HELVECARDINS A AND B, NOVEL GLYCOPEPTIDE ANTIBIOTICS

III. BIOLOGICAL PROPERTIES

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Helvecardins (HVCs) A and B were strongly active against aerobic and anaerobic Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), but they were inactive against Gram-negative bacteria and fungi. Though HVC A showed only slightly stronger antimicrobial activity than β-avoparcin (AVP), its *in vivo* protective activity against *S. aureus* infection in mice was greatly superior to AVP.

One of the serious problems in the treatment of infectious diseases is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), which are resistant to many antibiotics, including third generation β-lactam antibiotics[1,2]. Recently, glycopeptide antibiotics, which inhibit bacterial cell wall peptidoglycan synthesis, are being revalued because of their strong antimicrobial activity against Gram-positive bacteria, including MRSA, and their low host toxicity.

As reported previously, new glycopeptide antibiotics, helvecardins (HVCs) A and B, were found in our screening program for cell wall synthesis inhibitors[3,4]. Their respective structures are β-avoparcin 2′-*O*-methylated on rhamnose and a demannosyl derivative of HVC A. In this paper the biological properties are reported in comparison with β-avoparcin (AVP) and vancomycin (VCM).

Materials and Methods

Antimicrobial Activities

MICs were determined by the agar dilution method, using Mueller-Hinton agar for aerobic bacteria and GAM agar for anaerobic bacteria. Overnight cultures of the organisms were diluted to 10^6 cells/ml and layered on the surface of plates. MBCs were defined as the lowest concentrations that resulted in a 99.9% decrease in the viable cell number, according to the method reported by SHANHOLTZER *et al.*[5].

In Vivo Protection Assay

Staphylococcus aureus 56 was grown in 10 ml Nutrient broth (Eiken) at 37°C overnight without shaking. After harvesting and washing with ice-cold 0.85% NaCl, cells were suspended in 20 ml of 0.85% NaCl containing 5% mutin. *dd*Y Female mice were infected intraperitoneally with 1×10^8 cells of the organism and treated with antibiotics subcutaneously twice, immediately and again 4 hours after infection. ED_{50}s were determined on day 7 after infection.

Pharmacokinetics

Mice were injected subcutaneously with antibiotics, and at the indicated times, serum, feces, and urine samples were collected. For determination of concentrations of the antibiotics in the samples, a paper-disc agar diffusion assay was employed using *Bacillus subtilis* PCI 219 as the test organism, after dilution of the samples with 0.1 M phosphate buffer, pH 7.2.

Table 1. Antimicrobial activities of HVCs A, B, AVP and VCM.

Test organism	Medium	MIC (μg/ml)			
		HVC A	HVC B	AVP	VCM
Staphylococcus aureus FDA 209P	A	1.56	0.78	1.56	0.78
S. aureus 56	A	3.13	3.13	6.25	0.78
S. aureus 507 (MRSA)	A	6.25	3.13	6.25	0.78
S. aureus 1-1 (MRSA)	A	3.13	1.56	3.13	0.78
Enterococcus faecalis 452	A	0.78	0.78	0.78	0.78
Streptococcus agalactiae ATCC 13813	A	0.78	0.78	0.78	0.78
Micrococcus luteus ATCC 9341	A	0.20	0.20	0.39	0.78
Corynebacterium diphtheriae IID 527	A	0.20	0.39	0.39	0.78
Bacillus cereus IID 872	A	1.56	0.78	0.78	0.78
B. subtilis ATCC 6633	A	0.20	0.39	0.39	0.39
Peptostreptococcus parvulus VPI 0546	B	0.39	0.39	0.78	1.56
Propionibacterium acnes ATCC 11828	B	0.20	0.20	0.20	1.56
Clostridium difficile ATCC 9689	B	0.39	0.78	0.78	1.56
C. perfringens ATCC 13124	B	0.20	0.20	0.20	1.56
Escherichia coli NIHJ JC-2	A	>100	>100	>100	>100
Klebsiella pneumoniae IID 865-2	A	>100	>100	>100	>100
Serratia marcescens IAM 1184	A	>100	>100	>100	>100
Proteus vulgaris IID 874-2	A	>100	100	>100	>100
Pseudomonas aeruginosa IID 1117-2	A	>100	>100	>100	>100
Bacteroides fragilis GM 7000	B	>100	100	50	50

Medium A: Mueller-Hinton agar, B: GAM agar.
Inoculum size: 10^6 cells/ml.

Results

Antimicrobial Activities of HVCs A and B

MICs of HVCs A and B with a comparison to those of AVP, which is used as a feed additive for growth promotion of livestock[6], and VCM, which has been mainly used in the treatment of infectious diseases caused by methicillin-resistant Staphylococci and pseudomembranous colitis caused by *Clostridium difficile*[7], are shown in Table 1. HVCs A and B are active against aerobic and anaerobic Gram-positive bacteria, such as *S. aureus*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *Corynebacterium diphtheriae*, *Bacillus subtilis*, *Propionibacterium acnes*, and *C. difficile*, whereas they are inactive against Gram-negative bacteria and fungi. The order of activity against Gram-positive aerobic bacteria was VCM > HVC B = HVC A = AVP, whereas it was HVC A = HVC B = AVP > VCM against anaerobic Gram-positive bacteria. It is noteworthy that HVC A was as active against MRSA as methicillin-sensitive *S. aureus* (MSSA), and that it was more active than VCM against such anaerobic bacteria as *C. difficile* and *Clostridium perfringens*.

Table 2. *In vivo* efficacy of HVCs A, B, AVP and VCM against bacterial infection in mice.

Antibiotics	*Staphylococcus aureus* 56 ED_{50} (mg/kg)	*S. aureus* 1-1 ED_{50} (mg/kg)
HVC A	14.5	5.2
HVC B	15.2	—
AVP	29.0	—
VCM	6.1	3.4

ED_{50}s were determined according to Materials and Methods.
Challenge: ip.
Treatment: sc (0, 4 hours).

The values of MBCs of HVCs A and B against *S. aureus* 56 were the same as those of MICs.

Toxicity

LD_{50}s of HVCs A and B in mice injected intravenously were >500 mg/kg and 325 mg/kg, respec-

Table 3. Pharmacokinetics of HVCs A and B in comparison with AVP and VCM.

Antibiotics	Serum concentrations[a]				Urinary recoveries[b]	
	30 minutes	60 minutes	120 minutes	240 minutes	24 hours	48 hours
HVC A	6.5	3.7	3.1	0	56.7	1.7
HVC B	9.0	8.0	5.0	0	52.4	0
AVP	9.0	5.0	2.0	0	41.7	0
VCM	7.5	8.6	1.5	0	64.2	2.6

[a] Expressed in mg/ml.
[b] Expressed as % of administered drug.

tively. These values were comparable to that of AVP (500 mg/kg). The toxicity of HVC A was slightly lower than that of VCM (470 mg/kg).

In Vivo Protective Activities of HVCs A and B

As shown in Table 2, HVCs A and B protected mice from infection by *S. aureus*. Their ED_{50}s against *S. aureus* 56 infection in mice were 14.5 and 15.2 mg/kg, respectively, whereas the ED_{50}s of VCM and AVP were respectively 6.1 and 29.0 mg/kg. It is interesting that the protective activity of HVC A against *S. aureus* 1-1 infection which is methicillin-resistant is better than that against MSSA.

Pharmacokinetics in Mice

The concentrations of HVCs A and B in serum were respectively 6.5 μg/ml and 9.0 μg/ml at 30 minutes and 3.1 μg/ml and 5.0 μg/ml at 2 hours after subcutaneous injection at doses of 20 mg/kg in mice as shown in Table 3. VCM and AVP also showed similar patterns of serum concentrations to HVCs A and B.

The urinary recoveries of HVCs A, B, AVP and VCM were respectively 56.7%, 52.4%, 41.7%, and 64.2% at 24 hours after injection.

Discussion

Many glycopeptide antibiotics have been found, modification studies on them have been performed, and much information about their structure-activity relationships has been accumulated[7], but the *O*-methylation of the sugar moiety in HVCs is the first reported example. It is very interesting that the protective activity of HVC A was twice more strong than AVP, though the antimicrobial activity was not so much improved by 2′-*O*-methylation of rhamnose.

HVC B may be more toxic than HVC A or AVP, possibly due to its insolubility in water.

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