MERSACIDIN, A NEW ANTIBIOTIC FROM *BACILLUS*

IN VITRO AND *IN VIVO* ANTIBACTERIAL ACTIVITY

SUKUMAR CHATTERJEE, DIPAK KUMAR CHATTERJEE, RAJENDRA H. JANI, J. BLUMBACH and B. N. GANGULI†

Microbiology Department, Hoechst Centre for Basic Research, Mulund, Bombay 400 080, India

NORBERT KLESEL, MICHAEL LIMBERT and GERHARD SEIBERT

Pharma Research, Hoechst Akteingesellschaft, 6230 Frankfurt am Main 80, Germany

(Received for publication October 7, 1991)

Mersacidin is a new peptide antibiotic of the proposed lantibiotic family. It is active *in vitro* and *in vivo* against Gram-positive bacteria including the methicillin-resistant Staphylococci. Its *in vitro* activity is less than those of vancomycin and erythromycin but it shows much higher activity in the *in vivo* system than can be expected from the *in vitro* testing results. A water soluble potassium salt has been prepared which has an activity profile similar to that of mersacidin, but has better *in vivo* activity against *Streptococcus pyogenes* than the parent compound.

A new peptide antibiotic mersacidin[1,2], has been isolated from fermentations of a *Bacillus* species HIL Y-85,54728. The antibiotic contains the unusual amino acid methyllanthionine, and hence may be grouped in the class termed lantibiotics. Peptides of this group, besides being antibiotics, have also shown other interesting biological activities. For example, nisin[3] has been reported as an antimalarial agent, ancovenin[4] as an enzyme inhibitor, Ro 09-0198[5] as an immunopotentiator and lanthiopeptin[6] has been reported to be active against Herpes simplex virus I. Mersacidin is interesting from another perspective; its activity in experimentally infected animals (mice) is significantly higher than would be predicted from its moderate *in vitro* activity. A similar phenomenon has been reported concerning the antibiotic spiramycin[7] which is less active than erythromycin in the *in vitro*, but shows much better activity than erythromycin in the *in vivo* model.

In the present communication the antibacterial activities of mersacidin and its water soluble derivative, potassium mersacidin, are reported.

Materials and Methods

In Vitro Antibacterial Activity

Mersacidin used in the experiments was isolated and purified in our laboratories, while erythromycin and vancomycin were obtained commercially. The *in vitro* antibacterial activity of mersacidin was assessed against strains of *Staphylococcus* including both methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*, coagulase-negative Staphylococci, quinolone-resistant Staphylococci, and also against Enterococci. The bacterial strains were recent clinical isolates from various hospitals. For comparison, two standard antibiotics, vancomycin and erythromycin were also included in the tests.

Antibiotic activity was assessed by the agar dilution method using Mueller-Hinton agar. The

† Departmental Head.

inoculum volume was 5×10^5 colony forming units (cfu) per spot in the case of *Staphylococcus* strains and 5×10^4 cfu/spot in the case of Enterococci.

MIC values were determined after an incubation period of 24 hours at 37°C or 48 hours at 30°C in case of methicillin-resistant strains.

In Vivo Antibacterial Activity

Experimental Infection with *S. aureus* SG 511: Male or female Swiss albino mice weighing 18~22 g were used as experimental animals. The mice received normal pelleted food and tap water *ad libitum*. An 18-hour culture of *S. aureus* SG 511 grown in Tryptic Soya Agar (Difco) was used as the source of the infecting organisms. The inoculum contained 1.75×10^9 cfu/mouse (1 MLD). Minimum lethal dose (MLD) is defined as the minimum inoculum which caused all the infected animals to die within 20 hours of infection. Each mouse was infected by the intraperitonial (ip) route with 1.75×10^9 cfu in 0.5 ml of 5% hog gastric mucin. Untreated mice died within 20 hours post infection. At least 4 mice were used for each of the serial 2-fold dose concentrations of the compounds tested. Treatment was initiated by subcutaneous administration of mersacidin in three doses at 0, 2 and 24 hours post infection. ED_{50} and ED_{90} are defined as the doses which protected 50 and 90% of the animals, respectively.

Experimental Infections with Methicillin-resistant *S. aureus* Strains: All experimental conditions were the same as for *S. aureus* SG 511. The inoculum size for *S. aureus* E 710 was 2×10^{10} cfu/mouse (1 MLD), and the inoculum size for *S. aureus* C 31153 was 2×10^9 cfu/mouse (1 MLD).

Experimental Infection with *S. pyogenes* A 77: *S. pyogenes* A 77 was cultured in AC broth (Difco) overnight. A log phase culture was prepared and mice were infected intraperitonially. After 4 hours, the heart blood was asceptically drawn and transferred to crystal violet blood agar medium and incubated overnight. Typical *S. pyogenes* colonies were picked up and inoculated in fresh AC broth. After 5 hours the medium was diluted 1:2 with 15% sterile skimmed milk and cryopreserved in liquid nitrogen. The cryopreserved culture was used to determine the MLD. Swiss albino mice (18~22 g) of either sex received 4×10^3 cfu/0.5 ml (1 MLD) by the ip route for chemotherapeutic studies.

Results

Mersacidin inhibits 50% of the methicillin-sensitive *S. aureus* strains at a concentration of about 5 μg/ml while the corresponding values for vancomycin and erythromycin are around 0.3 μg/ml and 0.2 μg/ml, respectively (Fig. 1). The same relationship exists when coagulase-negative Staphylococci are

Fig. 1. Antibacterial activity *in vitro* against *Staphylococcus aureus* (Strain number = 20).

● Erythromycin, ▲ vancomycin, ■ mersacidin.

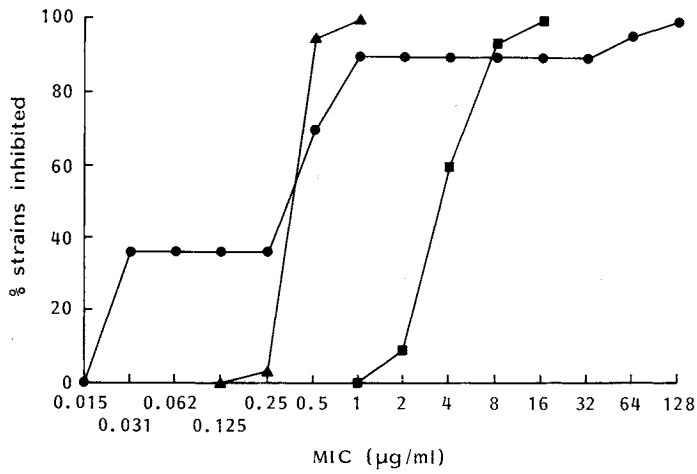


Fig. 2. Antibacterial activity *in vitro* against coagulase-negative *Staphylococcus* strains (Strain number = 26).

● Erythromycin, ▲ vancomycin, ■ mersacidin.

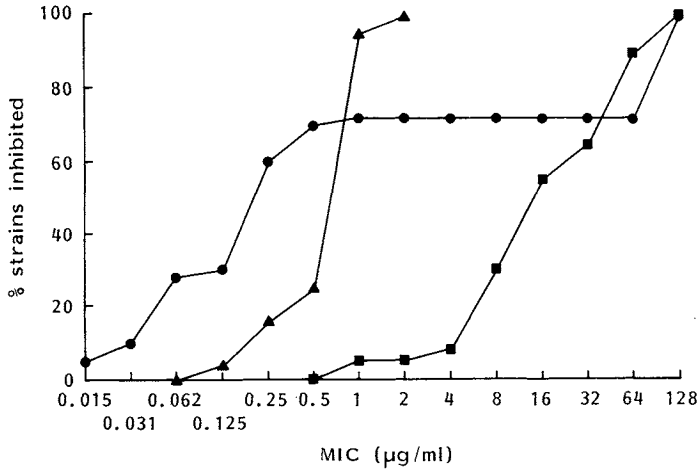
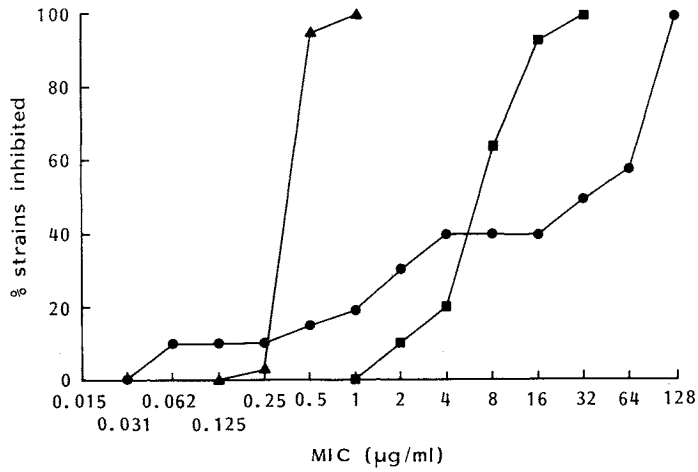


Fig. 3. Antibacterial activity *in vitro* against methicillin-resistant *Staphylococcus* strains (Strain number = 19).

● Erythromycin, ▲ vancomycin, ■ mersacidin.



considered. Mersacidin inhibits 50% of the strains at a concentration of 16 μg/ml while the corresponding values for vancomycin and erythromycin are 0.5 μg/ml and 0.2 μg/ml, respectively (Fig. 2). However, when MIC_{90} values are considered, mersacidin and erythromycin are approximately equally active. Vancomycin remains the best. Against methicillin-resistant Staphylococci, mersacidin has a somewhat better activity than erythromycin concerning the inhibition of more than 50% of the strains. However vancomycin still maintains its superiority (Fig. 3). Against quinolone-resistant Staphylococci, vancomycin is again the best (Fig. 4). Erythromycin is also better than mersacidin against most Staphylococci but to achieve the MIC_{90} the concentrations of erythromycin required are higher than

Fig. 4. Antibacterial activity *in vitro* against quinolone-resistant *Staphylococcus* strains (Strain number = 20).

● Erythromycin, ▲ vancomycin, ■ mersacidin.

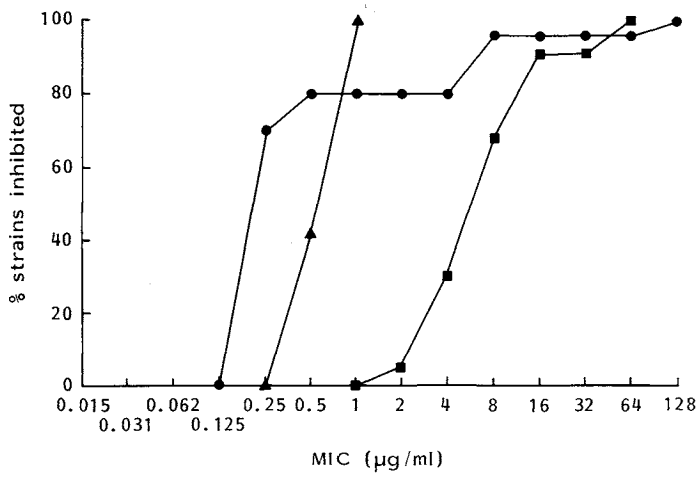
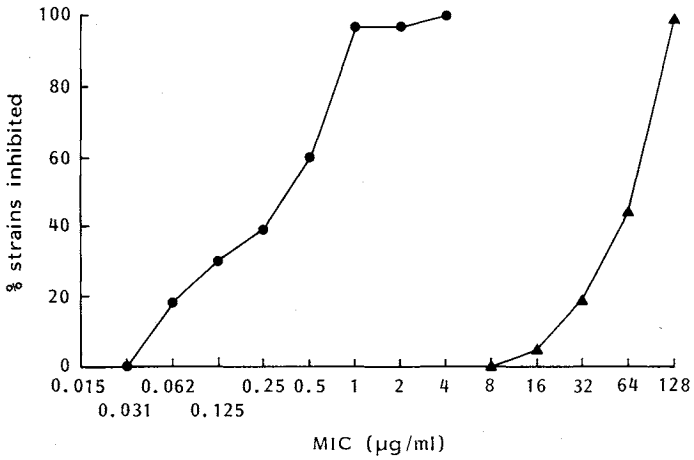


Fig. 5. Antibacterial activity of mersacidin and vancomycin against Enterococci (Strain number = 20).

● Vancomycin, ▲ mersacidin.



those of mersacidin. Against Enterococci, mersacidin, with MIC values between 16~128 μg/ml is much less active than vancomycin which inhibits more than 90% of the Staphylococci at concentrations of 1 μg/ml or less (Fig. 5). The *in vitro* activity of potassium mersacidin which has been tested against a limited number of strains is shown in Table 1. Its activity is comparable to that of mersacidin.

The *in vivo* activity of mersacidin, against both the methicillin-sensitive *S. aureus* SG 511 and the methicillin-resistant strains, *S. aureus* E 710 and *S. aureus* C 31155 is superior to that of vancomycin (Table 2). The ED_{50} and ED_{90} values are both lower. Against *S. pyogenes* A 77 mersacidin is less active than vancomyicin (Table 3). This deficiency has been overcome by the water soluble derivative, potassium mersacidin, with ED_{50} and ED_{90} values as good as those obtained with vancomycin (Table 4). While the

Table 1. *In vitro* antibacterial activity of potassium mersacidin and mersacidin.

Organism	MIC values (μg/ml)		Organism	MIC values (μg/ml)	
	Potassium mersacidin	Mersacidin		Potassium mersacidin	Mersacidin
Staphylococcus aureus 209 P	2.00	1.56	*S. epidermidis* 823	6.00	6.25
S. aureus R 85	2.00	0.78	*Streptococcus faecalis* 21777	25.00	50.00
S. aureus 710	1.00	0.78	*Bacillus subtilis* ATCC 6633	1.00	0.78
S. aureus 3066	10.00	25.00			
S. aureus E 88	10.00	12.50	*Micrococcus luteus* ATCC 9341	0.20	0.195
S. aureus SG 511	1.00	0.78			

Table 2. *In vivo* activity of mersacidin against *Staphylococcus aureus* infection in mice compared with vancomycin.

Compound	Pathogen	Dose mg/kg × 3 sc	No. of animals survived/total (% cured)	Approximate ED_{50}, ED_{90}* mg/kg × 3
Mersacidin	*S. aureus* SG 511	9.37	53/53 (100)	2.59, 5.38
		6.25	38/40 (95)	
		3.12	18/27 (67)	
		1.60	3/24 (12)	
Vancomycin	*S. aureus* SG 511	18.75	71/71 (100)	7.20, 9.37
		12.50	59/61 (97)	
		9.37	48/53 (91)	
		6.25	13/44 (29)	
Mersacidin	*S. aureus* E 710 (MRSA)	25.00	12/12 (100)	10.81, 19.59
		12.50	10/16 (63)	
		9.37	4/10 (40)	
Vancomycin	*S. aureus* E 710	37.00	6/ 6 (100)	18.98, 32.01
		25.00	14/21 (67)	
		12.50	3/11 (27)	
Mersacidin	*S. aureus* C 31153 (MRSA)	18.75	10/10 (100)	9.32, 12.65
		12.50	8/10 (80)	
		9.37	6/10 (60)	
Vancomycin	*S. aureus* C 31153	25.00	9/10 (90)	15.82, 20.98
		12.50	7/10 (70)	
		6.25	0/ 6 (0)	

* ED (Effective dose) 50 and 90 denote minimum dose required to cure 50% and 90% of the infected animals, respectively.

Table 3. *In vivo* activity of mersacidin against *Streptococcus pyogenes* infection in mice compared with vancomycin.

Compound	Pathogen	Dose mg/kg × 3 sc	No. of animals survived/total (% cured)	Approximate ED_{50}, ED_{90} mg/kg × 3
Mersacidin	*S. pyogenes* A 77	6.25	5/ 6 (83)	2.66, 6.86
		3.12	8/11 (73)	
		1.56	2/11 (18)	
Vancomycin	*S. pyogenes* A 77	0.80	9/10 (90)	0.5, 0.6
		0.40	6/ 8 (75)	
		0.20	0/ 4 (0)	

Table 4. *In vivo* activity of potassium mersacidin.

Pathogen	Dose mg/kg × 3 sc	No. of animals survived/total	Approximate ED_{50}, ED_{90} mg/kg × 3
Staphylococcus aureus SG 511	9.37	6/ 6	3.51 (7.20), 5.73 (9.37)
	6.25	9/12	
	3.12	3/ 6	
S. aureus E 710 (MRSA)	25.00	12/12	7.93 (18.98), 14.42 (32.01)
	12.50	4/ 6	
	6.25	8/18	
S. aureus C 31153 (MRSA)	25.00	11/11	6.93 (15.82), 12.83 (20.98)
	12.50	11/17	
	6.25	7/12	
Streptococcus pyogenes A 77	1.56	6/ 6	0.50 (0.5), 0.80 (0.6)
	0.80	9/10	

Comparative figures for vancomycin are given in the parenthesis.

activity of this derivative against *S. aureus* (both methicillin-sensitive and -resistant strains) remains comparable to that of mersacidin.

Discussion

Mersacidin is a new member of the group called lantibiotics, and has some interesting properties not hitherto reported for any other member of this group. It is a narrow spectrum antibiotic active only against Gram-positive bacteria. Its *in vitro* activity is poor as compared to clinically useful antibiotics like vancomycin and erythromycin. However, in the *in vivo* system the picture is totally reversed where mersacidin shows an overall better profile than vancomycin. Only against *Streptococcus* is mersacidin less active than vancomycin in the animal system. This disadvantage has been overcome by making the water soluble derivative, potassium mersacidin. And it is interesting to note that this improvement has taken place not at the cost of its activity against *Staphylococcus*, where the activity against both methicillin-sensitive and methicillin-resistant strains remains almost the same as with mersacidin. The phenomenon of an antibiotic of having significantly higher *in vivo* activity than would be predicted from *in vitro* activity is rare. Recently, a similar phenomenon was reported with spiramycin[7] which has 2 to 4 times poorer *in vitro* activity than erythromycin, whereas the ED_{50} in the septicemia model is 5 to 15 times superior. It has been explained that this paradox can be, at least partly, due to the ability of spiramycin to achieve intracellular and tissue concentrations that exceed serum concentrations by a factor of 10 or more. In case of mersacidin the mechanism by which it shows higher activity in the *in vivo* system is not yet known.

Acknowledgments

We thank Mrs. AMANDA NOGUEIRA for the preparation of this manuscript.

References

1) CHATTERJEE, S.; S. CHATTERJEE, S. J. LAD, M. S. PHANSALKAR, R. H. RUPP, B. N. GANGULI, H.-W. FEHLHABER & H. KOGLER: Mersacidin, a new antibiotic from *Bacillus*. Fermentation, isolation, purification and chemical characterization. J. Antibiotics 45: 832～838, 1992
2) LIMBERT, M.; D. ISERT, N. KLESEL, A. MARKUS, G. SEIBERT, S. CHATTERJEE, D. K. CHATTERJEE, R. H. JANI & B. N. GANGULI: Chemotherapeutic properties of mersacidin *in vitro* and *in vivo*. First International Workshop on Lantibiotics, Abstract No. 36. Bad Honnef, Apr. 15～18, 1991
3) HURST, A.: Nisin. Adv. Appl. Microbial. 27: 85～123, 1981
4) KIDO, Y.; T. HAMAKADO, T. YOSHIDA, M. ANNO, Y. MOTOKI, T. WAKAMIYA & T. SHIBA: Isolation and

characterization of ancovenin, a new inhibitor of angiotensin I converting enzyme, produced by actinomycetes. J. Antibiotics 36: 1295~1299, 1983

5) KESSLER, H.; S. STEUERNAGEL, M. WILL, G. JUNG, R. KELLNER, D. GILLESEN & T. KAMIYAMA: The structure of the polycyclic nonadecapeptide Ro 09-0198. Helv. Chim. Acta 71: 1924~1929, 1988
6) WAKAMIYA, T.; K. FUKASE, N. NARUSE, M. KONISHI & T. SHIBA: Lanthiopeptin, a new peptide effective against *Herpes simplex* virus: Structural determination and comparison with Ro 09-0198, an immunopotentiating peptide. Tetrahedron Lett. 29: 4771~4772, 1988
7) SMITH, C. R.: The spiramycin paradox. J. Antimicrob. Chemother. 22 (Suppl. B): 141~144, 1988