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Retraction

New antimicrobial peptide active against Gram-positive pathogens

A. Jasir, F. Kasprzykowski*, V. Lindström, C. Schalén** & A. Grubb

*Department of Clinical Chemistry, University Hospital, S-22185 Lund, Sweden, *Department of Chemistry University of Gdansk, Gdansk, Poland & **Department of Medical Microbiology Dermatology & Infection University Hospital, S-22185 Lund, Sweden*

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***Background & objectives*: Human and animal cystatins have been shown to inhibit the replication of certain viruses and bacteria, though it is not directly demonstrated that the effects are due to protease inhibitory capacity of the cystatins. We report antibacterial properties of a novel antimicrobial peptidyl derivative, (*2S*)-2-(N^{a}-benzyloxycarbonyl-arginyl–leucylamido)–1–[(*E*)–cinnamoylamido]-3-methylbutane, structurally based upon the aminoterminal segment of the inhibitory centre of the human cysteine protease inhibitor, cystatin C.**

***Methods*: Clinical isolates of group A, B, C and G streptococci were collected. The antibacterial activity of Cystapep 1 derivative was tested by agar well diffusion method.**

***Results*: Cystapep 1, displayed antibacterial activity against several clinically important Gram-positive bacteria. It displayed minimal inhibitory and bactericidal concentrations of about 16 µg/ml for both *Staphylococcus aureus* and *Streptococcus pyogenes*. In radial agar diffusion assays, groups A, B, C and G streptococci as well as staphylococci were generally susceptible to the action of Cystapep 1, whereas pneumococci and enterococci were less susceptible. No activity against Gram-negative bacteria was observed.**

***Interpretation & conclusion*: Cystapep 1 also showed high activity against methicillin-resistant *Staph. aureus* (MRSA) and multi-antibiotic resistant coagulase negative staphylococci (CNS), suggesting its mechanism of action to be different from most currently used antibiotics.**

Key words Antimicrobial peptide - cystatin C - *Staphylococcus aureus* - *Streptococcus pyogenes*

A new group of potential antibacterial agents was described in 1989 when an oligopeptidyl derivative, N-benzyloxycarbonyl-leucyl-valyl-glycyl-diazomethane (Z-Leu-Val-Gly-DAM), structurally based upon the inhibitory centre of human cystatin C, was found to suppress the growth of *Streptococcus pyogenes*[1]. The recently described cystatin superfamily of proteins comprises both eucaryotic and procaryotic cysteine protease inhibitors[2]. Indeed, human cystatins C, D and S, rat cystatins A and S, chicken cystatin and oryzacystatin have been described to inhibit the replication of certain viruses and bacteria[3] although it has not yet been directly demonstrated that these effects are due to the protease inhibitory capacity of the cystatins[4,5].

The inhibitory centre of cystatin C comprises three peptide segments, Arg^{8}-Leu^{9}-Val^{10}-Gly^{11}, Gln^{55}-Ile^{56}-Val^{57}- Ala^{58}-Gly^{59} and Pro^{105}-Trp^{106} [6,7]. This peptidyl derivative is based upon the aminoterminal segment of the inhibitory centre. While retaining capacity to inhibit cysteine proteases, it shows a narrow antibacterial spectrum by selectively inhibiting the growth of the cysteine protease producing bacterial species *Streptococcus pyogenes*[8]. Subsequent analysis of a number of related compounds, however, showed that their antimicrobial effect was probably not ascribable to any protease inhibition[8]. In addition, the antimicrobial spectrum of both linear and cyclic compounds within this class of compounds was found to differ from that of

Z-Leu-Val-Gly-DAM by comprising several Gram-positive pathogens. For one of the most promising linear derivatives, here named Cystapep 1, comparatively low MIC and MBC values for both *S. pyogenes* and *S. aureus* were recorded[8], and a strong mouse protective capacity of some of the compounds against lethal streptococcal infections was noted[8]. In the present work we studied the antimicrobial effect of Cystapep 1 against a larger collection of clinically important pathogens, including strains resistant to currently used antibiotics.

Material & Methods

Clinical isolates of group A, B, C and G streptococci, *S. aureus*, CNS, *Enterococcus faecalis*, *E. faecium*, *Streptococcus oralis*, *S. pneumoniae*, *Listeria monocytogenes*, *M. catarrhalis* and *Escherichia coli* were obtained from our Clinical Microbiology Department. The antibacterial activity of Cystapep1, synthesized as described[8], was tested by agar well diffusion on PDM II agar. Holes of 5 mm diameter were punched and 50 µl of a solution of Cystapep 1 (1.0 g/L) in DMSO was applied in each hole. The antibacterial effect was classified arbitrarily based upon inhibition zone diameters.

Results

A large number of Gram-negative isolates were found to be resistant to the action of Cystapep 1. In contrast, most examined Gram-positive species showed good susceptibility (Table). Thus, all strains of *S. aureus* and CNS, including those being methicillin- or multi-resistant, were susceptible to Cystapep 1. Also b-haemolytic streptococci, including macrolide and tetracycline resistant strains, turned out susceptible; however, the inhibitory effect on group A was stronger than on group B, C and G strains. Pneumococci were slightly less inhibited by Cystapep 1. Out of a-haemolytic streptococci both susceptible and resistant strains were found. *E. faecalis* strains were non-susceptible. In contrast, *L. monocytogenes* strains were clearly susceptible to Cystapep 1.

Table. Susceptibility of various Gram-positive species to Cystapep 1

Species (no. strains)	Inhibition zone diameter (mm)			
	<7	8-11	12-15	>16
MSSA (57)	0	0	29	28
MRSA (97)	0	0	1	96
CNS (56)	0	0	16	40
GAS (63)	0	0	0	63
GBS (33)	0	0	11	22
GCS (25)	0	0	21	4
GGS (25)	0	0	22	3
S. pneumoniae (34)	0	33	6	0
a- streptococci (64)	15	4	33	12
L. monocytogenes (11)	0	0	11	0
Total	15	32	150	268

By broth dilution, two strains each of GAS and *S.aureus* showed MBC and MIC of 16 mg/l, whereas single strains of GBS, GCS, and GGS had MBC/MIC of 32 mg/l for Cystapep 1.

Discussion

Cystapep 1 was as effective against various antibiotic resistant staphylococci and streptococci as against antibiotic susceptible strains of these species. Presently, resistant staphylococci represent leading agents in nosocomial and biomaterial-associated infections posing significant therapeutic problems due to shortage of effective antibacterial agents. In addition, the susceptibility of b-haemolytic streptococci to Cystapep 1 may prove useful due to treatment problems both for invasive and superficially located infections.

The activity of Cystapep 1 appears limited to Gram-positive bacteria, a selectivity in its anti-bacterial spectrum which may be advantageous from an ecological point-of-view. The compound bears little resemblance to those of previously reported naturally occurring antimicrobial peptides, *e.g.*, defensins, since Cystapep 1 is much smaller than these, contains extensively modified amino acid residues, and mimics the active centre of a human major protease inhibitor, though not retaining any protease inhibitory properties[8]. Its mode of action therefore may differ from that of known membrane pore forming peptides but is still elusive. Chemical modification of Cystapep 1 in order to allow preparation of stable water solutions of it will probably be needed for its development into a clinically useful anti-bacterial drug.

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Reprint requests: Dr A. Jasir, Department of Clinical Chemistry, University Hospital, S-22185 Lund, Sweden
e-mail: aftab.jasir@mmb.lu.se