TITLE: Antibacterial activity optimization of peptides originating from Bothropstoxin-I

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ABSTRACT:

Despite advances in the treatment of infectious diseases, pathogenic microorganisms remain a threat to public health due to their ability to adapt and resist antibiotics. The scarcity of therapeutic options increases the interest in new antimicrobial compounds. Antimicrobial peptides, such as Bothropstoxin-I, are being investigated mainly with this purpose: the C-terminal region (residues 115-129) of this protein and some analogs have activity against pathogenic bacteria. This study aims to optimize this peptide action by proposing molecule modifications and evaluating antimicrobial action, hemolytic activity, and biofilm reduction. We evaluated antimicrobial action by determining the minimum inhibitory concentration by microdilution method for eight bacteria: *S. epidermidis* ATCC 35984, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *E. faecium* ATCC 700221, *K. pneumoniae* ATCC 700603, *E. coli* ATCC 25922, *A. baumannii* ATCC 19606 and *P. aeruginosa* ATCC 27853. Hemolytic activity was evaluated with healthy human red blood cells and analogs microdilution. Biofilm reduction was assessed using *S. epidermidis* ATCC 35984, a well-known biofilm-forming strain. The analogs were incubated with the pre-formed biofilm at 512 mg/L, subjected to crystal violet staining and read at OD600 nm. The reduction was determined by comparing treated and non treated biofilm. The analogs (NA1307)K (sequence $(KKYRYHLKPF)_2K$) and E(NA1307) (sequence $E(KKYRYHLKPFCKK)_2$) allowed us to investigate whether dimerization in C-terminal of the peptide using lysine or in N-terminal using glutamate would have a role in antimicrobial activity. (NA1307)K was the most active against the tested strains, with no hemolysis. We synthesized a third analog (TL1815-KK, sequence $(YRYHLKPF)_2K$) by removing two initial lysines, which greatly reduced the antimicrobial activity, emphasizing the importance of positively charged residues in the N-terminal region. The last two analogs (NA1896, sequence $(KKWRWHLKPF)_2K$ and NA1897, sequence $(KKWRWHLKPW)_2K$) were modifications of (NA1307)K, to increase the hydrophobic moment of the molecule. NA1897 were the most active peptide, but the hemolysis rate also increased with hydrophobicity. No intense antibiofilm action was observed, but (NA1307)K obtained the greatest reduction ((30 ± 9)%). NA1897 was the most promising peptide tested and has great potential for therapeutic applications. Our group will further characterize its antibacterial and cytotoxic activity.

Keywords: antimicrobial peptides, Bothropstoxin-I, multidrug resistant

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