. cepacia 296i	>100	>100
Acinetobacter sp. CMX 669	>100	50

Materials and Methods

Antibiotics

Spenolimycin and spectinomycin (Lot No. 46-673-CD) were prepared at Abbott Laboratories, North Chicago, Illinois.

Test Bacteria

The aerobic Gram-positive and Gram-negative bacteria including *Neisseria gonorrhoeae* were from the Abbott culture collection. These organisms are maintained at −70°C or lyophilized. *N. gonorrhoeae* strains 84M and F28 were obtained from the Center for Disease Control, Atlanta, Georgia, and are spectinomycin-sensitive and spectinomycin-resistant strains, respectively.

In Vitro Potency Determinations

The minimal inhibitory concentrations (MICs) of spenolimycin were determined using the agar dilution procedure[3] on brain heart infusion agar. *N. gonorrhoeae* was grown in a 7% CO_2 atmosphere on GC agar base (Difco) supplemented with 1% (v/v) IsoVitalex and 1% (w/v) hemoglobin.

Mouse Protection Tests

CF-1 female mice were infected by injecting 100 LD_{50} doses of *Escherichia coli* Juhl, 10 LD_{50} doses of *Klebsiella pneumoniae* 4508 or 1,000 LD_{50} doses of *Streptococcus pneumoniae* 6303 intraperitoneally. Hog gastric mucin (5% w/v) was mixed with the *E. coli* and *K. pneumoniae* prior to intraperitoneal injection. Groups of ten mice infected in this manner were treated with three different graded doses of spenolimycin or spectinomycin at 1 and 5 hours post-infection. The median effective dose (ED_{50}) was calculated on the basis of cumulative mortalities on the sixth day by a trimmed version of the Logit method[4].

Results and Discussion

In Vitro Potency

The MICs of spenolimycin and spectinomycin against a variety of aerobic Gram-positive and Gram-negative bacteria are shown in Table 1. In general, spenolimycin was two to four-fold more active against the streptococci than spectinomycin and was equal to or two-fold less active than spectinomycin against aerobic Gram-negative bacteria. The MICs of spenolimycin against several strains of *N. gonorrhoeae* are shown in Table 2. In general, spenolimycin was two to four-fold more active than spectinomycin against *N. gonorrhoeae*. The spectinomycin resistant strain, *N. gonorrhoeae* F28, was cross-resistant to spenolimycin.

Table 2. *In vitro* potency of spenolimycin against *Neisseria gonorrhoeae*.

Organism	MIC (μg/ml)	
	Spenolimycin	Spectinomycin
Neisseria gonorrhoeae CMX 556	16	32
N. gonorrhoeae CMX 557	16	32
N. gonorrhoeae CMX 558	16	32
N. gonorrhoeae CMX 591	8	32
N. gonorrhoeae CMX 638	16	64
N. gonorrhoeae CMX 664	16	32
N. gonorrhoeae 35F AMP I	32	32
N. gonorrhoeae 389 AMP R	16	32
N. gonorrhoeae 17	16	32
N. gonorrhoeae 84M	8	16
N. gonorrhoeae F28	>64	>64

Table 3. *In vivo* potency of spenolimycin in mouse protection tests.

Organism (Infecting dose)	Antibiotic	MIC (μg/ml)	ED_{50} (mg/kg/day)
Escherichia coli Juhl (100 LD_{50}s)	Spenolimycin	50	50.2 (25.4~99.1)
	Spectinomycin	12.5	20.7 (14.3~29.9)
Klebsiella pneumoniae 4508 (10 LD_{50}s)	Spenolimycin	25	23.6 (14.9~37.2)
	Spectinomycin	25	25.0 (15.8~39.6)
Streptococcus pneumoniae 6303 (1,000 LD_{50}s)	Spenolimycin	12.5	15.8 (7.3~34.2)
	Spectinomycin	25	30.5 (20.9~44.3)

In Vivo Efficacy

In vivo studies have not been conducted against *N. gonorrhoeae* as there is no rodent model suitable for determining the efficacy of antibiotics against *N. gonorrhoeae*. In order to determine if spenolimycin would be effective *in vivo*, *E. coli*, *K. pneumoniae* and *S. pneumoniae* were used in the standard mouse protection test. The effective doses of spenolimycin and spectinomycin in this test reflected the *in vitro* potencies of these compounds against all pathogens. Accordingly, for *E. coli* Juhl, where spectinomycin was more active than spenolimycin *in vitro*, the ED_{50} of spectinomycin was one-half the ED_{50} of spenolimycin. The ED_{50}s of spenolimycin and spectinomycin were similar against *K. pneumoniae* 4508, where the compounds had the same MIC. Spenolimycin was also somewhat better than spectinomycin in treating *S. pneumoniae* 6303 in mouse protection tests.

References

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