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Review Article

Dynamics of proteolytic activity and L-amino acid oxidase activity of viper viper (Macrovipera lebetina obtusa Linnaeus, 1758) venom depending on storage period

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Abstract:

The purpose of the presented work is the biochemical analysis of the venom of viper (Macrovipera lebetina obtusa Linnaeus, 1758) with different shelf life. Experimental studies were carried out to determine the proteolytic activity and L-amino acid oxidase activity of venom samples collected over the period of time from 1989-2015.The research material was whole viper venom, dried in a desiccator over calcium chloride vapor, and samples of poison with different shelf life. In the venom of viper collected in 2015, the level of L-amino acid oxidase activity is 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, 1.15 times than in samples collected in 1989, 1991, 1993, 2010, respectively. It was revealed that the PA content in viper venom collected in 2015 is 2.73, 2.0, 1.46, 1.71 times higher than in venom samples collected in 1989, 1991, 1993, 2010, respectively. Based on the results of experimental studies, it was revealed that the activity of proteases depends on both the shelf life and the diet of the snake.

Keywords: venom; enzymes; macrovipera lebetina obtusa linnaeus; l-amino acid oxidase activity; proteolytic activity

Introduction

Among the huge number of biologically active substances of natural origin, one of the central places is occupied by animal poisons. Toxicity and enzymatic activity are the main characteristics of the biological activity of a poisonous secretion. The toxicity of a venom is an integral characteristic and reflects the overall effect of the toxin on a living organism, while the enzymes of snake venoms have specific points of application and mechanisms of action [1, 2].

Venomous snake bites are a serious public health problem worldwide. Poisonous animals and their poisons are in the area of attention of scientists of various specialties [3-5]. Using Maldi-mass spectrometry and high-performance liquid chromatography, a search was made for new polypeptide compounds contained in cobra venom in small quantities. A number of new proteins with a molecular weight of 7-25 kDa, typical of known toxins from many snakes, have been characterized; in this case, the content of one of the polypeptides did not exceed 0.02% of the weight of the dry venom [6, 7]. The pharmacokinetics and distribution of viper venom have been studied. Using mathematical modeling methods, the main pharmacokinetic parameters were found: Vd - apparent volume of distribution (4.45 ml), t1/2 - half-life of elimination (0.88 min.), t1/2,a - half-life of absorption (0.31 min.), k01 - absorption rate constant (2.24 min-1), kel - elimination rate constant (0.79 min-1), ClT - total clearance (3.49 ml/min), AIS0 area under the pharmacokinetic curve (942.82 µg/min ml-1) [8-10].Significant variation in the content of serine proteases and metalloproteases has been revealed in snake venoms. In the venom of the common viper (Vipera berus), 75% of the total proteolytic activity is due to metalloproteases, 25% to serine proteases. In the venom of the viper (Vipera lebetina), metalloproteases account for 15% of the total proteolytic activity, while serine proteases provide 85% of the activity of the venom [11, 12]. Thus, one of the essential components of V.renardi venom are proteolytic enzymes. It is proteases that play the main role in the development of a complex picture of poisoning. The authors determined the activity of L-amino acid oxidase, which catalyzes the conversion of L-amino acids into α-keto acids, which determines the color of the poisonous secretion [13,14]. Recently, many functional properties of L-amino oxidase have been described, such as cytotoxicity,

anticoagulant and hemorrhagic effects, antibacterial activity and a number of other physiological processes [15, 16 17, 18,19]. The authors provide data on the determination of the proteolytic activity of steppe viper venom. It was noted that the average value of proteolytic activity and L-amino acid oxidase activity of the venom of steppe vipers Vipera renardi from Crimea is significantly higher than from the Left Bank of the Lower Volga [20, 21, 22].It should be noted that the wide range of peptides and proteins with different biological functions makes animal venoms a valuable source of new compounds, both for use in basic research and for the development of new drugs.The development and improvement of physicochemical and biochemical methods for identifying and standardizing snake venom will provide the pharmaceutical industry with a high-quality and environmentally friendly, natural product, with a given toxicity and pharmacological activity.

The purpose of the work: is a biochemical analysis of the venom of viper (Macrovipera lebetina obtusa Linnaeus, 1758) with different shelf life. Determination of proteolytic activity (PA) and L-amino acid oxidase (AO) activity of venom samples collected over the period of time from 1989-2015.

The research material was whole venom of the Transcaucasian viper (Macrovipera lebetina obtusa), dried in a desiccator over calcium chloride vapor and samples of venom with different shelf life under non-standard conditions.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 +5-6^0 C. With this method of drying and storage, the poison retained its biological activity for at least 3 years.The proteolytic activity (PA) and L-amino acid oxidase (AO) activity of standard samples of viper venom collected in 1989, 1991, 1993, 2010 and 2015 were experimentally determined.The activity of enzymes in samples of venom from the Transcaucasian viper was determined by the titrometric 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 37^{0}C for 30 minutes. Then 7 ml of the extraction mixture was added to all test tubes, shaken and kept at a temperature of 20^{0}C for 1 hour. Next, 3 ml of the solution of the upper layer was taken from this mixture from each test tube and placed in conical flasks with a capacity of 25 ml, 5 drops of a 0.2% solution of thymol blue in 95% alcohol were added and titrated from a microburette with a 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. Statistical processing of experimental data was carried out using Student's test.

Research results: In each individual sample, standard methods for determining enzyme activity were used: proteolytic activity (PA) was determined by hydrolysis of sodium caseinate (22); -L-amino acid (AO) oxidase activity – using L-phenylalanyl as a substrate (23). The sex and age of the snakes were not taken into account when analyzing the biochemical data. Possible seasonal changes in proteolytic activity were not taken into account.

The results of the analysis of experimental data are shown in Table 1. From the data presented in the table, it can be interpreted that a high level, that is, the maximum value of PA, is noted in the venom samples collected in 2015 and is 0.82 IU/mg. In snake venom samples collected in 2015, the PA content is 2.73, 2.0, 1.46, 1.71 times higher than in samples collected in 1989, 1991, 1993, 2010, respectively.

Year of poison collection	PA, IU/mg	*AO, IU/mg*
	M±m	*M±m*
1989	0,30±0.02	0.09±0.01
1991	0,41±0.01	0.11±0.02
1993	0.56±0,02	0.15±0.01
2010	0.70±0,03	0.26±0.02
2015	0.82±0,01	0.30±0.01

Table 1: Proteolytic activity (PA) and L-amino acid oxidase (AO) activity in viper venom sample

The table data shows that the level of activity of L-amino acid (AO) oxidase also undergoes changes when stored under non-standard conditions. In venom samples collected in 2015, the level of L-amino acid oxidase activity is 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, 1.15 times than in samples collected in 1989, 1991, 1993, 2010, respectively. In each individual sample, standard methods for determining enzyme activity were used: proteolytic activity (PA) was determined by hydrolysis of sodium caseinate (22); -L-amino acid (AO) oxidase activity – using L-phenylalanyl as a substrate (23). The sex and age of the snakes were not taken into account when analyzing the biochemical data. Possible seasonal changes in proteolytic activity were not taken into account. Thus, based on the results of experimental studies, it follows that snake venom not only serves to kill and immobilize prey, but also performs some functions of digestive juice. Therefore, it is logical to assume that the activity of proteases also depends on the diet of the snake. From the above it follows that the average values of the enzymatic activity of the venom collected in 1989 turned out to be significantly lower than the enzyme activity in the samples of viper venom collected in 2015.It is known that the color of the poisonous secretion is determined by the presence in it of the coenzyme oxidase -L-amino acids (AO) - flavin adenine nucleotide. Accordingly, in colorless samples of the poison, AO is close to zero. It is logical to assume that the activity of proteases in venom may depend both on diet, conditions and storage period of venom samples.

Conclusions:

1. It has been experimentally established that in samples of viper venom collected in 2015, the PA content is 2.73, 2.0, 1.46, 1.71 times higher than in venom samples collected in 1989, 1991, 1993, 2010, respectively.
2. The level of L-amino acid oxidase (AO) activity was detected. In venom samples collected in 2015, the level of L-amino acid oxidase activity is 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, 1.15 times than in samples collected in 1989, 1991, 1993, 2010, respectively.

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