PHOLIPOMYCIN, A NEW MEMBER OF PHOSPHOGLYCOLIPID ANTIBIOTICS

III. BIOLOGICAL PROPERTIES

AKIO TORIKATA, HIROJI YOSHIKAWA, TOSHIAKI KATAYAMA,
and MAMORU ARAI

Fermentation Research Laboratories

MASAKI NAKAHARA and NORITOSHI KITANO

Central Research Laboratories, Sankyo Co., Ltd.
2–58, 1-chome, Hiromachi, Shinagawa-ku, Tokyo 140, Japan

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Pholipomycin, a new member of the phosphoglycolipid antibiotics, was primarily active against gram-positive bacteria including clinically isolated resistant bacteria. It differed from other members of the antibiotic family as it also demonstrated activity against gram-negative bacteria. Pholipomycin protected mice from infection with *Staphylococcus aureus* and was nontoxic to mice having an LD_{50} (i.v.) of 600 mg/kg. Besides possessing antimicrobiol activity, pholipomycin, when administered orally, appeared to promote growth in chickens and pigs.

As reported in the preceding papers,[1,2] pholipomycin, a new member of the phosphoglycolipid antibiotics, was produced by *Streptomyces lividoclavatus*, a previously undescribed species. Physicochemical characterization of pholipomycin revealed that it can be distinguished from other members of the antibiotic family.

This report describes the biological properties of pholipomycin.

Antimicrobial Spectrum

The minimum inhibitory concentrations (MIC) of pholipomycin against various microorganisms were determined by a serial two-fold agar dilution method. The results are presented in Table 1.

Pholipomycin was strongly active against gram-positive bacteria and weakly active against various gram-negative bacteria but inactive against fungi and yeasts. The antibacterial activity of pholipomycin, however, was significantly reduced by addition of horse serum to the medium. The effect of pholipomycin on bacteria was also dependent on the pH value of the test medium as shown in Table 2. The maximal antibacterial action on *S. aureus* FDA 209P was in the range between pH 5.0 and 6.5. A comparison of antibacterial activity of pholipomycin with other members of this family of antibiotics was performed by cylinder-plate method as shown in Table 3. Macarbomycin was the most active against *S. aureus* of the five antibiotics tested, whereas pholipomycin exhibited 3~9 times greater activity against *Escherichia coli* than did the others. Although the strain of *Pseudomonas aeruginosa* used in this comparison test was rather sensitive to this group of antibiotics, pholipomycin again showed the greater activity.

Chemotherapeutic and Toxicological Tests

Pholipomycin was effective in mice against infections with *S. aureus*. RFVL mice (5 mice

Table 1. Antimicrobial spectrum of pholipomycin

Test organism	Minimum inhibitory concentration (μg/ml)		
	Medium 1	Medium 2	Medium 3
Staphylococcus aureus FDA 209P JC-1	0.39	0.09	6.25
S. aureus 56 (Multi-resistant)	0.39	0.19	12.5
S. aureus 193 (Multi-resistant)	0.39	0.19	12.5
Bacillus subtilis PCI 219	50	0.09	>100
Micrococcus luteus PCI 1001	>100	12.5	>100
Corynebacterium xerosis B 58-3	>100	100	>100
Escherichia coli NIHJ JC-2	50	25	>100
E. coli K-12	12.5	12.5	>100
E. coli (ST-resistant)	25	25	—
Pseudomonas aeruginosa SANK 73860	3.12	0.78	50
Pseudomonas sp. SC-8452	100	25	>100
Proteus vulgaris OX 19	0.78	3.12	100
Klebsiella pneumoniae PCI 602	25	12.5	>100
Aeromonas liquefaciens Y-62	0.78	0.39	25
Mycobacterium smegmatis ATCC 607	50	—	—
	Medium 4		
Candida albicans YU 1200	>100		
Saccharomyces cerevisiae SANK 50170	>100		
Trichophyton mentagrophytes SANK 11868	>100		
Fusarium moniliforme SANK 10162	>100		
Pyricularia oryzae SANK 14758	>100		

Medium 1: Nutrient agar with 1% glycerol (37°C, 24 hours for bacteria; 26°C, 48 hours for fungi).
Medium 2: Heart infusion agar.
Medium 3: Heart infusion agar with 10% horse serum.
Medium 4: SABOURAUD dextrose agar.
ST: Streptothricin.

Table 2. Effect of pH on antimicrobial activity

pH	Minimum inhibitory concentration (μg/ml)					
	S. aureus 209P		*E. coli* NIHJ		*P. aeruginosa* SANK 73860	
	24 h.	48 h.	24 h.	48 h.	24 h.	48 h.
5.0	0.002	0.005	12.5	12.5	0.01	0.04
5.5	0.01	0.01	25	25	0.19	0.19
6.0	0.04	0.09	25	25	0.19	0.19
6.5	0.09	0.19	50	50	0.39	0.39
7.0	0.78	1.56	100	100	0.39	0.39
7.5	0.78	3.12	100	100	0.78	0.78
8.0	1.56	3.12	50	50	0.78	0.78
8.5	1.56	1.56	50	50	0.78	0.78
9.0	1.56	1.56	50	50	0.78	0.78

The pH of the medium, Antibiotic Medium 3, was adjusted with NaOH or HCl followed by filtration through a Millipore filter (GS 0.22 μ) for sterilization. A final concentration of the organisms at time of inoculation was 1.5×10^{-3} dilution of the overnight culture for *S. aureus* and *P. aeruginosa* and 1.5×10^{-4} for *E. coli*.

Table 3. Comparison of antibacterial activity of phosphoglycolipid-antibiotics

Antibiotic	*S. aureus* 209P	*E. coli* NIHJ	*P. aeruginosa*
Moenomycin (A+C)	131%	37.0%	48.3%
Macarbomycin	164	16.2	35.7
Diumycin A	99.3	11.6	27.7
Diumycin A′	118	30.5	73.4
Pholipomycin	100	100	100

Activity of each antibiotic was tested by cylinder-plate method using 12.5, 50 and 200 μg/ml of the antibiotic in M/15, pH 7.0 phosphate buffer. Percent activity was calculated using pholipomycin as a standard antibiotic.

Table 4. Effect of pholipomycin on growth of broilers (Field test)

	Pholipomycin group	Control group
Average initial body weight (g.)	41.0	40.9
Average 8-week body weight (g.)	1989±269*	1896±333*
Feed conversion ratio	2.32	2.68
Viability (%)	99	94

* Standard deviation. Chickens of Studler variety, 100 chickens in each group, were employed. The ration was a commercially available one (manufactured by Sumitomo Shiryo K. K., Japan).

$$\text{Feed conversion ratio} = \frac{\text{Total feed ingestion}}{\text{Total body weight}}$$

$$\text{Viability (\%)} = \frac{\text{Number of chickens surviving at final weighing}}{\text{Number of chickens initially used}} \times 100.$$

Table 5. Effect of pholipomycin on growth of pigs (Field test)

	Pholipomycin group	Control group
Average initial body weight (kg)	8.4	8.5
Average 75-day body weight (kg)	45.8	43.2
Feed conversion ratio	2.9	3.2

Pigs of 32-days of age with 8 pigs in each group were employed. The ration was a commercially available one. Pholipomycin was added at the rate of 5 ppm during the first 4 weeks and 2 ppm thereafter. Feed conversion ratio was calculated as indicated in Table 4.

per dose) were challenged with *S. aureus* No. 42 intraperitoneally (1×10^7 cells per mouse) and pholipomycin was administered subcutaneously at 0 and 4 hours after infection. Survival of 0, 1 and 5 mice was noted at doses of 50, 100 and 200 mg/kg, respectively. Pholipomycin was extremely low in its toxicity; the LD_{50} to mice was 600 mg/kg after intravenous administration. No effect on killifish was observed at 200 ppm of pholipomycin in aqueous solution.

Growth-promoting Activity

In addition to its antimicrobial activity, growth-promoting activity in animals was observed with pholipomycin as with other members of this group of antibiotics, *i.e.* the moenomycins[3,4] and the macarbomycins.[5] The addition of pholipomycin at 2.5 ppm to broiler diets resulted in consistent improvement in body weight gains. As shown in Table 4, at eight weeks of age, broiler weights were greater, feed conversion was improved and viability was increased in the group of birds supplemented with pholipomycin. The effect of pholipomycin on growth of pigs was also tested with first cross pigs (Hump X Rand), 32 days old, with the results shown in Table 5.

It is apparent from the results that pholipomycin is also effective in promoting the growth of pigs and in modifying, favorably, feed conversion.

Discussion

Strong antibacterial activity by the members of the phosphoglycolipid antibiotic family has already been reported.[5~7] Although these antibiotics are primarily active against gram-positive bacteria *in vitro* and *in vivo*, increased sensitivity of gram-negative bacteria carrying R factor was also observed.[8,9] Similarly, pholipomycin was active against gram-positive bacteria *in vitro* and

mice were protected from infection with *S. aureus* by subcutaneous administration of the antibiotic. Pholipomycin demonstrated extremely low toxicity to mice. Mode of action studies conducted on other members of this family have indicated that they inhibit cell wall biosynthesis in *S. aureus*.[10~12] We observed spheroplast formation of *E. coli* in isotonic media containing pholipomycin thereby confirming the same mechanism of action. In addition to its antimicrobial activities, pholipomycin was shown to promote growth in animals, as reported for other members of the family. Moenomycin, when added to the diet of broilers at a concentration of 1~4 ppm, resulted in a 3% increase in body weight at eight weeks of age.[4] A similar improvement, a 5% increase at 2.5 ppm, was observed in the present trials with pholipomycin. The fact that gram-negative bacteria are more sensitive to pholipomycin than to other members may offer an advantage to this antibiotic as a feed additive.

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