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Research Article

Dynamics of Changes in the Activity of Phospholipase A2 In the Venom of Viper (Macrovipera Lebetina Obtusa Linnaeus, 1758) Venom Depending on Storage Period

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Received Date: May 16, 2024 | Accepted Date: June 03, 2024 | Published Date: August 20, 2024

Citation: Sh.A.Topchiyeva (2024), Dynamics of changes in the activity of phospholipase A2 in the venom of viper (Macrovipera lebetina obtusa Linnaeus, 1758) venom depending on storage period, *Clinical Trials and Case Studies*, 3(4): **DOI: 10.31579/2835-835X/071**

Abstract:

The article presents experimental data and studies of the dynamics of changes in the activity of phospholipase A2 from the venom of vipers (Macrovipera lebetina obtusa Linnaeus, 1758) with different storage periods. A decrease in the enzymatic activity of viper venom was revealed during long-term storage in the period from 1989 to 2015 from 42 IU/mg to 30.5 IU/mg. In all likelihood, as a result of biochemical changes, there is a decrease in enzyme activity from 100% to 72.61, 77.38, 82.38, 95.24%, respectively, which must be taken into account when producing and storing preparations based on snake venom. Thus, the obtained results of experimental studies can be used in storing, identifying, standardizing and determining the biological activity of both whole venom of the Transcaucasian viper and preparations based on viper venom.

Keywords: venom, Macrovipera lebetina obtuse; phospholipasa A2.

Introduction

Snake venoms are a complex complex of biologically active substances that have extremely diverse properties and the ability to affect the main integrating systems of the body: the blood and the nervous system. Their toxic and medicinal properties have been known to man since ancient times. However, only in the twentieth century did their scientifically based use for therapeutic and diagnostic purposes begin [1-4]. Currently, due to the intensive development of pharmacology and pharmacy, a colossal amount of medicines has been created for the treatment of human diseases. There is no doubt that the poison of the Transcaucasian viper can be classified as a group of natural biologically active substances. Snake venoms contain compounds that act on various cellular targets. Thus, the venoms of snakes of the Elapidae family contain well-known α-neurotoxins that have a high affinity for nicotinic cholinergic receptors (nAChRs) and a small group of toxins that block Ca2+ and K+ channels. Very little is known about the presence of nAChR antagonists andvoltage-activated Ca^{2}+ channel blockers in venoms of snakes of the family Viperidae. It has been established that venoms from snakes of the Viperidae family have the ability to block nAChRs and voltage-activated Ca2+ channels. The efficiency of nAChR blocking is significantly lower than that of the cobra venom N. kaouthia, which has a high content of -neurotoxins (classical nAChR blockers), but the blocking of Ca2+ channels by Viperidae and cobra venoms is similar. The data obtained suggest that the studied venoms of Viperidae contain toxins that block targets of both types [5-8]. Modern methods of fractionation of mixtures of organic compounds (electrophoresis, ultracentrifugation, ultrafiltration, fixation on ion-exchange resins, etc.) have made it possible to separate the proteins that make up snake venoms and determine their biochemical properties and toxicity. It turned out that snake venoms contain from 5 to 11 different protein fractions. Snake venom proteins have the properties of either enzymes or enzymatic poisons. On this basis, a currently generally accepted view has emerged, according to which the main active principles of snake venoms are simple or complex proteins that have the properties of enzymes or enzymatic poisons [9]. The authors analyzed the properties of the poisonous secretion using the activity of proteolytic enzymes and L amino acid oxidase. Determination of protein concentration in venom solutions was carried out using the Lowry method. As is the case with proteolytic activity, the largest range is observed in representative samples. According to the authors, for the Middle Volga region the minimum variant of amino acid L oxidase activity is 1.2, and the maximum is 47.1, and the range of variation was 45.9 U/mg protein min [10]. It has been shown that in common vipers Vipera berus from Samara, venom output increases after wintering in May and decreases in September before leaving for the winter. A statistically significant summer increase in the activities of proteases (in June) and L-amino acid oxidase (in July) in venom was revealed [11]. The LD values of the poisonous secretions of common and eastern steppe vipers were determined using various experimental animals. At the same time, statistically significant differences in the LD values determined in animals of the same species of different masses could not be identified [12]. As studies of the proteome of venoms of 85 species of snakes of the viper family have shown, the bulk of the venoms were representatives of three groups of proteins (all of them are enzymes): 1) phospholipase A, 2) serine proteinases, 3) metalloproteinases [13]. Phospholipase A_2 is the most important “biochemical weapon” not only of vipers, but also of other higher snakes, as well as some poisonous animals of other classes (wasps, bees, scorpions, cone snails). The content of phospholipase A2 in the

venoms of snakes of the Viperidae subfamily (data on 20 species) varied from 5 to 64%, and from the Crotalinae family (data on 53 species) – from 0.5 to 91% [14-17]. The maximum enzyme content was described for the representative of viperids (true vipers) Vipera nicolskii (65%) [18] and for the representative of crotalids (rattlesnakes) Crotalus durissus cascavella (90.9%) [19]. In a particular species of snake, the enzyme concentration did not change very significantly depending on activity and age [20-22]. Phospholipases A_2 (PLA_2) catalyze the hydrolysis of fatty acid esters in the 2-position of 3- sn phospholipids and are found in intracellular and secreted forms. Within the same snake venom, several PLA_2 can be found, which present differences in their ability to hydrolyze phospholipids in biological bilayers. In this study, Chinese cobra, Naja naja atra (N. n. atra) venom was purified by Size Exclusion Chromatography using a Superdex 75 and cation exchange using a column packed with Carboxymethyl Cellulose (CM-Cellulose) to pre-separate protein components from the venom. PLA_2 activity was assayed against human erythrocytes and monomeric substrate to determine differences in hydrolytic activity. Primary sequence alignment shows 94% homology, however Band E presented 40% less activity against human erythrocytes, despite having 100% active site homology. Molecular modeling of both sPLA2s revealed differences at the Cys-19 and Trp-19 position and spatial orientation differences at the Trp-61, Tyr-63 Phe-64, and Lys-65 positions. Due to amino acid differences at the BRS, differences in hydrolytic activity may be linked to the anchorage of $sPLA_2$ to the erythrocyte surface [23]. The extensive protein superfamily of phospholipases A2 consists of six types of enzymes, 15 groups and numerous subgroups [24]. Phospholipase A2, snake venoms belong to the type of small secretory PLA_2. Enzymes of this group, as a rule, are single-chain proteins consisting of 115-125 amino acid residues with a mass of 13-15 kDa. Phospholipases A_2 from snake venoms are one of the aggressive toxic proteins, often playing a major role in immobilizing and killing the victim of a snake bite [25-27]. Snake venom phospholipase A2 enzymes have a wide range of pharmacological effects, but there is no direct relationship between these pharmacological effects and phospholipase activity. According to literature data, the molecular weight of PLA_2 is approximately 13-15 kDa. There are several group A phospholipases; most often they are an integral part of many tissues and secretions of living organisms. Phospholipase A_2 is the active component of snake venom with hemolytic action [28] Based on the above, the purpose of this work is to study the dynamics of changes in the activity of phospholipase A_2 in the venom of viper (Macrovipera lebetina obtusa Linnaeus, 1758) with different storage periods.

Material and research methods

The research material was the whole venom of the Transcaucasian viper (Macrovipera lebetina obtusa), dried in a desiccator over calcium chloride vapor, and venom samples with different shelf life for the periods when venom samples were collected in 1989, 1991, 1993, 2010, 2015. The poisonous secretion of the Transcaucasian viper was dried under standard conditions in a desiccator over calcium chloride at room temperature for at least 10-12 days. Next, the crystalline poison was collected and analyzed. Samples of viper venom were stored in glass containers in the refrigerator at a temperature of $+5\text{-}6^0$ C. With this method of drying and storage, the poison retained its biological activity for at least 3 years. Determination of phospholipase A_2 activity in the venom of the Transcaucasian viper was carried out using the titrimetric method.

Research results and discussion

Research results We studied the enzymatic activity of phospholipase A2 in standard samples of viper venom collected in 1989, 1991, 1993, 2010 and 2015. The activity of the phospholipase A2 enzyme in the venom of the Transcaucasian viper was determined by the titrimetric method. To do this, 1 ml of the drug (0.05 g of poison + 0.9% potassium chloride solution + water), 1 ml of reagent 1 (0.1 g of albumin + 80 mg of 0.05 mol of Tris buffer solution pH 8.0 + 2 ml of a 0.05 mol solution of Trilon B + 0.4 ml of a 50% solution of calcium chloride and the volume of the solution was adjusted to 100 ml with a 0.05 molar solution of Tris buffer and 1 ml of a solution of Z-alphalecithin in absolute alcohol. The tubes were kept in a thermostat at a temperature of 37C for 30 minutes. Then 7 ml of the extraction mixture was added to all test tubes, shaken and kept at a temperature of 20C for 1 hour. Next, from this mixture, 3 ml of the solution of the upper layer was taken from each test tube and placed in conical flasks with a capacity of 25 ml, 5 drops of 0.2% solution of thymol blue in 95% alcohol were added and titrated from a microburette with 0.01 molar solution of potassium hydroxide to transition of yellow color to blue. At the same time, a control experiment was carried out, where instead of viper poison they took water and proceeded as described above, table 1.

Statistical processing of experimental data was carried out using Student's test. **Table 1.**

Year of venom collection	Enzyme activity (IU/mg)
1989	30,5±1,8
1991	32,5±1,5
1993	34,6±0,9
2010	40,0±2.2
2015	42,0±1,8

Phospholipase A2 activity in samples of viper venom (IU/mg)

As can be seen from Table 1, the activity of phospholipase A_2 during the storage period since 1989, with an extension of the storage period, decreased by 11.5 IU/mg compared to the poison sample collected in 2015. In all likelihood, as a result of biochemical changes, there is a decrease in enzyme activity from 100% to 72.61, 77.38, 82.38, 95.24%, respectively, which must be taken into account when producing and storing preparations based on snake venom. Thus, from the above it follows that the duration of storage of snake venom has a significant impact on the enzymatic, namely phospholipase activity of zootoxin. During long-term storage of viper venom samples from the storage period from 1989, 1991, 1993 to 2010, a significant decrease in the activity of the phospholipase A_2 enzyme is noted and amounts to 72.61, 77.38, 82.38, 95.24%, respectively. The results of experimental data can be used in the identification, standardization and determination of the biological activity of the venom of the Transcaucasian viper.

Conclusions

1. Experimentally revealed a decrease in the enzymatic activity of viper venom during long-term storage in the period from 1989 to 2015 from 42 IU/mg to 30.5 IU/mg.

2. A decrease in phospholipase A_2 activity was detected at 1.38. 1.29, 1.21 and 1.05 times, respectively, compared to samples collected in 2015, which is associated with biochemical processes in samples of viper venom, depending on the conditions and storage period.

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