Summary of doctoral dissertation

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"Design, chemical synthesis and analysis biological activity of RTD-2 analogues against breast cancer cell lines"

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The subject of the doctoral dissertation is related to the synthesis and biological evaluation of antimicrobial peptides with potential anticancer activity. Research proves that some peptides modulate the immune system, fight infection and display broad cytotoxicity against cancer cells. Antimicrobial peptides are part of the innate immune response, stimulate wound healing, regulate inflammation and initiate acquired immunity. My research focuses on defensins that are active against gram-positive and gram-negative bacteria, viruses, fungi and parasites. Their broad spectrum of action makes them extremely interesting molecules in the development of a new type of peptide drug. Unfortunately, a significant problem is the low efficiency of chemical synthesis and obtaining of the correct disulfide bridge pattern.

The aim of the study was to assess the ability of simplified, biologically active analogues of θ-defensin to selectively target various types of breast cancer cells. The synthesis of peptides containing many cysteine residues is quite difficult and unprofitable. Therefore, the synthesized peptides contained only one disulfide bond and are an attractive alternative to compounds with potential pharmaceutical application.

The experimental part of my work included:

1. Designing and synthesis of novel RTD-2 analogues which will serve as tools to elucidate its anticancer properties,

2. Biological evaluation of cytotoxic activity toward breast cancer cells.

The synthesis steps were performed manually using Fmoc/tBu chemistry. After removing the peptides from the resin, I performed an oxidation reaction with iodine to form a disulfide bridge between the cysteine residues, followed by an intramolecular head-to-tail cyclization. The purity of the synthesized peptides and the correctness of the synthesis were checked by high-performance liquid chromatography. The molecular weights of the synthesized RTD-2 analogues were identified based on mass ions obtained on mass spectrometry with MALDI-TOF technique. Cytotoxic potential was measured with MTT assay. Moreover, θ-defensins analogues were subjected to three-dimentional cell culture systems which commonly used in the study of cancer cell biology. The next stage was to define of cellular localization of RTD-2 analogues. Time-dependent cellular localization of peptides was evaluated by fluorescence microscopy. For this purpose, breast cancer cells were incubated with fluorescent labelled RTD-2 ([$Ser^{3,7,12,16}$]-Ala(2-BAD)RTD-2; [$Ser^{3,7,12,16}$]-Lys(HOC)RTD-2). Additionally, I performed the synthesis of biotin-labeled RTD-2 analog ([$Ser^{3,7,12,16}$]-Lys(Bt)RTD-2). This was followed by immunoprecipitation of RTD-2 and possible interacting proteins which was a main point in research to identify possible molecular partner(s) interacting with RTD-2 analog.

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