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MECHANISM OF *VIPERA BERUS* VENOM ACTIVITY AND THE PRINCIPLES OF ANTIVENOM ADMINISTRATION IN TREATMENT

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ABSTRACT

In the present paper, the actual knowledge on the composition and toxic properties of the European common viper venom was analyzed. The organism response to the particular components and the methods of neutralization of venom toxin in bitten person were presented. On the basis of literature data, the clinical course of envenomation with its classification according to the severity of symptoms was characterized. In the paper the situations in which administration of antivenom is required to neutralize toxic properties of venom and its possible adverse reactions were also described.

Key words: *common European viper, viper envenomation, viper antivenom*

INTRODUCTION

There is only one representative of venomous snake families in Poland, i.e. common European viper (*Vipera berus*).

It is difficult to estimate the frequency of snakebites in Europe (1). It is assessed that the number of snakebites, especially of the representatives of the family *Viperidae* amounts to 15,000-25,000 annually (2). The majority of common European viper bites is of rather mild course. In 70-80% of patients, the bite may be accompanied by the absence of symptoms or purely local signs. Irrespective of the fact that severe envenomation occurs rarely and mortality is low, the children and elderly are the groups at high risk of complications or death (1). In Europe the mortality due to the bites of different snakes species is estimated to be 30 cases on an annual basis. The fatal cases occur especially in children, elderly and persons with cardiovascular diseases (2). The mortality due to the common European viper bites is low and accounts for less than 1%. The last fatal case due to the common European viper bite was reported in the year 2004 in Germany (3, 4, 5, 6). There are no detailed epidemiological data on the frequency of snakebites and their consequences in Poland. Nevertheless, the children and adult men are affected predominantly (2, 6).

Given its therapeutic properties, the common European viper venom has been used for a long time in the folk medicine and is still used in the medicine (7, 8). It constitutes the mixture including many components which provide analgesic and ant-inflammatory effects, decrease the coagulability of blood and play regenerative function in therapies following the injuries, contusions and haematoma. It is also used as the calefacient, the agent which reduces the muscle tension, in the prophylaxis of rheumatic disorders, degenerative joint diseases and inflammation of human musculoskeletal system. The properties of viper venom give the possibilities to use it in the supportive treatment of particular cardiovascular diseases and haematologic disorders (7, 9).

Apart from its therapeutic properties, the viper venom is also used in cosmetology, in "botox-like"cosmetic products that provide skin smoothing effects by relaxing and blocking the mimic muscles.

VENOM CHEMICAL COMPONENTS AND THEIR EFFECTS

The viper venom is a yellowish liquid containing an array of 25 proteins and peptides acting as enzymes and ligands which various effects result in immobilizing the "victim" and initial digestion of tissues surrounding the bite site (1, 2, 5, 7, 10, 11).

The European common viper venom is of haemolytic, proteolytic and cytotoxic activity contributing to the homeostatic imbalance (2, 7, 12, 13, 14, 15). The compounds present in the venom are phospholipases (including phospholipase A_2), hyaluronidases, toxic polypeptides, hydrolases of peptides (including metalloproteases), amino acids and carbohydrates (1, 2, 5, 11, 13, 16). Several of the venom components lead to the release of histamine, bradykinin, prostaglandins and serotonin (7, 13, 16).

The components present in the viper venom such as the hydrolases of peptides, hyaluronidases, phospholipases and proteases provide cytotoxic effects, lead to homeostatic imbalance, cause oedema, hypovolemia and extravasation of plasma to extravascular space as the result of endothelium damage. Apart from disrupting the endothelium, metalloproteases digest also the extracellular matrix elements (1, 2, 9).

Phospholipase A_2 is the mediator of inflammation process (2, 9, 15). It may have highly cytotoxic effect on plasma membranes and organelles in proper concentrations. As a consequence, it contributes to the paralysis of skeletal muscles, nerve terminals and endothelium damage. Thus, it disrupts the blood morphotic elements (erytrocytes, leukocytes, thrombocytes). As the result of erytrocytes membranes phospholipids disruption, intravascular haemolysis is observed (10, 12, 14, 16, 17, 18). It dissolves the platelet-activating factor (PAF) and activates the release of arachidonic acid from membrane phospholipids which mediates the inflammation process (2).

The hyaluronidase, which dissolves the hyaluronic acid, contributes to the decrease of tissues cohesion at the bite site. As the consequence, the tissues permeability and the level of venom absorption increase (2, 9, 14, 17, 19). Furthermore, hyaluronidases are responsible for local oedema, blisters, extravasation and necrosis (12, 15). After penetration to the cardiovascular system, the toxic polypeptides affect kidneys, lungs, heart (12).

SYMPTOMS AND CLINICAL COURSE OF ENVENOMATION

The viper venom spreads from the site bite to the systemic circulation via blood and lymph, contributing to local and then to systemic effects (15, 16). Its maximum concentration in plasma is observed from 0.5 to 4 hours after the bite and its biological half-life is estimated at 6-16 hours (3).

At the site of viper bite, the venom teeth marks are present. The bite is accompanied by pain, painful swelling that spreads quickly, sensation of tingling, tenderness, bruising at the bite site (the result of venom haemolytic activity), enlargement of regional lymph nodes. Sometimes petechiae and blisters containing serous exudate are noticeable. The gastrointestinal disorders, including abdominal pain, diarrhoea, nausea and vomiting are common. Simultaneously the general symptoms may appear, e.g. anxiety of different level, fever and coexisting increase of respiratory rate. The increased perspiration, thirst and from the central nervous system: sleepiness and confusion can occur (5, 6, 10, 11, 12, 16, 17, 20).

As the result of envenomation, an array of processes is activated. The proteinases are released and the proinflammatory cytokines are induced (TNF-α, IL-1, IL-6, IL-10, IFN-γ). In the presence of cytokines, decreased blood flow in organs is observed which may be accompanied by the disseminated intravascular coagulation (DIC) and vasculitis. As the result, the organs necrosis appears. Furthermore, the immunological response contributes to the synthesis of immunoglobulins. Then the kidneys are affected, which is due to the haemolysis (5, 9).

It is assumed that the venom may activate the complement in an alternative pathway which can cause the angiooedema (2, 6, 13, 21). C3 convertase is responsible for the cleavage of C3 human protein, element of complement system to C3a and C3b polypeptides. The C3b element may attach to the antigen membrane and function as opsonin (which enhances the phagocytosis) or create the complex with C3 convertase. As the result, the C5 convertase is formed which catalyzes the formation of C5a and C5b. These proteins participate in the early phases of inflammation process (22). Inflammation in the area of bite site may enhance the processes of distribution of toxic venom elements by the cells. This effect may be strengthened by the hyaluronidase (9, 23).

The thrombin like compounds activate the complement and contribute to the hemostasis imbalance. On the one hand they lead to fibrinolysis and on the other hand - contribute to the blood clot formation (20).

The coagulation disorders may result from pro- and anticoagulant effects on activity of V factor (1, 7, 9, 13, 16). In the presence of low venom volume-concentration, the coagulability of blood increases while in high concentration – the coagulability is decreased. It corresponds to the simultaneous changes of prothrombin time and APTT (activated partial thromboplastin time). In the laboratory findings, the leukocytosis and neutrophilia are often reported (2, 6, 24). Due to its effect on the erytrocytes membrane, the viper venom causes the decrease of sedimentation and thus the increase of haematocrit. Then the anaemia is observed as the result of haemolysis and extravasation of plasma to extravascular space (2, 9, 12, 15, 21). It may lead to the changes in erytrocytes shape, their ability to roll disappears and the osmotic resistance declines (13). The thrombocytopenia,

increase of creatine kinase, aminotransferases, urea and creatinine rarely occur (3, 10, 12, 15, 17).

The interaction of haemoragin, elements of antiplatelet and anticoagulant activity may contribute to the occurrence of disseminated intravascular coagulation (15, 16).

The increased permeability of the capillaries contributes to the shock, pulmonary oedema, renal insufficiency (9, 12). For the acute renal insufficiency are responsible, i.a. rhabdomyolysis and myoglobinuria which may be present rarely, especially in children (2, 9, 10, 14, 16, 25). The intravascular haemolysis, which is responsible for kidney impairment, lead to the formation of micro-clots in kidney, causing the acute necrosis of renal tubules (9).

In the envenomation of more severe course, the disorders of cardiovascular system characterized by electrocardiographic abnormalities (of which the most prevalent are: inversion of T wave, changes of ST segment), atrioventricular block of I and II degree, atrial fibrillation and cardiac muscle disorders may appear (10, 12, 21, 25).

The haemorrhagic oedema may lead to the decrease of blood pressure, as well as the shock may be present. In children (due to the low body mass) the symptoms of respiratory and cardiovascular systems may be more escalated. Thus, the course of envenomation is more severe, including the loss of consciousness and even death (3, 9, 12, 14, 16, 17, 20, 25).

Given the symptoms intensity, the severity of envenomation is classified into four-grade scale: grade 0 (G,-0) - "dry" bite without envenomation, the presence of venom teeth marks, G-1 – envenomation of mild course, only local oedema is present with the peak observed after 1-2 days, G-2 – moderate envenomation accompanied by regional swelling of limb and poorly noticeable general symptoms (moderate vomiting, abdominal pain, diarrhoea, moderate BP fluctuation, tachycardia, hypotonia), G-3 – severe envenomation characterized by extensive oedema spreading proximally and the presence of life-threatening general symptoms (significant or prolonged hypotonia, haemorrhagic shock) (3, 16, 21, 26). It is estimated that in approximately 10%-30% of cases, the severe envenomation is present. In about 80% of cases, G-0 or G-1 appear and 10%-20% envenomations are of no clinical significance (4, 5, 13, 14, 20).

The severity of envenomation is dependent on the volume of venom injected related to the body mass. Furthermore, the factors that should be considered are also health status and condition of patient at the moment of bite (fatigue, stress, potential coexisting disease, advanced age), physical activity following the bite and allergy to venom components (2, 3, 4, 8, 11, 15, 16, 19, 21). The early occurring symptoms such as oedema, gastrointestinal symptoms, hypotonia and hyperleukocytosis are indicative of severe envenomation (15, 24). Worth mentioning is the fact that in children more often the severe envenomation and possibility of shock occurrence within 16 hours after the bite appear, even in the absence of local symptoms (21). However, the recovery is observed earlier in children than adults (13).

In persons allergic to specific venom elements, apart from the direct toxic effects, the venom may induce anaphylactic reactions dependent on IgE antibodies (2, 15, 16, 18, 21). The allergic reactions may be of different intensity from nettle-rash, larynx swelling to anaphylaxis. It is referred especially to persons bitten several times (13, 18).

The common European viper venom does not provide, contrary to the venom of tropical snakes and several vipers, substantial neurotoxic effect (3, 15).

This venom is metabolized in the liver and then eliminated from the organism via kidneys with urine and via gastrointestinal tract with faeces (20).

ANTIVENOM ADMINISTRATION IN TREATMENT

The appropriate manner of venom neutralization in the organism of bitten person is antivenom administration. The antivenom manufacture consists in the immunization of animals, usually large ruminants, mainly horses with small doses of venom or the venom mixtures from single or various viper species. Then, the immunological serum is extracted (22). Given the fractionation type of active substance, whole IgG, F(ab') fragments and monovalent Fab are obtained (25, 27, 28). Antivenoms may be of liquid or more stable form, i.e. freeze-dried (28). Some producers have implemented the modification in processes, e.g. chromatography and pasteurization in order to improve the purity and/or viral safety. The WHO guideline on antivenoms production and control presents the recommendations aiming at standardization of manufacture and quality control (25, 28). The common efforts undertaken in the fields of research and development as well as the improvement of manufacture techniques are required to meet these recommendations (22, 25).

Apart from the traditional methods (antivenoms obtained from animal immunological serum), the alternative or complement methods are applied worldwide (1, 29, 30), including therapy of ovine-derived antibodies (CroFab), which causes significantly fewer allergic reactions in relation to the standard antivenoms (1, 16, 31) and treatment with purified polyclonal antibodies derived from chicken egg yolks of immunized hens (32). Furthermore, mainly in the folk medicine, the natural venom inhibitors, including the Brazilian plants extracts, compounds obtained from rodents and exotic sea

creatures and electroconvulsive therapy are employed (33). Each of the aforesaid treatment method has both advantages and disadvantages. The major advantage of alternative methods is minimization of the adverse reactions occurrence (mainly the allergic reactions) with simultaneous reduction of costs. But its efficacy is disputable. However, the methods employing the animals raise concerns of economic and ethic nature with the simultaneous risk of anaphylactic reaction occurrence (1). It should be noted that new technological methods may significantly improve the quality and safety of antivenom administration (22, 30, 34, 35).

The drug of choice, which is registered and used in our country, is antivenom of *Vipera berus* venom produced in BIOMED® Sera and Vaccines Manufacturing Company in Warsaw (2, 20). In the case of antivenom depletion or temporary deficiency, the Ministry of Health authorizes the temporary launch of immunological serum imported from other countries (Russia, Croatia, Romania). Such a situation was reported in 2011 when the transport of antivenom of viper venom produced by Microgen Institute of Immunology Inc. HR-10000 Zagreb Croatia was permitted.

In Poland, product of BIOMED® is available since 1938. Initially, it was produced by the Department of Sera and Vaccines of National Institute of Hygiene at the level of native serum and functioned as "the serum against viper venom". Since 1958, the manufacture was transferred to BIOMED® Sera and Vaccines Manufacturing Company in Warsaw, where the product was still produced under the name "serum". Since the years 60. of the XX century, the new stages of purification were introduced and the manufacture of "antivenom of viper venom" was commenced. The demand for antivenoms in the country is seasonal (dependent on the activity of vipers) and ranges from 2,500-3,500 (to 4,000) packages/year. The product is exported to Ukraine, Slovakia and the Czech Republic (36).

The product is derived from the serum of horses immunized with the venom of the common European viper. The specific equine IgG binds and neutralizes toxic activities of venom by specific bond: antibody (antivenom) – antigen (viper venom). Then, it enhances its redistribution from tissues and elimination from organism (2, 12).

In the case of the appearance of reactions to the venom (pain, increasing oedema), the serum should be administered to neutralize the venom. Thus, it blocks or reduces the possibility of severe envenomation (5, 10, 12, 21, 29, 31). The administration of antivenom provides the improvement in the terms of early symptoms and reduces also the frequency of late symptoms (4, 6, 15). The treatment with antivenom may be followed by the occurrence of adverse reactions, especially allergic reactions. Thus, it should be administered with the method of gradual dilution after the glucocorticoids test in hospital settings or in the centre supplied with anti-shock equipment with simultaneous monitoring of cardiovascular and respiratory systems function (4, 6, 15).

The antivenom of viper venom is the only effective antidote to the venom (12, 14). Its usage is mainly recommended when the following conditions are present: progressing oedema of limb is observed with the risk of trunk involvement, hemostasis imbalance, haemodynamic instability, disorders of consciousness, oedema of mucous membranes leading to the risk of respiratory track obturation, prolonged or recurrent gastrointestinal disorders as well as in the case of bites in children (even in the case of low-grade bites) and pregnant women (13, 15, 17, 21).

The persons who are bitten by the viper should be immediately administered the serum in the dosage of 500 units (1 vial). The antivenom is administered intramuscularly in larger doses (20 ml) when the person is bitten by several vipers or the hours passed since the moment of bite. The antivenom may be applied intravenously only when the patient life is in danger. In such the case the antivenom should be administered slowly (10 ml), but it should follow the previous intramuscular injection (20ml) after at least 1 hour (20). However, it should be noted that the antivenom which is available in Poland is not indicated to be routinely carried out in such way (3). The injection of antivenom should be repeated after several or dozen hours if the patient clinical status required that (2, 16). However, according to some reports, such procedure is not justified as the venom volume does not increase in the patient's organism and successive administration of serum may contribute to the increased risk of serum disease and its sequelae occurrence (20).

Given the xenogen protein present in the product, the anaphylactic reactions, including shock may appear after antivenom administration. The initial anaphylactic reactions are present within 10-180 minutes since injection. The following symptoms may be present: fever, itching, nettle-rash, dry cough, nausea, vomiting, colic, diarrhoea, tachycardia. In some patients the life-threatening symptoms such as bronchial spasm, hypotension, angiooedema (2, 7, 12, 15, 16, 17, 37) may occur.

Possible pyrogenic reactions are present within 1-2 hours after antivenom administration (12).

Late adverse reactions appear on average 7 days after the end of treatment, including fever, lymph nodes enlargement, nausea, diarrhoea, itching, recurrent nettle-rush, muscle and joints pain, nephritis. Furthermore, the immunologic complexes occurring in the course of anaphylactic reaction, given they are not degradated in the liver and eliminated by the kidney, may be deposited in

tissues and initiate the chronic inflammation processes of autoimmunological nature (12).

The serum disease should be also classified to the possible adverse reactions which may occur even 7-20 days after antivenom administration. The symptoms include oedema at the site of injection, enlargement of lymph nodes, fever, joints swelling, nettle-rash and in severe cases also kidney damage, neuritis (2, 7, 12, 14, 15, 17).

SUMMARY AND CONCLUSIONS

The toxic effects of viper venom activity can be life-threatening, especially in allergy sufferers and children.

The common European viper biochemical composition is diverse, i.e. constitutes a mixture of peptydes, proteins and enzymatic proteins. They are responsible for the complex response of organism of bitten person at the local (oedema) and systemic level (disorders of cardiovascular, respiratory, urinary, nervous system).

The immunological response induced as the results of contact with antigen (venom) is of complex nature (synthesis of immunoglobulins and proinflammatory cytokines, activation of complement in alternative pathway).

The effective treatment should consist in neutralization of venom components due to the safe administration of antivenom after considering the benefits and potential adverse reactions resulting from application of xenogen protein. However, it should be emphasized that antivenom of viper venom is the only effective antidote to the venom.

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