Summary of doctoral dissertation

Synthesis of theta-defensins analogues and evaluation of their antimicrobial and cytotoxic activity toward selected bacterial strains and cell lines

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θ-defensins are peptides presents in the neutrophils of Old World monkeys like macaques and baboons. They belongs to host defence peptides family as a subfamily of defensins. Cationic host defence peptides have been known for displaying broad cytotoxicity against cancer cells whereas being harmless toward normal mammalian cells. θ-defensins are the only known example of cyclic polipeptides present in animal proteome. They are characterized by the presence of six cysteines residues forming three disulphide bridges (Cys1-Cys6, Cys2-Cys5 and Cys3-Cys4). Research has shown that they have very strong biological activity including antibacterial, antifungal and antiviral properties however their activity toward cancer cells has not been studied yet. Cyclic backbone of θ-defensins is additionally stabilized by the presence of three disulphide bridges. Stability together with strong biological activity make θ-defensins an interesting leading structure for creation of new pharmaceutics. However synthesis of peptides with multiple disulphide bridges is difficult and not cost-efficient therefore development of θ-defensin analogues with similar biological activity but simplified structure is of great importance. The current study presents synthesis of RTD-1, RTD-2, RTD-3 and their simplified analogues together with evaluation of antibacterial and anticancer properties of synthesized compounds in the view of their future medicinal application.

The main aims of the research presented in this doctoral dissertation included:

1. manual synthesis of θ-defensins and their analogues using Fmoc/tBu strategy;
2. analysis of antimicrobial properties of synthesized compounds toward Gramm-positive and Gramm-negative bacteria strains;
3. evaluation of cytotoxic potential of synthesized compounds toward non-transformed cells and breast cancer cell lines.

Synthetic steps consisted of assembly of peptide chain on solid support, cleavage from the resin, formation of disulphide bridges and head-to-tail cyclization. Presence of cyclic, fully oxidized peptides was confirmed by RP-HPLC and MALDI-TOF MS analysis. To evaluate antimicrobial activity of synthesized compounds against Gramm-positive and Gramm-negative bacteria, broth dilution assays on *S. aureus* and *E. coli* strains were performed. Haemolytic effect of synthesized compounds was determined on erythrocytes from human blood. Cytotoxicity toward peripheral blood mononuclear cells, mammary epithelial cells (HB2) and breast cancer cell lines (SKBR3, MDA-MB-231) was assessed with modified MTT test. Additionally the effect of synthesized compounds on breast cancer cells was also evaluated in 3D cultures which more closely resemble tumour microenvironment.

Conducted research showed that replacement of cysteine residues with alanine residues results in lack of biological activity. Contrastingly analogues with serine residues do not induce haemolytic effect in erythrocytes from human blood and are not cytotoxic toward peripheral blood mononuclear cells. However they are potent against breast cancer cell lines. This discovery may lead to development of new θ-defensin based pharmaceutics with future potential application in cancer therapy.