INTRODUCTION

Venomous snakes and their toxic cocktails are distributed in a wide range all over the world and cause many human fatalities (>80,000) each year. The investigation of snake venoms is of much interest for the discovery of different kinds of new pharmacological drugs. The Transcaucasian Nose-horned Viper (Vipera ammodytes transcaucasiana), classified as an endemic subspecies distributed mainly in Anatolia within only population in Georgia, was first observed in the province Artvin, in the northeastern of Turkey. Afterwards several more localities were published along the Black Sea coast and from more inland of Anatolia. The recently new discovered habitat at the Sivas province extended the geographical habitat to more southern parts of Anatolia and is a quite unusual compared to previous observations. In this work, we show the first characterization of the venom proteome and the bioactivity screening of Vipera ammodytes transcaucasiana, the Transcaucasian Nose-horned Viper. The composition of the venom (V. a. transcaucasiana) was analyzed by bottom-up mass spectrometry, as well as intact protein mass profiling and peptides or proteins were identified via de-novo sequencing. Additionally cytotoxicity of the venom was tested on a panel of cancer cell lines together with normal cell lines by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT).

RESULTS AND DISCUSSION

Figure 1. Venom composition of Vipera ammodytes transcaucasiana. Venom is composed of various peptides and proteins: not annotated (27.47%), metalloproteases (0.29%), VEgap (11.96%), PLA2s (20.32%) and PL2 inhibitor (0.74%), SVEPs (2.87%), CRISP's (3.79%), SVTLE's (6.49%). C-type lectine (6.68%), vaspin (11.32%), LAAO (6.38%) and other (9.74%).

Figure 2. crude venom analysis. A) Representative semi-preparative HPLC separation of crude venom from Vipera ammodytes transcaucasiana. Crude venom (100 μg) was separated by a linear gradient of 5–40% B for 55 min, 40–70% for 20 min and finally 70% B for 10 min (A=water, B=acetonitrile). Peak detection was performed at λ=214 nm using a diode array detector (DAD). B) Coomassie stained SDS-gels from chromatographic fractions containing proteins. Bands were excised from the gel and subjected to in-gel reduction with 10 mM dithiobiotin (DTT), alkylation with 50 mM iodoacetamide and digestion with 66 ng sequencing-grade trypsin. The digested fractions were investigated by a LTQ Orbitrap XL mass spectrometer (Thermo, Bremen, Germany) and identified via de-novo sequencing. Venom composition from Vipera a. transcaucasiana is shown in Figure 1. C) Intact protein mass profiling of crude venom from Vipera ammodytes transcaucasiana. LC-ESI-HR-MS/MS experiments were performed on a LTQ Orbitrap XL mass spectrometer (Thermo, Bremen, Germany). D) Cytotoxicity assay by MTT with crude venom extracts from Vipera a. transcaucasiana. Viability of cancer and non-cancerous cell lines after crude venom treatment.

CONCLUSION

Venom analysis and bioactivity screening of the Transcaucasian Nose-horned Viper showed active venom fractions against human breast carcinoma epithelial cells (MDA-MB-231). Mass spectrometric analysis of bioactive fractions identified fraction 17 as an ammodytin (2A) variant, fraction 18 as a vaspin basic subunit variant and fraction 19 as a cysteine-rich venom protein. Fraction 1 is a small peptide with still unknown sequence.

REFERENCES


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