University of Medicine, Pharmacy, Sciences and Technology of Târgu Mureș
Doctoral School
PhD Thesis Summary

STUDIES REGARDING THE BIOLOGICAL ACTION OF SNAKE VENOMS

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Snake venoms are complex mixtures of proteins, peptides, small organic and inorganic molecules, all of them exerting a synergistic effect. Due to their content in enzymes and toxins with various biochemical and pharmacological effects, snake venoms have been used as sources for lead molecules in drug development for decades. The introduction into therapy of venom-derived molecules, such as captopril, eptifibatide or tirofiban proves the relevance of this area of research. The elucidation of individual biological actions of snake venoms is at the basis of an in-depth understanding of how they exert their complex effect on the human organism. Furthermore, obtaining and testing individual components from snake venoms allows the identification of lead molecules for drug development.

The present PhD thesis had two main objectives. The first objective was to determine the specific biological activities of snake venoms, namely the pro-inflammatory and the antibacterial activity. The second objective was the development and application of two methods for obtaining proteinaceous fractions or pure components from snake venoms.

Based on the hypothesis that snake venom proteins influence the gene expression of inflammatory mediators, the first study proposed qualitative and quantitative determination of the effect of *Vipera ammodytes ammodytes* on the human cytokine network. The study consisted in the determination of gene expression using a RT-qPCR method with a TaqMan® Array Human Cytokine Network Plate. The results showed a significant increase in the expression of several genes associated with inflammatory mediators, such as interleukins (IL1A, IL1B, IL6, IL16, IL10), chemokines (IL8), interferons (IFNA2, IFNB1) and tumor necrosis factors (TNF, LTA). A thorough understanding of the mechanism by which snake venoms exert their pro-inflammatory action can help in the development of specific antivenoms that could limit the severe local effects of snake venoms.

The second study focused on the determination of the antibacterial activity of selected snake venoms on different pathogenic bacterial strains. The specific objectives of the study were: the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of 11 different snake venoms, the determination of the protein composition of snake venoms by gel electrophoresis, and the correlation of MIC and MBC values with the determined protein composition. The results showed a significant difference in the effect of snake venoms from the Elapidae and Viperidae families, probably due to their different content in proteolytic enzymes. Based on the proteinaceous profile, the components responsible for the antibacterial activity of snake venoms include phospholipases A2, metalloproteinases and L-amino

acid oxidases. Furthermore, the results indicate that the venom of *Daboia siamensis* and the venoms of the two *Agkistrodon* species (*A. piscivorus leucostoma, A. piscivorus piscivorus*) have the most pronounced effect on susceptible bacterial strains. Fractionation and isolation of components from these venoms, along with the screening of the antibacterial activity of the individual components, could result in the identification of lead molecules for the development of new antibacterial agents.

The third experiment aimed to separate and purify different fractions from the venom of *Crotalus atrox*, using ion exchange and size-exclusion chromatography. Further objectives included the determination of fraction homogeneity by SDS-PAGE analysis and the identification of individual proteins based on available published data. By using successively ion exchange chromatography (both anion and cation exchange) and size-exclusion chromatography, several homogenous and heterogeneous fractions were successfully separated from the crude venom. Homogeneous fractions include: atrolysin B, obtained following the separation of the crude venom on an anion-exchange and cation-exchange column, and α-fibrinogenase, obtained following two consecutive separations on an anion-exchange column. Proteins from different families, such as P-I class metalloproteinases (atrolysin C, atrolysin D, atrolysin E), P-III class metalloproteinases (VAP-1, VAP-2), L-amino acid oxidases (apoxin-1) and serin-proteases (catroxobin, catroxase) were contained in heterologous fractions.

The objective of the fourth study was the development and optimisation of a heterologous expression method for the recombinant protein crotoxin B, isoform C (a phospholipase A2 present in the venom of *Crotalus durissus terrificus*). Furthermore, for the purification of the recombinant protein a separation method using affinity chromatography was developed. The coding sequence of crotoxin B, isoform C has been inserted in the pET-30b(+) plasmid to obtain the recombinant DNA. These vectors were transferred into *E. coli Rosetta™ (DE3)pLysS* host cells, used for protein expression. For heterologous expression method optimisation included the determination of the optimal incubation temperature, IPTG concentration and duration of effective expression. For affinity chromatography, optimisation of the separation method consisted in the use of different buffer systems and pH conditions. The obtained and purified recombinant protein can be used in biochemical, microbiological and pharmacological studies to determine its effects.

The original aspects of this thesis are:

- First evaluation of the effect of *Vipera ammodytes ammodytes* venom on the human cytokine network, using a RT-qPCR method and TaqMan® Array Plates.
- First use of a standardized microdilution method for the determination of the antibacterial activity of snake venoms on different bacterial strains.
- First use of pET-30b(+) plasmid and *Rosetta™ (DE3)pLysS* host cells for the expression of crotoxin B, isoform C (a phospholipase A2 found in a snake venom).