AN *IN VITRO* STUDY OF THE EFFECTS OF A FEW NOVEL SYNTHETIC CATIONIC ANTIMICROBIAL PEPTIDES ON HUMAN PLASMA COAGULATION

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The increasing spread of bacteria that have become resistant to current antibiotics has prompted the search for new-generation antibacterial agents. Cationic antimicrobial peptides (cAMPs) could be candidates for this purpose. A chemical synthesis of short peptides is considered a relatively cheap way to produce therapeutic forms of cAMPs. We have designed and synthesized a number of short cAMPs using a bioinformatic analysis of the medicinal leech genome: (P1) Phe-Arg-Ile-Met-Arg-Ile-Leu-Arg-Val-Leu-Lys-Leu; +4 charge; 10 μM MIC_{max}; (P2) Phe-Arg-Ile-Met-Arg-Ile-Leu-Arg-Val-Leu-Lys; +4 charge; 10 μM MIC_{max}; (P3) Arg-Trp-Arg-Leu-Val-Cys-Phe-Leu-Cys-Arg-Arg-Lys-Lys-Val; +6 charge; 17 μM MIC_{max}; (P4) Lys-Phe-Lys-Lys-Val-Ile-Trp-Lys-Ser-Phe-Leu; +4 charge; 90 μM MIC_{max}; (P5) Arg-Pro-Ile-Leu-Ile-Arg-Val-Arg-Arg-Ile-Arg-Val-Ile; +5 charge; 77 μM MIC_{max}; (P6) Arg-Leu-Lys-Arg-Phe-Lys-Arg-Val-Ala-Leu-Arg-Arg-Glu-Lys-Thr-Ala-Arg-Asn-Phe-Arg-Ser-Ile-Val-Ser; +9 charge; 11 μM MIC_{max}; (P7) Phe-Leu-Ile-Gly-Lys-Ala-Ile-Lys-Arg-Lys-Phe-Cys-Leu-Arg-Ser-Val-Trp-Asn-Ala; +3 charge; 7 μM MIC_{max}; (P8) Ser-Ala-Val-Ile-Tyr-Lys-Ile-Pro-Tyr-Asn-Ala-Ile-Ala-Ser-Arg-Trp-Ile-Ile-Ala-Pro-Lys-Lys-Cys; +4 charge; 12 μM MIC_{max}. The minimum inhibitory concentrations (MICs) for Gram-negative (*Escherichia coli*) and Gram-positive (*Bacillus subtilis*) bacteria were determined, and the higher one among these two MICs for each peptide is indicated above as MIC_{max}. The traditional biocompatibility assessment consists of determining cytotoxicity. However, as our earlier results have shown, the peptide-induced hemolysis can be inhibited by the addition of blood plasma to the hemolysis assay medium. This suggests peptide binding in plasma, with an affinity sufficient to prevent the peptide translocation to cells. Hence, the biocompatibility evaluation of cAMPs should include a study of their interaction with plasma components. The goal of the present research was to reveal whether cAMPs can affect plasma coagulation. The coagulation behavior of the peptides was investigated by measuring activated partial thromboplastin time (APTT) and prothrombin time (PT). APTT assesses the intrinsic and common pathways of coagulation cascade. PT assesses the extrinsic and common pathways. Plasma was pretreated with different peptide concentrations (25, 50, and 100 μM). Adding 25 μM peptide did not lead to significant changes in APTT and PT compared to control plasma. At higher concentrations, a profound effect was observed for P3 and P6. APTT was prolonged with P3 by 75±25% at 50 μM and 415±20% at 100 μM, and with P6 by about 120±20% and 350±50% at 50 μM and 100 μM, respectively. P8 exerted a milder APTT prolongation (by 50±15% at 100 μM). Like it was with APTT, PT was considerably prolonged with P3. The PT increase was 310±30% and 730±50% at 50 μM and 100 μM P3, respectively. It was shown in an additional experiment that P3 can bind to fibrinogen, a coagulation factor involved in the common pathway of coagulation. The combined effect of P3 on APTT and PT may be ascribed to this binding. By contrast to P3, P6 had no effect on PT, which suggests that its influence on coagulation occurs at the intrinsic pathway level, probably through binding to an intrinsic coagulation. Other peptides had no effect or caused non-significant prolongation of plasma coagulation time even at the highest concentration used. The results obtained revealed the possibility of cAMPs' side effects on plasma coagulation.

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