ANTIMICROBIAL PROPERTIES OF MANNOPEPTINS

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Mannopeptins show *in vitro* antimicrobial activity against gram-positive and some gram-negative bacteria. The antimicrobial activity is unaffected by the addition of serum, and potentiated by alkaline pH or decrease in inoculum size. The antibiotics exert bectericidal effect at doses twice as high as the minimum inhibitory concentration. When the antibiotics were injected into mice through either intravenous, intraperitoneal, intramuscular or subcutaneous routes, the antimicrobial activity appeared within 15 minutes in the serum of mice and was slowly excreted in the urine. However, the antibiotics were poorly absorbed by the oral route. The antibiotics were capable of protecting mice from lethal infection produced by the intravenous injection of *Staphylococcus aureus*, *Streptococcus pyogenes* and the intraperitoneal injection of *Shigella* sp. and *Escherichia coli*, but ineffective against *Salmonella typhosa*.

As reported in a previous paper[1], new antibiotics, mannopeptins A and B were isolated from the culture filtrate of *Streptomyces platensis* strain FS-351. The antibiotics are produced in the presence of excess ferrous ion, although Fe^{++} is absent in the molecule. Mannopeptins are glycopeptides related to ristocetin[2], vancomycin[3] and LL-AV290[4]: This paper deals with an evaluation of their biological properties.

Materials and Methods

Mannopeptins A and B were prepared as reported in a previous paper[1]. The positive control agents, lincomycin, erythromycin and cephalexin, were commercial products.

Antimicrobial activity testing: The minimum inhibitory concentration of mannopeptins was estimated by the two-fold serial dilution method with heart infusion agar as an assay medium. The test organisms were previously cultured for 16 hours on nutrient agar slope and loopfuls of the culture were suspended in sterile water to make $10^7 \sim 10^8$ viable cells per ml. The suspensions were streaked on agar plates containing given amounts of the antibiotics. The plates were incubated at 37°C and the minimum inhibitory concentrations (MIC) were determined routinely 24 hours later. The MIC was defined as prevention of visible growth of the test organisms.

Bioassay of mannopeptins: A paper disc agar diffusion method was used for the quantitative determination of the antibiotics using *Staphylococcus aureus* FDA 209P as a test organism.

Bactericidal activity: The viability of *Staph. aureus* FDA 209P in the presence of the antibiotics was determined by the plate count technique. An 18-hour culture of the organism was diluted 10^5 times with nutrient broth and the antibiotics were added to give concentrations of 1.56, 3.12, 6.25 12.5 μg/ml. Aliquots were taken from each tube prior to incubation and at 3, 6, 9 and 24 hours after incubating at 37°C. Platings were made in duplicate at several dilutions to ensure reliable counts. Colony counts were made after 24 hours.

Therapeutic effect in mice: Male, *ddY* mice, weighing 18~22g, were used. Intraperitoneal infection was caused with 0.5 ml of bacterial suspension in 5 % mucin solution containing 100 MLD of challenging organisms. In cases of intravenous infection, 0.2 ml of bacterial suspen-

sion containing 10 MLD challenging organism in physiological saline was injected through the tail vein. One hour after challenging, treatment was made by single subcutaneous administration of the antibiotics. The 50% effective dose (ED_{50}) was calculated from the survival rate of the mice at 7 days later by the method of REED and MUENCH[5].

Results and Discussion

The minimum inhibitory concentration of mannopeptins is demonstrated in Table 1. The spectra differ from those of known glycopeptide antibiotics in activity against some gram-negative bacteria; mannopeptin A as well as B is equally effective against *Escherichia*, *Salmonella* and *Shigella*, whereas ristocetin, vancomycin and LL-AV 290 are not.

As illustrated in Table 2, the sensitivity distribution of mannopeptins shows a single peak against bacteria including clinical isolates resistant to other chemotherapeutic agents. *Staphylococci* were the most sensitive being inhibited at concentrations of 1.25~12.5μg/ml. Mannopeptins

Table 1. Antimicrobial spectra of mannopeptins

Organisms tested	MIC (μg/ml)	
	Mannopeptin A	Mannopeptin B
Staphylococcus aureus FDA 209 P	6.25	6.25
Staphylococcus aureus Terajima	6.25	6.25
Staphylococcus aureus B-43 (Pc)	12.5	12.5
Staphylococcus aureus r-24 (Pc, SM, TC, SA)	1.56	6.25
Streptococcus hemolyticus T-3	25	25
Streptococcus hemolyticus T-11	25	25
Bacillus subtilis PCI-219	0.78	3.12
Bacillus cereus strain Ch	25	25
Sarcina lutea ATCC-9341	3.12	3.12
Escherichia coli G-1	50	50
Escherichia coli K-12	25	25
Escherichia coli (SM, CM, TC, SA)	50	50
Salmonella typhosa TA	25	25
Salmonella typhosa TD-4	25	25
Salmonella paratyphi A	50	50
Shigella dysenterae Shiga	50	50
Shigella flexneri 3a	12.5	12.5
Shigella sonnei Ch	50	50
Klebsiella pneumoniae 1	>100	>100
Proteus vulgaris OX-2	>100	>100
Pseudomonas aeruginosa Ps-1	>100	>100
Candida albicans c-a-8	>100	>100
Trichophyton asteroides T-4	>100	>100
Mycobacterium tuberculosis H37RV	>100	>100

The medium for bacteria was heart infusion agar, 37°C, for 48 hours. The medium for fungi was SABOURAUD medium, at 28°C for a week. The medium for *Mycobacterium* was KIRCHNER medium, 28°C for a week.

Resistant property to chemotherapeutics is expressed in parenthesis; abbreviations are as follows; Pc=penicillin G, SM=streptomycin, TC=tetracycline, SA=sulfonamides CM=chloramphenicol.

Table 2. The distribution of mannopeptins sensitive bacteria

Organisms	Mannopeptins	MIC (μg/ml)								
		>100	100	50	25	12.5	6.25	3.12	1.56	0.78
Staphylococcus aureus (29 strains)	A					13	13	2	1	
	B					11	16		2	
Streptococcus hemolyticus (3 strains)	A		1	1	6					
	B		1	1	6					
Bacillus (3 strains)	A				1	1				1
	B				1		1	1		
Sarcina lutea (1 strains)	A									1
	B									1
Eschericha coli (22 strains)	A		5	15	2					
	B		5	14	3					
Salmonella (6 strains)	A		3	5	3					
	B		4	3	4					
Shigella (6 strains)	A			3	1	2				
	B			1	3	2				
Klebsiella (3 strains)	A	3								
	B	3								
Proteus (7 strains)	A	7								
	B	7								
Pseudomonas (6 strains)	A	6								
	B	6								

A and B inhibited the growth of *Streptococci* at 25~100 μg/ml and this weak activity differentiates them from ristocetin and vancomycin; the latter two are very effective against this pathogen. The antibiotics are effective against *Escherichia*, *Shigella* and *Salmonella*, although the MIC's are relatively high, and no resistant strains were found among them.

Acute toxicity of the antibiotics was determined using mice. The following LD_{50}'s were estimated; 97mg/kg (iv), 125 mg/kg (ip), over 500 mg/kg (im, sc and po). The antibiotics are necrotoxic, since necrotic regions were noted at the injected sites when they were administered either subcutaneously or intramuscularly.

As shown in Table 3, the effects of serum, pH and inoculum size on antibacterial activity were determined using *Staphylococcus aureus* FDA 209 P as a test organism. The antibacterial activity was unaffected by the addition of horse serum up to 20% in the assay medium. The activity was enhanced either in

Table 3. Effect of pH, inoculum size and serum on the antimicrobial activity of mannopeptins

		MIC (μg/ml)	
		Mannopeptin A	Mannopeptin B
pH	6	6.25	6.25
	7	6.25	6.25
	8	3.12	3.12
	9	1.56	1.56
Inoculum size (cells/ml)	1×10^3	3.12	3.12
	1×10^4	6.25	6.25
	1×10^5	6.25	6.25
Horse serum (%)	0	6.25	6.25
	10	6.25	6.25
	20	6.25	6.25
	40	6.25	6.25

Test organism; *Staphylococcus aureus* FDA 209 P

alkaline media or with smaller inoculum size. The MIC at pH 9 was one fourth of that at pH 7; this is a common property of the basic water-soluble antibiotics. The inoculum size also affected the antibacterial activity, since a slight increase in activity was observed as the inoculum size diminished.

As shown in Fig. 1, the viable cell count rapidly decreased in media containing mannopeptin at twice the MIC level; at the MIC, the bacterial counts transiently decreased and then, returned to the starting level 24 hours after incubation. It is evident that the antibiotics are bacteriocidal rather than bacteriostatic.

Absorption, distribution and excretion of the antibiotics were determined with the use of mice (Fig. 2). When the antibiotics were injected subcutaneously, the bioactivity appeared within 15 minutes in the blood and the serum level was maintained for relatively long periods, suggesting slow excretion of the antibiotics from the kidney. The peak height of mannopep-

Fig. 1. Effect of mannopeptins on viability on *Staphylococcus aureus*.
(a) Mannopeptin A. (b) Mannopeptin B.

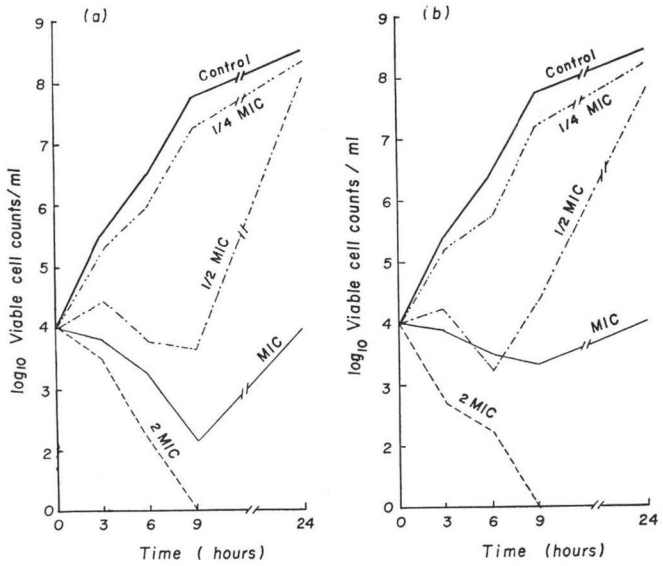
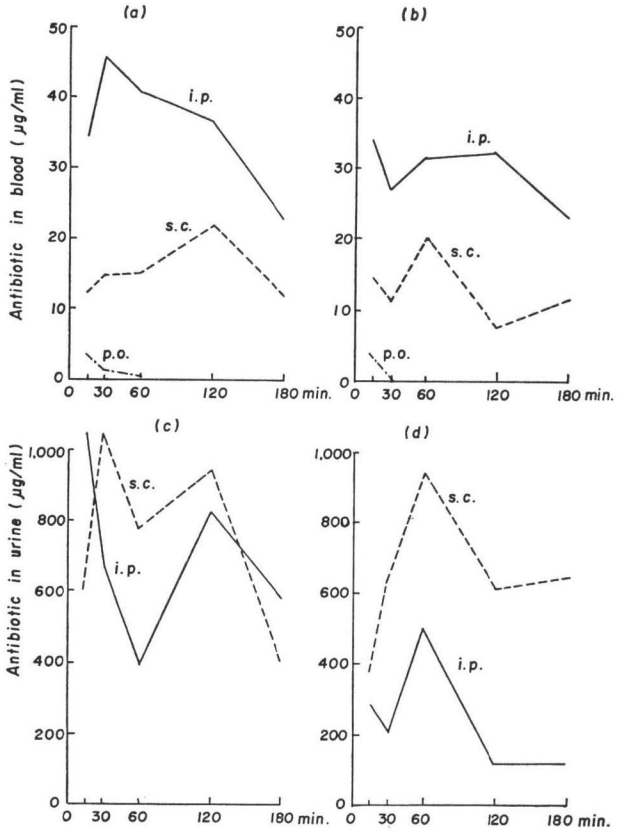


Table 4. ED_{50} of mannopeptins against lethal infection of some bacteria

Bacteria infected	Route of infection	Challenging dose (MLD)	ED_{50} (mg/kg)		
			Mannopeptin A	Mannopeptin B	Positive control agents
Staphylococcus aureus Ch	iv	1	12.3	30.4	30.7 (Lincomycin)
Staphylococcus aureus r-9	iv	10	11.6	42.2	>200 (Lincomycin)
Streptococcus hemolyticus G36M5	ip	100	108.0	>200	88.1 (Erythromycin) 79.0 (Cephalexin)
Escherichia coli No 11	ip	100	45.4	68.0	—
Shigella sp. st-r-91	ip	100	30.0	>200	—
Salmonella typhosa type WT-1	ip	100	>200	>200	—

Chemotherapeutic agents were administered subcutaneously 1 hour after infection.

Fig. 2. Serum and urine levels of mannopeptins in mice. The serum and urine levels of mannopeptin A are expressed in Fig. 2 (a) and (c), and those of mannopeptin B in Fig. 2(b) and (d). Mannopeptins were administered at a dose of 50 mg/kg. Abbreviations; i.p.=intraperitoneal administration, s.c.=subcutaneous administration, p.o.=oral administration.



tin A in the serum is higher than that of mannopeptin B; this fact may explain the better chemotherapeutic effect of mannopeptin A than B in mice (Table 4).

The antibiotics were capable of protecting mice from lethal intravenous infections of *Staphylococcus aureus* strain Ch and *S. aureus* strain r-9 (Table 4); the latter is resistant to macrolide antibiotics, so that a positive control agent, lincomycin was completely ineffective. The antibiotics were effective against lethal infections of *Streptococcus hemolyticus*, *E. coli* and *Shigella* sp., although the ED_{50}'s were relatively high.

In conclusion, the strong points of the mannopeptins are the potent bactericidal effect against *Staphylococci* and their ability to protect mice efficiently from lethal infections of the pathogen. On the other hand, severe necrotoxicity like that of ristocetin and vancomycin at the site of injection is the weakest point of the mannopeptins.

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