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VIPERA BERUS BERUS(COMMON VIPER) VENOM: BIOCHEMISTRY ANTOXICOLOGY

Abhik Roy[*1]

[*1]Ramakrishna Mission Vivekananda Centenary College, Kolkata, India.

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ABSTRACT

The isolating and characterizing process of each snake venom materials are necessary for understanding the pathophysiology of the envenomation and to improve the therapeutic procedures for the patients. It may also lead to the discovery of the novel toxins. The different compositions of venom, toxicological and immunological characteristics of the common vipers (Vipera berus berus) have been discussed. The common vipers venom contains proteins and polypeptides that belong to 10-15 toxin families like L-amino acid oxidase (LAAO), hyaluronidase, 5′ -nucleotidase, glutaminyl-peptide cyclotransferase etc. and many more. The differences in V.berus venom composition depend on age, habitat, sex and the food diet of the snakes. These variability in the venom lead to the decrease in the efficiency of the anti venom against snakebites. This review represents an overview and several proteins or toxins that have been isolated and characterized from the V.b. berus venoms and also their toxic actions and biochemical properties.

I. INTRODUCTION

Family Viperidae contains 374 species of vipers and this family is divided into three sub-families: Viperinae (True Vipers, 101 species), Crotalinae (Pit Vipers, 271 species), Azemiopinae (2 species).The Vipera berus is a small, stout-bodied snake that is distributed over the large area of Europe and Asia. Vipera berus is divided into five sub-species: Vipera berus berus (named by LINNAEUS 1758), Vipera berus bosniensis (named by BOETTGER 1889), Vipera berus barani (named by B¨ohme and Joger, 1983), Vipera berus sachalinensis (named by TZAREVSKY 1917), Vipera berus marasso (named by POLLINI 1818).

Vipera berus is the most widely distributed terrestrial snake on the planet that occupies Eastern Europe, Western Europe, Central Europe, Central Asia, and East Asia.

The phylogenetic approach of the analysis of the variation in the mitochondrial DNA sequence in 918 bp of the non-coding control region and in the 1043 bp of the cytochrome b gene lead to the divison of V.berus into three major clades: a Balkan, an Italian and a Northern clade.

1. Toxicological Characterization

V. berus is the most largely distributed viper in whole Europe and leads to more snakebites than the other species under the genus Vipera. Approximately 70% among all the registered V. berus bites forms almost no to very little effects in humans and deaths are very rare in these cases. After the bite and entering of the venom inside the body, some significant local damaging effects in tissues like hemorrhage, oedema and myonecrosis have been seen.Some gastrointestinal problems are also observed like recurrent vomiting ,hypotension etc. The envenoming is only seriously harmful for children, causing vomiting, nausea, diarrhea etc. in early stages and in later stages kidney failure and compartment syndrome can be seen. V. berus does not show any neurotoxic activity in animal and human beings. Sometimes The venom of the viper is seem to be contaminated with radioactive isotopes, which snakes are collected from the parts of the Earth, which are contaminated by radioactive isotopes.

2. Antivenoms

According to the WHO (World Health Organization), the best cure for envenomation is the application of an antivenom serum. This antivenom is made up of polyclonal antibodies, which are isolated from hyperimmune animal serum or plasma. Viper antivenom may be monovalent or polyvalent, depending upon the polyclonal antibodies inside them. The viper antivenom is mainly derived from some host sources like horse, donkey and sheep. These hosts contain humoral immune response, which make them advantageous for using for antivenom production. The most effective antivenom produced so far, against the viper venom is the ViperaTAb , which is a monovalent Fab antivenom which is isolated from hyperimmunized against V. b. berus venom. ViperaTAb is

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licensed by the European Medicines Agency, and this antivenom shows a huge response for treating the after snakebite effects.

3. Proteomic Studies

The toxins, available in venom are divided into two separate protein families with a diverse mechanism of action and these make them a rich source for so many drugs that can target human proteins. Proteomic studies have shown that common viper venom is made up of Serine Proteases, metalloproteinases , PLA2, L-amino acid oxidases, phosphodiesterases, phosphomonoesterases, 5′ -nucleotidases, hyaluronidases , NGF, trypsin and chymotrypsin inhibitors, disintegrins, cysteine-rich secretory protein. The proteomic study and DNA sequencing helps in the quantitative comparison between different snake venom compositions.

V. berus venoms have been undertaken proteomic studies to make the envenomation therapy more efficiency, though there are discrimination in these studies are available. These discrepancies, according to scientists, may be due to the geographical variability of the source of venoms. Two different studies are popular; one showing 10 distinct protein families: serine proteases, metalloproteinases, natriuretic peptides, phospholipases A2, aspartic proteases, cysteine-rich secretory proteins, C-type lectins (snaclecs), L-amino-acid oxidases, disintegrins, and Kunitz-type protease inhibitors; and the other other one shows 15 protein/ peptide families: Phospholipases A2, serine proteinases, metalloproteinases, bradykinin-potentiating peptides, C-type natriuretic peptides, cysteine-rich secretory proteins, L-amino acid oxidase, C-type lectin-like (snaclecs), vascular endothelial growth factor, dimeric disintegrin, nerve growth factor, Kunitz-type protease inhibitors, 5′ -nucleotidase, phosphodiesterase and hyaluronidase. (Both these studies are made on V. berus, collected from Russian origin).

The Proteins isolated from the viper venom is divided into two broad groups:

4. Non-Enzymatic Proteins

There are a total of 12 non-enzymatic proteins (proteins, which can carry out such functions that require the capacity to bind but these proteins cannot catalyze a reaction like an enzyme). These proteins are disintegrins, nerve growth factor, cysteine-rich secretory proteins, snaclecs/C-type lectins, vascular endothelial growth factor, SVMP inhibitor, Kunitz-type inhibitors of trypsin and alpha-chymotrypsin, B-and C-type natriuretic peptides, bradykinin potentiating peptides, angiotensin-like peptides. Five of these proteins are discussed below.

i. Disintegrins:

Disintegrins are a family of integrin antagonists (such protein, that blocks any biological response), distributed in Viperid venoms (usually in Viperidae and Crotalidae). Disintegrins are potentially the inhibitors for platelet aggregation. Integrins can also bind to the surface of the surface of the malignant cells and the angiogenic endothelial cells, those are associated with cancer. Disintegrins are separated into four distinct groups, based on their length (38-100 residues) and the number of disulfide bonds (4-8). A Heteromeric disintegrin, VB7 is detected in V. berus venom and its appearance is proved by proteomic study. This VB7 was isolated by reverse phase HPLC (High performance liquid chromatography) and seems to be consisting of 64 amino acid residues, which are connected with a 63 amino acid residue subunit B via interchain disulfide bonds. VB7 displays a RGD motif (Arginyl-glycyl-aspartic acid motif) in subunit A and KGD motif in subunit B. It inhibited the adhesion of K562 cells, expressing the integrin α5β1, to immobilized fibronectin, a component of blood plasma and extracellular matrix. Fibronectin is an important component in healing of wound and formation of a blood clot to stop the bleeding and protect the tissue after envenomation, VB7 probably hinders these procedures.

ii. Nerve Growth Factor (NGF):

Nerve growth factor or NGF is a type of protein that helps in the differentiation and maintenance of embryonic and sympathetic sensory neurons. (Discovered by Cohen and Levi-Montalcini in 1956, got their Nobel Prize in 1986). NGF also plays a role in the growth of nerve tissue but what is the reason behind the presence of this protein in snake venom, is still unclear and yet a big question. An In vitro Bioassay was done with 8-day chick embryo gangliatic cells to detect the function of NGF within V. berus venom. The NGF causes pheochromocytoma PC12 cells to differentiate. Monoclonal antibodies which are linked to M. l. turanica. venom NGF was isolated and these antibodies were then linked with BrCN-activated agarose. This procedure was

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performed for the purification of NGF from ten different snake venoms including V. berus . Anti- M. l. turanica NGF antibodies were used for a syudy to cross-react with 21 more snake venoms and all these venoms (including V. berus) consisted of NGF and also the molecular masses of these venoms were determined.

iii. Cysteine-rich venom protein (CRISP-Vs)

Cysteine-rich proteins that are found in animal venoms (CRISP-Vs) belong to a large family of cysteine-rich proteins or CRISPs. CRISP-Vs contain a single polypeptide chains that have a molecular weight of 23-23 kDa and this chain contains 16 cysteine residues leading to the formation of 8 disulfide bridges. The cDNAs, which encode CRISP-Vs were cloned and sequenced. The cDNAs were isolated from the venom of V. berus and V. nikolski. The mature CRISP-Vs amino acid chains were made up of 220 amino acid residues. The only distinct feature of these two proteins is the presence of Lys92 in V. berus, instead of Glu92. The snake venom CRISPs inhibit the ion channels and the growth of the newly formed blood vessels or inhibits angiogenesis. They promote the inflammatory responses in leukocyte and neutrophil infiltration and also increase the vascular permeability.

iv. Kunitz- type serine protease inhibitors

The Kunitz- type serine protease inhibitors were isolated from the venoms of Vipiridae and Elapidae. These proteins consist of 60 amino acid long peptides and they are characterized by the presence of 6 conserved cysteine residues, which are engaged in three disulfide bonds. From the venom of V. berus, inhibitors of two classes are isolated, class I and II respectively, both of these classes have molecular masses of about 7000 Da and isoelectric points of greater than 10 and 9.9. The inhibitor I prefers alpha-chymotrypsin (Ki = 4.6 × 10 10 M) for the formation of an enzyme inhibitor complex at a molar ratio of 1:1. The inhibitor II prefers trypsin (Ki = 6.7×10^{-11} M), forms an EI-complex at a molar ratio of 1:2, but also inhibits alpha-chymotrypsin (Ki = 1.4×10^{-9} M) and hog pancreatic kallikrein (Ki = 1.6×10^{-8} M). The structural and functional properties, the pathophysiological significance and the possible therapeutic applications of these protease inhibitors of snake venom were presented by Thakur and Mukherjee (2017).

v. Other non-enzymatic proteins and peptides

V. b. berus contains some non-enzymatic proteins, those are not yet isolated and characterized but in the proteomic study of the viper venom, these proteins are detected to be functional. These proteins are: angiotensin-like peptide , NP , BPP, VEGF, snaclecs. The viper venom has two distinct C-type lectins depending upon their structural and functional properties. These are C-type lectin like proteins and sugar binding snake lectins. C-type lectin like proteins contains homologous heterodimers forming several momomers and oligomers and these proteins show several biological functions like platelet aggregation. Clemetson named the Snaclecs (Snake venom c-type lectins) and identified 8 different snaclecs in V. b. berus venom and this components constituted almost 5.5% of the whole venom proteins pool. Though snaclecs are not isolated and characterized from the venom on today's date.

5. Enzymatic proteins

The major enzymatic proteins that are detected and isolated from V. b. berus venom are usually; phospholipases A2, snake venom serine proteases, metalloproteinases, L-amino acid oxidases, phosphodiesterase, phosphomonoesterase, 5′ - nucleotidase, hyaluronidases, ribonucleases . Till today phosphodiesterases, 5′ -nucleotidases, ribonucleases and hyaluronidases are not still isolated from V. b. berus venom. Some of the most important enzymatic proteins that are detected through proteomic studies are as follows:

i. Phospholipases A_2

Phospholipases A_2 caharcteristically catalyze the hydrolysis of the ester bond, present at the sn-2 position in the glycerophospholipids and lead to the formation of free fatty acids and lysophospholipids. This protein is the most found protein in Viperidae snake venom. Though there are so many similarities in the structure and catalytic properties, phospholipase A_2 exhibits a wide range of pharmacological and toxicological functions, like myotoxicity, cardiotoxicity, neurotoxicity, anticoagulant and many hemolytic activities. These proteins have several mechanisms to show toxic effects. The first toxic PLA_2 was found by Delori in 1971. An anticoagulant factor was associated with PLA_2, isolated from the V. b. berus venom. This factor is a single chain protein that

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consists of 119 amiono acid residues and a molecular weight of 13400 dalton and an isoelectric point of 9.2. The Hungarian snake venom showed 4 to 7 isoforms of PLA_2, The Russian venom showed 3 isoforms and the Austrian venom showed a total of 5 isoforms, depending upon the preperative isoelectric points. The basic and most toxic PLA_2 was isolated from the Russian snake venom and the primary structure of the venom was established, consisting of a single chain protein with 14 Cys in positions, which became a characteristic property for the PLA_2 sub group II. The DNA sequences responsible for encoding the PLA_2 in the common viper venom was determined using the PCR- based method. Scientists cloned and sequenced some phospholipase transcripts from 21 fifferent European vipers and they also deduced the amino acid sequences out of it. The results were completely identical with the results of the basic snake venom type. Some scientists also studied the effect of inhibition of PLA_2 on human platelets, four different bacterial strains (gram-negative Escherichia coli and Vibrio fischeri; gram-positive Staphylococcus aureus and Bacillus subtilis) and on five types of cancer cells (PC-3, LNCaP, MCF-7, K-562 and B16–F10) in vitro. The protein inhibited the platelet aggregation induced by collagen in human and the growth of gram positive Bacillus subtilis , while it showed no inhibitory effect on the growth of gram negative Escherichia coli, which lead to the conclusion that the effect of the PLA_2 was completely cell type dependent. The enzyme inhibited the viability of K-562 cells and the cell death was apoptotic, i.e, induced by the cell itsef, while it exhibited no inhibitory effect on LNCaP cells and only some effect (8%–20%) towards other studied cells (Samel et al., 2013). Recently, Siniavin et al. (2021) demonstrated that snake venom PLA2s exhibit strong antiviral activity against SARS-CoV-2 at nanomolar concentrations inhibiting the viral spike glycoprotein interaction with ACE2 of Vero E6 cells. V. nikolskii venom dimeric PLA2 and its subunits manifested especially potent virucidal effects, which were related to their phospholipolytic activity, and inhibited cell–cell fusion mediated by the SARS-CoV-2 spike glycoprotein. Snake PLA2s are seem to be applicable as the antiviral drugs that can target the viral envelope and could also can be used as tools to study the interaction of viruses with host cells .

ii. Proteolytic enzymes

The proteolytic enzymes, isolated from the venom from V. b. berus were mainly metallo and serine proteinases that have the capacity to catalyze the digestion of tissue proteins and peptides.The two types of proteases are as follows:

2.1) Snake venom metalloproteinases (SVMP)

SVMPs are responsible for the development for some symptoms like haemorrhage, hypotension, hypovolemia, inflammation, oedema and necrosis. SVMPs are Zn-dependent enzymes, which are largely found in the Viperidae venoms. These are multi domain precursors and stored in the venom gland as inactive zymogens. SVMPs are mainle divide into three classes namely PI, PII, PIII, depending upon their multi-domain structure.

2.1.1) Haemorrhagic metalloproteinase (HMP)

The occurance of haemorrhage is one of the most common consequences of envenomation on viperid venoms. The metalloproteinase, isolated from the V. b. berus venom was demonstrated to have haemorrhagic activity with a minimum haemorrhagic dose of about 4 micrograms in mouse. The caseinolytic activity of HMP was inhibited by EDTA, but not by PMSF. HMP is a glycoprotein with mol. mass of 56.3 kDa. Enzyme contains one zinc atom per molecule of protein. HMP hydrolyses casein, fibrinogen and splits the insulin B chain at the positions Ala14-Leu15, Tyr16-Leu17, His10-Leu11. In oxidized insulin B chain HMP digests the same bonds as HR-l proteinase from Agkistrodon blomhoffi venom. It digests completely the A alpha chain and slowly the B beta chain of fibrinogen, the gamma chain is not digested.

2.1.2) Factor X activating enzymes

Human coagulation factor X is a serine protease zymogen, which circulates in blood as a two-chain molecule. A variety of factor X activators have been detected in snake venoms. Viperidae and Crotalidae venom activators are mainly metalloproteinases (Siigur and Siigur, 2006, 2010, reviews). Three factor X activating enzymes have been isolated from V. b. berus venom: VBFXAE (Samel and Siigur, 1995), VBFXAEI (Siigur et al., 2002) and VBFXAEII (Samel et al., 2003). VBFXAE is a single-chain glycoprotein with isoelectric points in the pH range of 3.5–4.5 containing 2 Ca2+ions per mole. The activator is inactive on synthetic substrates, on casein, prothrombin, and fibrinogen. VBFXAEI and VBFXAEII enzymes are high molecular mass proteinases. The

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specificity studies of factor X activating enzymes from V. b. berus venom demonstrate that these enzymes are nonspecific.

2.2) Serine Proteinases (SVSP)

Snake venom serine proteinases belong to a group of highly studied toxins, widely found in the venom of snakes from Viperidae, Elapidae, and Crotalidae families. They are complex and multifunctional enzymes, acting primarily on haemostasis (Serrano and Maroun, 2005). A few serine proteinases have been detected in V. b. berus venom. Two glycosylated arginine ester hydrolases, designated EI and EII have been isolated and characterised from V. b. berus venom (see Table 1). Both enzymes were active towards the arginine esters BAEE and TAME. EI and EII differ in their activity towards kininogen, EII having high kinin-releasing activity, while EI has only weak activity against kininogen. Arginine ester hydrolases showed similar actions on Pro-Phe-Arg-MCA (Samel et al., 1987). Nedospasov and Rodina (1992) investigated age changes of amidolytic activity of V. berus venom using mixtures of chromogenic peptide substrates. Quantities of the venom (total protein content) and its proteolytic activity from snakes of different ages were compared. The venom composition of newly born adders was considerably different from the venom composition of young (12-month) adders of the same population.

2.3) L-amino acid oxidases (LAAO)

L-amino acid oxidase (L-amino acid: O2 oxidoreductase) is a flavoenzyme that catalyses the stereospecific oxidative deamination of an L-amino acid to produce α-ketoacid, hydrogen peroxide and ammonia:

$$RCH(NH_3^+)\,COO^- + O_2 + H_2O \rightarrow RCOCOO^- + NH_4^+ + H_2O_2$$

LAAO is responsible for the yellowish colour of venoms. A yellow colour has been detected in the fraction of preparative isoelectric focusing (pI 4.8) of V. b. berus venom and an L-amino acid oxidase isoform has been isolated. The enzyme is a non-covalently bound glycosylated homodimer with a monomeric molecular mass of 57.7 kDa. The purified protein catalysed oxidative deamination of L-amino acids; the most specific substrate is L-Phe.

II. CONCLUSION

The envenoming with V. b. berus venom is not usually associated with serious harm to the patients except for children. Venom composition is highly variable among the different populations throughout the area of distribution of snakes. Proteomic and functional analyses of V. b. berus venom indicate the presence of proteins belonging to at least 15 protein/peptide families, with predominance of PLA2s, serine- and metalloproteinases. PLA2s, SVMPs, SVSPs, LAAO, disintegrins, NGF, CRISP, Kunitz-type proteinase inhibitors have been isolated and characterised from V. b. berus venom. The high complexity, considerable enzymatic, antibacterial, and cytotoxic activities of V. berus venom imply as a promising source for new antibacterial and cytostatic agents. The toxins present in V. berus venom have also potential to serve as a basis for the design of new molecules with potential biotechnological application.

III. REFERENCES

[1] Al-Shekhadat, R.I., Lopushanskaya, K.S., Segura, ´A., Guti´errez, J.M., Calvete, J.J., Pla, D., 2019. Vipera berus berus venom from Russia: venomics, bioactivities and preclinical assessment of microgen antivenom. Toxins 11 (2), 90

[2] Archundia, I.G., de Roodt, A.R., Ramos-Cerrillo, B., Chippaux, J.P., Olguín-P´erez, L., Alag´on, A., Stock, R.P., 2011. Neutralization of Vipera and Macrovipera venoms by two experimental polyvalent antisera: a study of paraspecificity. Toxicon 57, 1049–1056

[3] Casewell, N.R., Al-Abdulla, I., Smith, D., Coxon, R., Landon, J., 2014. Immunological cross-reactivity and neutralisation of European viper venoms with the monospecific Vipera berus antivenom ViperaTAb. Toxins 6, 2471–2482

[4] Calder´on, L., Lomonte, B., Guti´errez, J.M., Tarkowski, A., Hanson, L.A., 1993. Biological and biochemical activities of Vipera berus (European viper) venom. Toxicon 31, 743–753.

[5] Cui, S., Luo, X., Chen, D., Sun, J., Chu, H., Li, C., Jiang, Z., 2016. The adder (Vipera berus) in Southern Altay Mountains: population characteristics, distribution, morphology and phylogenetic position. PeerJ 4, e2342

[6] Delori, P.J., 1973. Purification and physiochemical, chemical, and biological properties of a toxic A2 phospholipase isolated from the venom of viperidae snakes: Vipera berus]. Biochimie 55, 1031–1045

[7] Fry, B.G., Vidal, N., van der Weerd, L., Kochva, E., Renjifo, C., 2009. Evolution and diversification of the Toxicofera reptile venom system. J. Prot. 72, 127–136.

[8] García-Arredondo, A., Martínez, M., Calder´on, A., Saldívar, A., Soria, R., 2019. Preclinical assessment of a new polyvalent antivenom (Inoserp Europe) against several species of the subfamily Viperinae. Toxins 11 (3), E149

[9] Guillemin, I., Bouchier, C., Garrigues, T., Wisner, A., Choumet, V., 2003. Sequences and structural organization of phospholipase A2 genes from Vipera aspis aspis, V. aspis zinnikeri and Vipera berus berus venom. Identification of the origin of a new viper population based on ammodytin I1 heterogeneity. Eur. J. Biochem. 270, 2697–2706.

[10] Hermansen, M.N., Krug, A.H., Tjønnfjord, E., Brabrand, M., 2019. Envenomation by the common European adder (Vipera berus): a case series of 219 patients. Eur. J. Emerg. Med. 26, 362–365.

[11] Jan, V.M., Guillemin, I., Robbe-Vincent, A., Choumet, V., 2007. Phospholipase A2 diversity and polymorphism in European viper venoms: paradoxical molecular evolution in Viperinae. Toxicon 50, 1140–1161

[12] Kovalchuk, S.I., Ziganshin, R.H., Starkov, V.G., Tsetlin, V.I., Utkin, Y.N., 2016. Quantitative proteomic analysis of venoms from Russian vipers of pelias group: phospholipases A2 are the main venom components. Toxins 8 (4), 105.

[13] Kriˇzaj, I., Siigur, J., Samel, M., Cotiˇc, V., Gubenˇsek, F., 1993. Isolation, partial characterization, and complete amino acid sequence of the toxic phospholipase A2 from the venom of the common viper, Vipera berus berus. Biochim. Biophys. Acta 1157, 81–85.

[14] Lamb, T., Stewart, D., Warrell, D.A., Lalloo, D.G., Jagpal, P., Jones, D., Thanacoody, R., Gray, L.A., Eddleston, M., 2021. Moderate-to-severe Vipera berus envenoming requiring ViperaTAb antivenom therapy in the UK. Clin. Toxicol. 21 (10), 992–1001

[15] Latinovi´c, Z., Leonardi, A., ˇSribar, J., Sajevic, T., ˇZuˇzek, M.C., Frangeˇz, R., Halassy, B., Trampuˇs-Bakija, A., Pungerˇcar, J., Kriˇzaj, I., 2016. Venomics of Vipera berus berus to explain differences in pathology elicited by Vipera ammodytes ammodytes envenomation: therapeutic implications. J. Proteonomics 146, 34–47

[16] Malenyov, A.L., Bakiev, A.G., Shurshina, I.V., Zaitseva, O.V., Zinenko, A.I., 2006. Proteolytic activity of common viper's venom from some populations in Russia and Ukraine. In: Proceedings of Samara scientific centre of Russian Academy of Science [Известия СаМарского научного центра Российской акадеМии наук, 8, pp. 1040–1044 (Russian).

[17] Morjen, M., Kallech-Ziri, O., Bazaa, A., Othman, H., Mabrouk, K., Zouari-Kessentini, R., Sanz, L., Calvete, J.J., Srairi-Abid, N., El Ayeb, M., Luis, J., Marrakchi, N., 2013. PIVL, a new serine protease inhibitor from Macrovipera lebetina transmediterranea venom, impairs motility of human glioblastoma cells. Matrix Biol. 32, 52–62

[18] Paolino, G., Di Nicola, M.R., Pontara, A., Didona, D., Moliterni, E., Mercuri, S.R., Grano, M., Borgianni, N., Kumar, R., Pampena, R., 2020. Vipera snakebite in Europe: a systematic review of a neglected disease. J. Eur. Acad. Dermatol. Venereol. 34 (10), 2247–2260

[19] Pla, D., Quesada-Bernat, S., Rodríguez, Y., S´anchez, A., Vargas, M., Villalta, M., Mes´en, S., Segura, ´A., Mustafin, D.O., Fomina, Y.A., Al-Shekhadat, R.I., Calvete, J.J., 2020. Dagestan blunt-nosed viper, Macrovipera lebetina obtusa (Dwigubsky, 1832), venom. Venomics, antivenomics, and neutralization assays of the lethal and toxic venom activities by anti-Macrovipera lebetina turanica and anti-Vipera berus berus antivenoms. Toxicon X 6, 100035.

[20] Samel, M., Vija, H., Kurvet, I., Künnis-Beres, K., Trummal, K., Subbi, J., Kahru, A., Siigur, J., 2013. Interactions of PLA2-s from Vipera lebetina, Vipera berus berus and Naja naja oxiana venom with platelets, bacterial and cancer cells. Toxins 5, 203–223.

[21] Siigur, J., Siigur, E., 2010. Activation of factor X by snake venom proteases. In: Kini, R. M., Clemetson, K.J., Markland, F.S., Morita, T. (Eds.), Toxins and Hemostasis. Springer Science+Business Media B.V. 2010, pp. 447–464

[22] Siniavin, A.E., Streltsova, M.A., Nikiforova, M.A., Kudryavtsev, D.S., Grinkina, S.D., Gushchin, V.A., Mozhaeva, V.A., Starkov, V.G., Osipov, A.V., Lummis, S.C.R., Tsetlin, V.I., Utkin, Y.N., 2021. Snake venom phospholipase A2s exhibit strong virucidal activity against SARS-CoV-2 and inhibit the viral spike glycoprotein interaction with ACE2. Cell. Mol. Life Sci. 78 (23), 7777–7794

[23] Sjostrom, L., Karlson-Stiber, C., Persson, H., Al-Abdulla, I.H., Smith, D.C., 1996. Development and clinical application of immunoassays for European adder (Vipera berus berus) venom and antivenom. Toxicon 34, 91–98

[24] Tadokoro, T., Modahl, C.M., Maenaka, K., Aoki-Shioi, N., 2020. Cysteine-rich secretory proteins (CRISPs) from venomous snakes: an overview of the functional diversity in A large and underappreciated superfamily. Toxins 12 (3), E175

[25] Valenta, J., Stach, Z., St˘r´ıteský, M., Mich´alek, P., 2014. Common viper bites in the Czech Republic-epidemiological and clinical aspects during 15 year period (1999–2013). Prague Med. Rep. 115, 120–127

[26] Varga, C., Malina, T., Alf˙oldi, V., Bilics, G., Nagy, F., Ol´ah, T., 2018. Extending knowledge of the clinical picture of Balkan adder (Vipera berus bosniensis) envenoming: the first photographically documented neurotoxic case from South- Western Hungary. Toxicon 143, 29–35.

[27] Westerstr¨om, A., Petrov, B., Tzankov, N., 2010. Envenoming following bites by the Balkan adder Vipera berus bosniensis - first documented case series from Bulgaria, 1510-1505 Toxicon 56

[28] Zinenko, O., Tovstukha, I., Korniyenko, Y., 2020. PLA2 inhibitor varespladib as an alternative to the antivenom treatment for bites from Nikolsky's viper. Vipera berus nikolskii. Toxins (Basel). 12 (6), E356.