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Animal venoms in the production of medicines

Przemysław Szabat[1], Julita Poleszak[1*], Marta Szabat[1], Grzegorz Boreński[1],

Magdalena Wójcik[1], Joanna Milanowska[2]

[1]Student Science Club at the Department of Applied Psychology, Medical University of Lublin
[2]Department of Applied Psychology, Medical University of Lublin

* E-mail adres: julita.poleszak@wp.pl

ORCID ID:

Przemysław Szabat https://orcid.org/0000-0001-5796-1900
Julita Poleszak https://orcid.org/0000-0002-5166-6262
Marta Szabat https://orcid.org/0000-0001-6309-2027
Grzegorz Boreński https://orcid.org/0000-0002-5359-7555
Magdalena Wójcik https://orcid.org/0000-0002-0999-6284
Joanna Milanowska https://orcid.org/0000-0001-9741-1583

ABSTRACT

Introduction: Toxicity of animal venom is a defense mechanism known to mankind for centuries for many species of animals. We are still learning the possibilities of its use as a substance with therapeutic potential, because on its basis several drugs known and commonly used in medicine were created.
Aim of the study: To review research on the known and potential use of animal toxins as medicinal substances.
Material and method: Standard criteria were used to review literature data. Search for articles in the PubMed database was carried out using the following keywords: animal venoms, medical use, snake venoms, drugs from venom,

Description of the state of knowledge: Animal venoms have been used by man since ancient times. Due to many different mechanisms, they are able to modulate the tissue functions of the human body. In the form of already known drugs have an effect, among others. on the cardiovascular, nervous and circulatory systems. Research is also being carried out on their use in the treatment of other diseases such as autoimmune diseases and cancer.
Summary: Due to the variety of substances in animal venom and their therapeutic properties, it is possible to use them for the production of medicines. However, many studies are needed to better understand them.

Key words: animal venoms, medical use, snake venoms

1. INTRODUCTION

The world around us is one big organism made of billions of cells, which are prokaryotes, protists, fungi, plants, animals. This is the classification of organisms proposed by Robert Whittaker in 1969. Each of these organisms has developed organs, mechanisms and behaviors that allow them to survive in the course of evolution. This also applies to many species belonging to the animal kingdom that have developed venom production capacity during their evolution. It is used to deter, paralyze or kill other animals by acting as a defensive element or tool to obtain food. It is produced by many species of animals, which include chordates (mammals, birds, fish, amphibians and reptiles), ringworms, arthropods (hymenoptera, scorpions, spiders), molluscs, echinoderms (starfishes, sea urchins) and sandworms (sea anemones, jellyfish) [1]. These toxins can be produced both in the tissues of the animal's body as well as in specially formed glands. By using organs such as stings, stinging cells, canines or other specialized organs, toxins are introduced into the victim's body, where they cause various symptoms from those less serious, such as itching, burning or pain, through cardiovascular disorders up to the death of the individual.

Animal venom has been used by man since the earliest centuries. Toxins from frogs, snakes and spiders have been used to treat many diseases, including chickenpox, leprosy or fever, as well as to accelerate wound healing [2]. The first information on poisonous substances and animals producing them was written by Aristotle (384–322 BC). Their potential action was already known, which in 326 B.C.E. was used to poison arrows using Russell's viper venom (Daboia russelii) and led to the death of Alexander the Great's soldiers [3]. However, this is not the only possible application. Research on snake venom began in Italy in the 17th and 18th centuries, then animal experiments with using venom from snakes was began [4]. However, the strong development of research into animal poisons dates back to the second half of the 20th century. In the 1950s and 1960s, two independent research groups isolated proteases and α-bungarotoxins found in snake venom [5, 6]. That was one of the first discoveries. Further research led to the isolation of substances that were then used in

medicine. Currently, the components of animal venom used for the production of new drugs are increasingly being isolated.

Animal venom is a solution that contains many substances that perform a variety of functions, and its composition depends on the species. In most cases, its toxicity is mainly caused by proteins and peptides, the content of which may be even over 90% in the case of venom of some snakes [7]. Protein substances can be enzymes belonging to oxidases or hydrolases and enzymatically inactive proteins. So far, only L-amino acid oxidase has been distinguished among oxidases, while in the class of isolated hydrolases we can find phospholipases A2, proteinases / peptidases, acetylcholinesterases and hyaluronidases [2]. Among the proteins without enzymatic activity, we can find, among others disintegrin, neurotoxin [8].

2. MOLECULAR MECHANISMS OF ANIMAL VENOM ACTIVITY

For toxicity of venom are responsible the substances they contain, which when introduced into the body cause a series of reactions through which the animal achieves its purpose for which this venom was applied. Among them we can find neurotoxic substances. Synapses and channels and receptors present in them become their target. These are particles that strongly affect the neuromuscular system, causing symptoms such as paralysis, muscle spasms and weakening of their function. Peptide toxins acting on ion channels are mainly responsible for this action. They can cause inhibition of ion movement within these channels [9]. A study by J. Craig and M. McIntosh using cone snail venom showed the presence of two peptides responsible for neurotoxicity, these are GVIA ω-conotoxin (produced by Conus geographus) and MVIIA ω-conotoxin (produced by Conus magus) [10]. The mechanism of action of these toxins is based on blocking neuromuscular transmission within the presynaptic termination by inhibiting the release of acetylcholine. Scientists have proven that these toxins work by selectively blocking Ca2 + channels in the posterior corners of the spinal cord, which inhibits pain conduction to the upper floors of the central nervous system. L and N type Ca2 + channels are blocked in a strong and durable way, while the effect of these substances on T type channels is weak and is reversed very quickly. This activity has been demonstrated for sympathetic neurons and those in the hippocampus of rats [11]. Myotoxic activity is also associated with the effect of venom on Ca2 + channels, which has been proven using the Plesiotrigon iwame and Potamotrigone sting venom. After injecting the toxin into the skeletal muscle of mouse, it was observed that they develop inflammation followed by necrosis and consequent rhabdomyolysis [12]. This effect has also been noted for the Scatophagus argus venom, after use of which has been seen to increase creatine kinase activity in mouses [13].

Researchers have also proved the occurrence of venom affecting the cardiovascular system. Symptoms of their action include changes in blood pressure and smooth muscle relaxation of the walls of blood vessels, as well as effects on the strength of heart contraction and its frequency. Cardiotoxins bind to the cells of the heart causing blockage of its contraction [14]. The results of the study by Carlson et al. indicated that the mechanism of action of the toxin contained in the venom of Scorpaena guttata is the release of acetylcholine from muscarinic receptors. This study also indicates the role of acetylcholine and

catecholamines in attenuating heart response [14]. The effect of toxin on vessels is associated with their relaxation caused by the release of NO from the vascular endothelium, however, scientists' opinions differ in terms of the involvement of muscarinic receptors in this process [15, 16]. Carlson et al. also demonstrated the effect of scorpion venom on respiratory disorders, which may even lead to its arrest in cats, rabbits and dogs [14]. Based on study using venom derived from Synanceja trachynis, it has been shown that bronchospasm caused by toxin activity can be associated with the release of substance P and its effect on neurokinin 1 receptors [17]. However, we can conclude that the effect of toxins from venom may vary depending on the species of animal on which the experiment was conducted, as well as the species of animals from which venom was collected.

Among the venom of animals, we can also find venom with hemolytic activity. These include almost all the venom of fish and the venom of other animals such as snakes, jellyfish, sea anemones, bees, spiders and scorpions. Khoo et al. demonstrated the presence of venom Synanceia horrida stonustoxin (SNTX) in rabbits and rats that causes erythrocyte hemolysis and inhibition of ADP or collagen dependent platelet aggregation [18].

Many enzymes are responsible for the cytotoxic activity of substances contained in animal venoms. Among them, we can find a Cardiotoxin-3 (CTX) polypeptide that causes cell apoptosis. The mechanism of induction of cell death has not been fully understood, scientists suspect mediation of the pathway associated with caspase 12 and c-Jun N-terminal kinases initiated by the influx of Ca2 + ions, increased levels of Ca2 + ions and GRP78 protein or inactivation of signaling pathways of EGFR; P13-K / Akt and JAK / STAT3 [19, 20, 21]. The group of cytotoxins include also disintegrin, for which it has been shown that they inhibit the development of cancer. They impede cell adhesion, also affecting the inhibition of tumor metastasis [22, 23]. Phospholipase A2 also has a cytotoxic effect by hydrolysing of bindings to form of lysophospholipids and free fatty acids that cause disruption of stability of cell membrane [24]. Examining Macroviper lebentin venom, it was shown that it may have anti-integrin effects; it was observed that it inhibits cell adhesion and migration, disrupts actin skeleton and release of αvβ3 integrin. In addition, amino acid L-oxidase, similarly to disintegrin and CTX, has antiproliferative activity. It induces cell apoptosis using the Fas-dependent pathway. drCT-1 - a protein found in the venom of the Russell indian viper (Daboia ruselli russell) also has cytotoxic and antiproliferative properties. In a study on human leukemia cells, it was shown to cause inhibition of the tumor process by inducing apoptosis in the G1 phase of the cell cycle [25].

Toxins contained in animal venoms also have a fibrinolytic effect. Metalloprotease and to a greater extent serine proteases, which in addition to fibrinolytic activity, also have fibrinogenolytic activity are responsible for them. The effect on the coagulation system is manifested by systemic bleeding and coagulopathy, which lead to hypovolemia and the development of hemodynamic shock [26]. These are enzymes that degrade extracellular matrix protein [27]. A fibrinogenolytic activity test showed that serine proteases hydrolyze the fibrinogen α chain and then its β chain leading to coagulation disorders [28]. The proteases contained in venom also affect the complement system by activating it, which leads to the development of inflammation, as well as their effect is visible in the processes of cell

differentiation, increasing the permeability of cell membranes and hemostatic activity of the organism [29].

3. MEDICINAL PRODUCT BASED ON ANIMAL VENOM

With the development of knowledge about animal venoms, the possibilities of their use have increased. This was an incentive to try to use them in medicine, where they became the basis for the production of some drugs. Captopril and enalapril - angiotensin converting enzyme inhibitors were among the first approved drugs based on animal venom. They were discovered in the 1970s on the basis of bradykinin enhancer peptides (BPF) isolated from Bothrops jararaca venom [30]. These peptides are short proline-rich chains that bind to the ACE substrate binding pocket, thereby inhibiting the production of vasoactive angiotensin [31]. During the studies, it was shown that they help reduce the amount of angiotensin II, thereby inhibiting its action, as well as their effect on the vessels is visible by enhancing the action of bradykinin which is a vasodilatation. In 1981, captopril in the form of an oral tablet was launched [32].

In addition to captopril, other drugs based on a substance derived from animal venom are eptifibatide and tirofiban. Based on a toxin derived from the venom of rattlesnake (Sistrusus miliarius barbouiri) eptifibatide, a cyclic heptapeptide, was produced. The second one, tirofiban is a product based on echistatin derived from the sand viper venom (Echis carinatus) [33]. Both substances are included in the group of antiplatelet agents. The mechanism of their action is based on preventing fibrinogen, von Willebrand factor and other adhesion molecules from binding to GP IIb / IIIa receptors, reversibly inhibits platelet aggregation. Both compounds were developed to resemble the Arg-Gly-Asp sequence recognized by the GPIIb / IIIa integrin receptor, thereby blocking the receptor [34, 35]. These drugs are used in patients with cardiovascular thromboembolic events, including patients with unstable angina and STEMI infarction, reducing the risk of death or recurrent myocardial infarction [36].

A compound derived from animal venom is also used in pain therapy. This substance is a neurotoxic peptide - ω-conotoxin, derived from the cone snail venom. The mechanism of action of this toxin has been mentioned above. An important study, during which the first results were obtained about the possibility of using it in the treatment of pathological pain, is the study by G. Milijanich. The study showed that this substance binds to the dorsal horns of the spinal cord, which contain the neurons responsible for sensing and transmitting pain information. Further research conducted by the scientist and his colleagues confirmed initial assumptions. In 2004, this substance was approved for medical use as a ziconotide, used in the treatment of severe and chronic pain [37].

Exenatide is a synthetic drug based on the hormone exenatide-4 found in the saliva of Gila monster (Heloderma suspectum) [38]. It is a substance with a high degree of homology to human GLP-1 peptide, therefore it was approved for use in the treatment of type 2 diabetes in 2005 [39]. GLP-1, and thus exenatide, works by induction of an increase in

intracellular cAMP resulting in an increase in insulin production. This leads to a decrease in blood glucose and glucagon and a slowed gastric emptying [40].

Batroxobin is also an FDA approved drug that has been discovered and synthesized on the basis of venom. It was isolated from Bothrops atrox moojeni venom and is a thrombin-like serine protease. It causes fibrinogen to coagulate by binding the γA / γA form and the γA / γ 'variant fibrinogen with a γ chain in the central E-region. This is a different way of binding fibrin than thrombin, which can cause greater affinity of batroxobin for fibrin and its prothrombotic properties. Batroxobin also reduces bleeding time and whole blood clotting time in vivo. It is used in the presence of severe thrombolysis and bleeding after previous administration of heparin [41].

In 2017, bee venom (Apitox®) was approved for medical use [42]. Due to the very rich composition, it has many activities, including anti-inflammatory and relieving swelling and pain. Melitin and adolapine are responsible for its anti-inflammatory effect. The first compound inhibits the enzymatic activity of phospholipase and causes an increase in cortisol production by stimulating the pituitary to secretion of ACTH [43]. The other one inhibits pro-inflammatory substances, including TNF-α, PGE-2, cytokines and enzymes such as COX-2 [44]. Therefore, it is used in the treatment of multiple sclerosis and rheumatoid arthritis in the form of injections [45, 46].

In addition, substances derived from animal venom are used in other aspects of medicine. For example, ecarin from the snake Echis carinatus is used to monitor the activity of direct thrombin inhibitors, while Russell's viper venom factor X activator (RVV-X) isolated from the Daboia russelli venom is used to determine the X coagulation factor [47]. Also based on a toxin derived from snake venom, fibrin glue was produced, which can be used in periodontal disease. The component of this glue is a serine protease isolated from the venom of the rattlesnake (Crotalus durissus terrificus).

4. SUBSTANCES IN THE RESEARCH PHASE

The development of science is conducive to the isolation of new substances derived from venom, whose impact on the human body is in the phase of clinical research. These include vipegitide and its pegylated counterpart vipegitide-PEG2, whose matrix is a C-type lectin protein found in the venom of an Israeli viper. They are peptide antagonists of the α2β1 integrin having anti-platelet activity. It inhibits platelet aggregation by blocking collagen binding to the α2β1 integrin subunit. This integrin is involved in the formation of clots, and under pathological conditions it can participate in the formation of thrombi causing or example acute coronary syndrome. Vipegitide and vipegitide-PEG2 can be used as new therapeutic substances that, thanks to their anticoagulant effect, can be used to treat and prevent thrombotic events, particularly acute coronary syndromes [48].

Among the clinical studies that have been discontinued is the study on α-conotoxin Vc1.1. This substance was isolated from the Conus victoriae cochlear venom and is an antagonist of nicotinic cholinergic receptors of the subtype a9a10 associated with analgesia [49]. It also activates the $GABA_B$ receptor, leading to inhibition of calcium channels in

nociceptive neurons [50]. For this reason, it was considered that α-conotoxin Vc1.1 may eliminate neuropathic pain; however in the second phase of the clinical trial it was found to be ineffective in humans [51]. Despite this, research was still being carried out into its use in the treatment of neuropathic pain [52]. RgIA α-conotoxin, isolated from another cone snail species, Conus regio, was also studied. The mechanism of this toxin is similar to α-conotoxin Vc1.1, but the first one has a much stronger effect in humans.

Studies on the inhibition of neuropathic pain also concerned a toxin from snails of the species Conus marmoreus - Mr1A χ-conopeptide, on the basis of which homologous substances were produced. These substances worked by inhibiting the reuptake of noradrenaline, resulting in the elimination of neuropathic pain in animals [53]. In humans, limited dosing potential has been demonstrated due to its toxicity [54].

Irish scientists are investigating the use of a substance derived from the venom of the rattlesnake (Crotalus durissus terrificus) in cancer therapy. This substance, known as CB24, contains the composition of isolated crotoxin, a substance consisting of an inactive protein and cytotoxic phospholipase A2 [55]. Studies on the use of crotoxin against lung cancer [61] and leukemia have shown the positive effect of the toxin, which inhibits the development of the disease [62] and the reduction in tumor size [56, 57].

On the basis of compound derived from the sea anemone Stichodactyla helianthus, a substance - ShK-186 was produced, which selectively blocks the Kv1.3 subtype of voltage-activated potassium channels. Due to the potential involvement of channels in immune diseases due to T-cell activation, researchers are exploring the possible use of ShK-186 in the treatment of autoimmune diseases [58, 59]. For these diseases, scientists are also investigating the HsTX1 toxin, derived from the scorpion venom Heterometrus spinnifer, which, like ShK-186 is a blocker of voltage activated potassium channels - Kv1.3 [60]. During studies in which this compound was administered through the buccal mucosa and the respiratory tract, it was shown that the plasma concentration of substances administered by these routes reached the level necessary to maintain the therapeutic effect. Its pegylated counterpart has also been shown to be effective in the treatment of arthritis [61, 62, 63].

The cosmetic properties of venom toxins, which are used in various creams and ointments, have also been known for a long time. Substances with such properties were isolated from the secretions of the Tropidolaemus wagleri snake and the snail from the species C. consors [64, 65]. Waglerin-1 and two similar peptides derived from T. wagleri venom act in a mechanism similar to botulinum toxin, blocking nicotinic acetylcholine secretion receptors [66]. They also have a modulating effect on GABAA receptors [67]. On the other hand, the substance contained in C. consors m-CnIIIC cochlear secretions, blocks the NaV1.4 channel and nicotinic acetylcholine secreting receptors [68]. The effect of the cosmetic application of the above substances is smoothing wrinkles and skin rejuvenation.

5. SUMMARY

This review provides information on the use of animal venoms as potential substrates for the production of drugs. Substances contained in venoms through numerous mechanisms

exert influence on many tissues of the human body. The greatest impact can be observed on the nervous system and in it on the ion channel system and on the blood system and in particular on the coagulation and fibrinolysis systems. As a result of research into animal venom, substances with proven medical properties have been isolated or obtained. This led to the creation of such medicinal substances as captopril, exenatide or tirofiban, which have been used in the treatment of many diseases. Due to the large number of species that produce venom, many substances have not yet been tested. This brings great research potential. Current methods used in research on substances with therapeutic potential are developing at a blistering pace. The combination of new techniques and the potential of nature, which are animal products, such as venom, brings many possibilities. As you can see in above article, venom-based drugs have become widely used and despite the passage of time they are still useful and effective medications like for example captopril. Among the less known drugs of animal origin, we can find ziconotide an analgesic drug that is used in the treatment of chronic pain syndromes. It is an alternative for people in the terminal stages of cancer who, despite numerous symptoms of the underlying disease, are often forced to use opioids to control pain in doses that cause side effects that increase their discomfort. Further research on the properties and substances contained in animal venom will allow a deeper understanding of the mechanisms by which they affect on human organism and it is possible that they will contribute to the discovery of a remedy for autoimmune diseases or cancer.

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