A-16686, A NEW ANTIBIOTIC FROM *ACTINOPLANES*

II. BIOLOGICAL PROPERTIES†

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A-16686, a new glycoproteide antibiotic obtained from fermentation of an *Actinoplanes* strain, is active against Gram-positive aerobic and anaerobic bacteria; MIC values ranged from 0.016 to 2.0 μg/ml. A-16686 is bactericidal for growing cells of *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus faecalis*, *S. faecium*, *S. mutans*, *S. mitis* and *S. sanguis*.

There is no cross-resistance with clinically used antibiotics. A-16686, administered subcutaneously, is very effective in experimental *S. pyogenes* and *S. pneumoniae* septicemias in the mouse.

Antibiotic A-16686, a complex of three closely related polypeptides containing chlorinated phenyl moieties, was obtained from fermentation of *Actinoplanes* sp. ATCC 33076[1]. In this report we present data on the *in vitro* and *in vivo* antibacterial activity of this antibiotic.

Materials and Methods

Antibiotics

A-16686 was dissolved in distilled water. Its activity was compared with those of cephaloridine, ampicillin, erythromycin and vancomycin.

Organisms

Reference strains and 60 Gram-positive clinical isolates were tested; the clinical isolates were obtained from various hospitals and most of them were resistant to one or more clinically used antibiotics, including methicillin.

Media

For susceptibility testing, Oxoid Iso-Sensitest broth was used for most strains and Difco Todd-Hewitt broth for streptococci; the latter medium was employed for killing curves. Difco brain heart infusion agar and Wilkins-Chalgren agar[2] were used for *Actinomyces* and anaerobes, respectively.

Supplemented thioglycolate medium without indicator (BBL-135C) was used to prepare the inoculum of anaerobic bacteria[3].

Susceptibility Testing

MIC Determination: MIC were determined by either the broth or the agar serial two-fold dilution method, with concentrations ranging from 0.002 to 128 μg/ml. For most strains, overnight broth cultures were diluted to obtain inocula of approximately 10^4 colony forming units (cfu)/ml. The tubes were incubated at 37°C for 18~24 hours.

For anaerobic bacteria, plates containing antibiotic were inoculated, using a multipoint inoculator (H 400 Denley Instruments Ltd), with 48-hour broth cultures, diluted 1: 10. Incubation was for 48 hours in an atmosphere of N_2: CO_2: H_2 (80: 10: 10).

The MIC was taken as the lowest concentration of antibiotic which prevented visible growth.

† Some of these data were presented at the 13th International Congress of Chemotherapy, Vienna, August 28, 1983.

Effect of pH, Serum and Inoculum Size on MIC: The broth dilution method was used. pH's ranged from 6 to 9 and bovine serum concentrations from 10 to 70%; inoculum sizes ranged from 10^4 to 10^7 cfu/ml.

Cross-resistance Determination: Cross-resistance was evaluated by determining MIC of A-16686 for mutants of *Staphylococcus aureus* ATCC 6538 resistant to various antibiotics.

Bactericidal Activity

Killing Curves:

Growing Cells: Various concentrations of A-16686, ampicillin and vancomycin were added to growing cells of *S. aureus* L1532, *S. epidermidis* L1578, *Streptococcus faecalis* L1362, *S. faecium* var. *durans* L1584, *S. mitis* L796, *S. mutans* L659, *S. bovis* L1325 and *S. sanguis* L667.

At intervals during incubation at 37°C, samples were removed and plated to titer surviving bacteria.

Stationary Cells: In this case, the antibiotic was added to overnight broth cultures of *S. aureus* ATCC 6538, which were then further incubated at 37°C for 24 hours.

Experimental Infections

The ED_{50}'s were determined as described previously[4].

Results

Susceptibility Testing

MIC Determination

A-16686 was very active against Gram-positive aerobic and anaerobic bacteria (Table 1); it had no activity (MIC: $\geq$128 μg/ml) on aerobic or anaerobic Gram-negative bacteria, *Candida albicans, Trichophyton mentagrophytes, Mycobacterium tuberculosis* H37Rv, *Mycoplasma gallisepticum,* or *Trichomonas vaginalis* (data not shown).

MIC of A-16686 for clinical isolates of staphylococci and streptococci are shown in Table 2, in comparison with those of cephaloridine, ampicillin, erythromycin and vancomycin. A-16686 was very active against the strains tested (MIC range 0.016 to 2 μg/ml). *S. pyogenes* and *S. pneumoniae* were particularly sensitive to A-16686 (median MIC 0.125 and 0.032 μg/ml, respectively); for *S. pneumoniae,* its activity was comparable to those of cephaloridine, ampicillin and erythromycin. Cephaloridine and ampicillin had the best activity against *S. pyogenes*; against other streptococci of various serological groups, A-16686 showed activity comparable to that of ampicillin but it was slightly less active than cephaloridine and erythromycin.

Table 1. *In vitro* activity of A-16686 against selected organisms.

Organism	MIC (μg/ml)
Staphylococcus aureus ATCC 6538	0.5
S. aureus TOUR	1.0
S. epidermidis ATCC 12228	0.5
Streptococcus pyogenes C 203 SKF 13400	0.063
S. pneumoniae UC 41	0.032
S. faecalis ATCC 7080	0.25
S. faecium ATCC 10541	0.125
S. bovis ATCC 9809	0.125
S. agalactiae ATCC 7077	0.063
S. dysgalactiae ATCC 9926	0.125
S. mutans ATCC 27531	0.5
Corynebacterium diphtheriae type mitis ATCC 11051	1.0
Clostridium perfringens ISS	0.25
C. difficile ATCC 9689	1.0
Propionibacterium acnes ATCC 6919	0.25
Actinomyces viscosus ATCC 19246	0.125
A. naeslundi ATCC 12104	0.5

Particularly noteworthy is A-16686's activity against *S. faecalis* and *S. faecium* (MIC range 0.5~2 μg/ml).

A-16686 had good activity against clinical isolates of staphylococci, most of them resistant to one or more antibiotics including methicillin.

Influence of pH, Inoculum and Serum on the MIC of A-16686

The *in vitro* activity of A-16686 against clinical isolates of *S. aureus, S. epidermidis, S. faecalis*

Table 2. *In vitro* activity of A-16686 against clinical isolates in comparison with other antibiotics.

Organism (No. of strains)	Median MIC and range (μg/ml)				
	A-16686	Cephaloridine	Ampicillin	Erythromycin	Vancomycin
S. aureus (10)	1 (0.5~2)	0.38 (0.063~1)	>128 (2~>128)	0.25 (0.125~>128)	1 (1~2)
Staphylococci coagulase-negative (10)	0.75 (0.5~1)	0.094 (0.032~0.5)	2 (0.25~64)	1.13 (0.125~>128)	1 (0.5~2)
S. pyogenes (10)	0.125 (0.063~0.25)	0.008 (0.004~0.016)	0.016 (0.008~0.032)	0.032 (0.016~0.063)	0.5 (0.5~1)
S. pneumoniae (10)	0.032 (0.016~0.063)	0.024 (0.016~0.25)	0.032 (0.016~1)	0.016 (0.002~0.063)	0.5 (0.25~0.5)
S. faecalis-faecium (10)	1 (0.5~2)	12 (8~32)	2 (1~16)	1 (0.125~>128)	2 (1~2)
Other streptococci (10)*	0.125 (0.063~0.5)	0.016 (0.004~0.125)	0.125 (0.032~0.5)	0.032 (0.032~0.5)	0.75 (0.5~1)

* Including *S. mutans* (2), *S. salivarius* (2), *S. bovis* (1), *S. mitis* (1), *S. sanguis* (2), *S. agalactiae* (2).

Table 3. Influence of inoculum size, serum, or pH of the medium on the activity of A-16686.

Organism		MIC (μg/ml)										
		Inoculum: cfu/ml			Bovine serum (%)				pH of the medium			
		10^5	10^6	10^7	0	30	50	70	6	7	8	9
S. aureus	L 1508	1	2	4	1	1	1	1	0.5	1	1	0.5
S. epidermidis	L 1521	1	2	2	1	1	1	1	0.5	0.5	1	0.5
S. faecalis	L 1331	1	1	2	1	1	2	2	1	1	1	2
S. pyogenes	L 1317	0.25	0.5	0.25	0.25	0.25	0.125	0.125	*	0.25	0.125	*

* No growth.

and *S. pyogenes* was not significantly affected by the pH of the medium, the addition of bovine serum or the inoculum size. Only in *S. aureus* there was a small (four-fold) increase in the MIC when the inoculum was increased from 10^4 to 10^7 cfu/ml (Table 3).

Cross-resistance

No cross-resistance between A-16686 and various clinically used antibiotics was observed on *S. aureus* ATCC 6538 mutants (data not shown).

Bactericidal Activity

Killing Curves

A-16686 was bactericidal for growing cells but not for non-proliferating cells. After 3~5 hours of contact with a concentration five times the MIC, 99.9% of growing cells of *S. aureus, S. epidermidis, S. faecalis, S. faecium, S. mitis* and *S. mutans* were killed; killing of *S. bovis* was less marked (Figs. 1~7). In contrast, even at 50 times the MIC (10 μg/ml) A-16686 had no bactericidal activity for non-proliferating cells of *S. aureus* ATCC 6538.

The bactericidal action of A-16686 appeared to be slower against *S. sanguis* (Fig. 8); after 5 hours hardly any effect was observed, but by the next time point (24 hours) 99.9% killing was obtained.

The bactericidal action of A-16686 is largely independent of the concentration used; good killing was obtained at concentrations as low as two times the MIC and more rapid killing was observed at concentrations of ten times the MIC against some strains.

Fig. 1. Bactericidal activity of A-16686, ampicillin and vancomycin against *S. aureus* L1532.

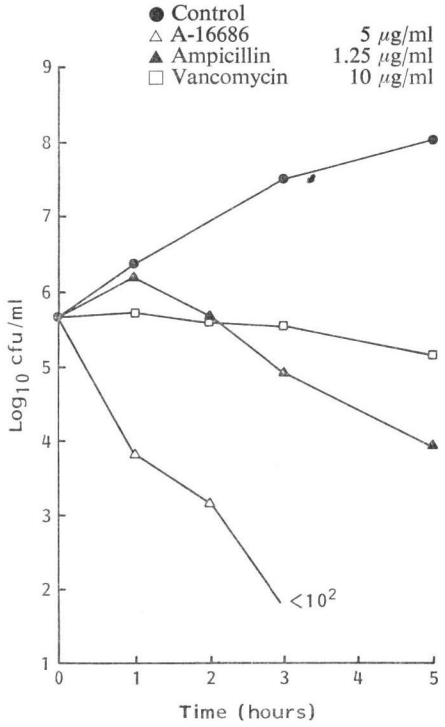


Fig. 2. Bactericidal activity of A-16686 and vancomycin against *S. epidermidis* L1578, ampicillin-resistant.

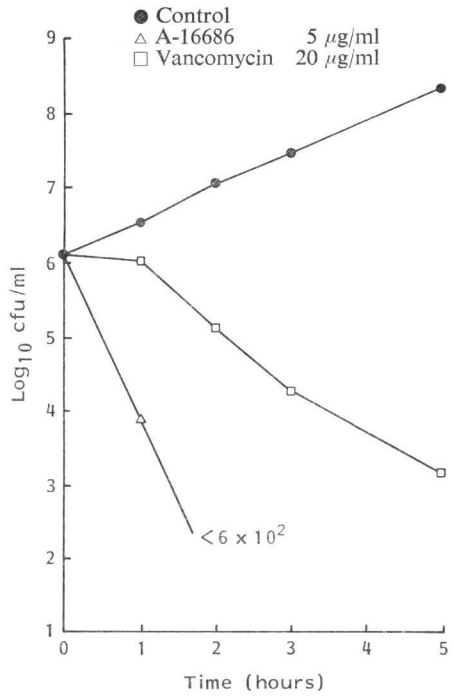


Fig. 3. Bactericidal activity of A-16686, ampicillin and vancomycin against *S. faecalis* L1362.

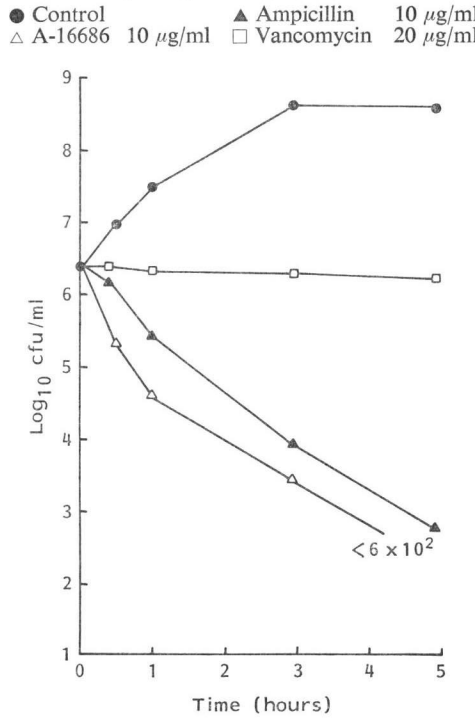


Fig. 4. Bactericidal activity of A-16686 and vancomycin against *S. faecium* var. *durans* L1584, ampicillin-resistant.

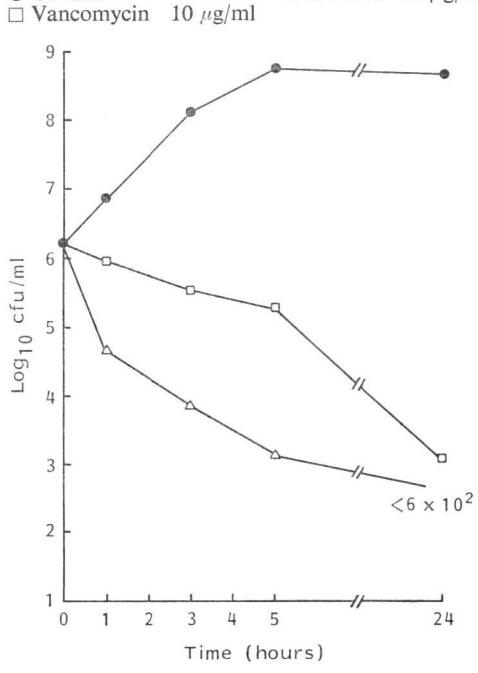


Fig. 5. Bactericidal activity of A-16686, ampicillin and vancomycin against *S. mitis* L796.

● Control ▲ Ampicillin 1.25 μg/ml
△ A-16686 5 μg/ml □ Vancomycin 5 μg/ml

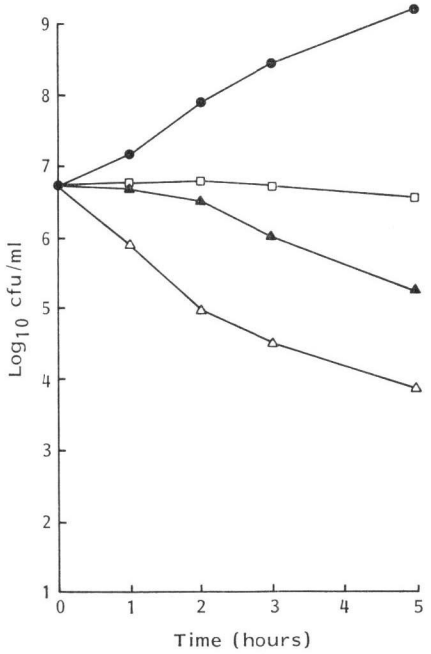


Fig. 6. Bactericidal activity of A-16686, ampicillin and vancomycin against *S. mutans* L659.

● Control ▲ Ampicillin 0.32 μg/ml
△ A-16686 5 μg/ml □ Vancomycin 5 μg/ml

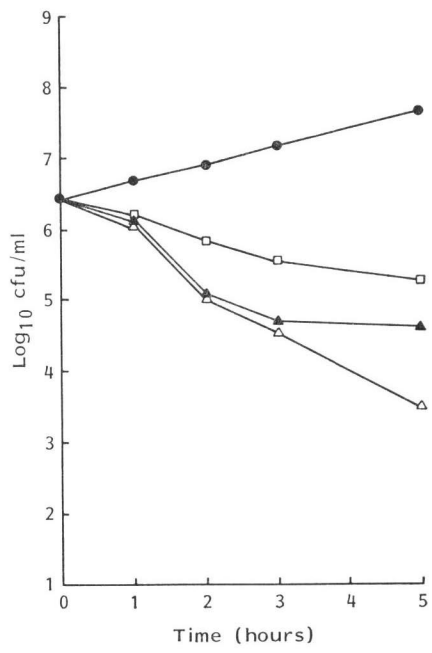


Fig. 7. Bactericidal activity of A-16686, ampicillin and vancomycin against *S. bovis* L1325.

● Control ▲ Ampicillin 0.625 μg/ml
△ A-16686 5 μg/ml □ Vancomycin 2.5 μg/ml

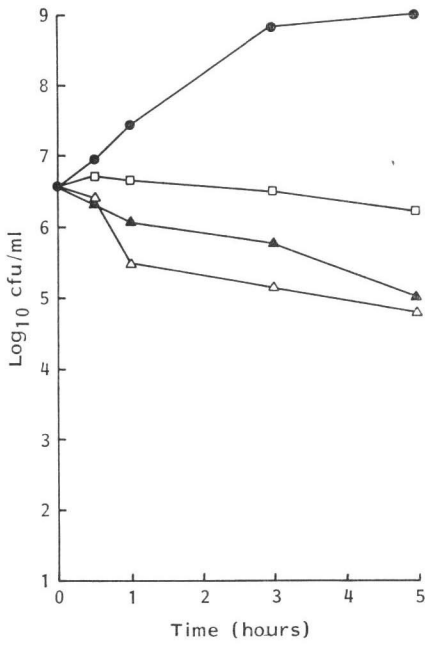
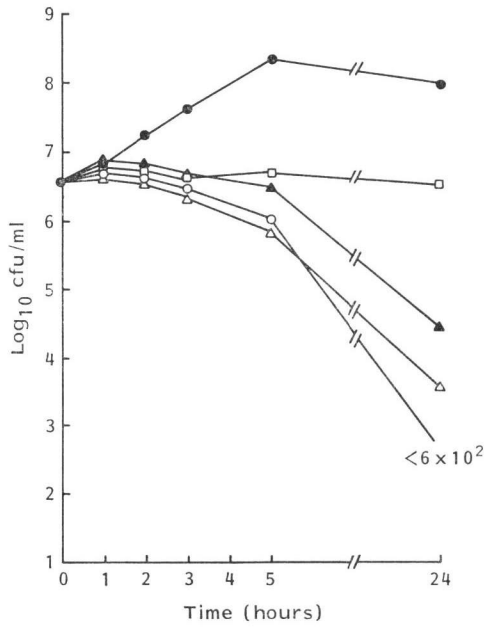


Fig. 8. Bactericidal activity of A-16686, ampicillin and vancomycin against *S. sanguis* L667.

● Control ▲ Ampicillin 5 μg/ml
○ A-16686 10 μg/ml □ Vancomycin 10 μg/ml
△ A-16686 5 μg/ml



Experimental Infections

Subcutaneously administered A-16686 was very effective in curing mice experimentally infected with *S. pyogenes* and *S. pneumoniae* (Table 4). In these infections, its efficacy was greater than those of the other antibiotics tested, with the exception of cephaloridine in the *S. pyogenes* infection.

A-16686 was less effective in the *S. aureus* infection, where it protected 50% of the treated animals at a dose of about 25 mg/kg; this result is comparable to those obtained with erythromycin and benzylpenicillin in the same experimental conditions.

When administered orally in water solution containing either 0.5% Methocel or 7.5% Cetomacrogol 1000, which reportedly increases oral absorption[5], A-16686 was not effective at up to 200 mg/kg in the *S. pyogenes* infection.

Table 4. Efficacy of A-16686 in experimental infections in mice in comparison with other antibiotics.

Antibiotics	sc ED_{50} (mg/kg/day)		
	S. aureus L165	*S. pyogenes* L49	*S. pneumoniae* L44
A-16686	~25	0.081	0.14
Ampicillin	8.1	0.1	4.1
Cephaloridine	2.8	0.03	0.93
Erythromycin	28	0.44	26
Lincomycin	57	0.66	76
Benzylpenicillin	25	0.29	20
Vancomycin	7.2	0.58	1.9

Toxicity

A-16686 had LD_{50}'s of 328 mg/kg ip and 122 mg/kg iv in mice and 2,000 mg/kg po in rats.

Activity of Different Components of A-16686

The *in vitro* and *in vivo* activities of the three components[1] of the complex, A1, A2 and A3, were determined. Each of them had *in vitro* activity similar to that of the complex. In experimental septicemias, A1 and A2 showed activity similar to that of the complex, while A3 was slightly less effective (Table 5).

Table 5. *In vitro* and *in vivo* activity of A-16686 complex and its components.

A-16686	*S. pyogenes* L49		*S. pneumoniae* L44	
	MIC (μg/ml)	sc ED_{50} (mg/kg/day)	MIC (μg/ml)	sc ED_{50} (mg/kg/day)
Complex	0.012	0.068	0.025	0.23
A1	0.012	0.041	0.025	0.15
A2	0.012	0.048	0.025	0.18
A3	0.012	0.14	0.025	0.41

Conclusions

These studies show that A-16686 has good *in vitro* activity against staphylococci (including methicillin-resistant isolates), streptococci, *Actinomyces* and Gram-positive anaerobes. Although less active than cephaloridine, ampicillin and erythromycin against most strains of streptococci, it is generally more active against *S. faecalis* and *S. faecium*.

Of particular interest is its marked and rapid bactericidal activity against several species including staphylococci, *S. faecalis, S. faecium, S. mutans* and *S. mitis* and its good, if less rapid, killing of *S. sanguis*. Other interesting features are its excellent therapeutic activity in curing experimental septicemias in the mouse and the lack of cross-resistance with clinically used antibiotics.

A-16686 appears to be a promising antibiotic for the therapy of infections caused by Gram-positive bacteria.

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