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THE THERAPEUTIC ACTION OF THE LEECH *Hirudo medicinalis*

©Tilyakhodjaeva G., *ORCID: 0000-0002-6891-3530, Ferghana Medical Institute of Public Health, Ferghana, Uzbekistan, gul.tilyakhodjaeva@gmail.com*

ЛЕЧЕБНОЕ ДЕЙСТВИЕ ПИЯВКИ *Hirudo medicinalis*

©Тиляходжаева Г. Б., *ORCID: 0000-0002-6891-3530, Ферганский медицинский институт общественного здоровья, г. Фергана, Узбекистан, gul.tilyakhodjaeva@gmail.com*

Abstract. The article under discussion examines the mechanism of the therapeutic effect of the medicinal leech *Hirudo medicinalis*. Today, studying the composition of the saliva of medicinal leeches is of the greatest relevance. The secretion of leech saliva contains a whole complex of biologically active substances — it contains more than a hundred medicinal components, enzymes, hormones, vitamins and amino acids, as well as a special substance hirudin, which makes the blood more fluid, which prevents the formation of unnecessary platelets. This is an indispensable way to prevent heart attacks, strokes and thrombosis. The author of the article believes that proof of the medicinal properties of specific components of saliva can lead to the development of new drugs or therapeutic methods.

Аннотация. Рассматривается механизм лечебного действия медицинской пиявки *Hirudo medicinalis*. На сегодняшний день изучение состава слюны медицинских пиявок представляет наибольшую актуальность. В секрете слюны пиявки содержится целый комплекс биологически активных веществ — более сотни лекарственных компонентов, ферментов, гормонов, витаминов и аминокислот, а также особое вещество гирудин, делающее кровь более жидкой, что предотвращает образование ненужных тромбоцитов. Это незаменимый способ профилактики инфарктов, инсультов и тромбозов. Автор статьи считает, что доказательство лечебных свойств конкретных компонентов слюны может привести к разработке новых лекарственных препаратов или терапевтических методов.

Keywords: biologically active substances, *Hirudo medicinalis*, hirudin, destabilase, orgelase, antistasin, decorzin, viburnum, eglin.

Ключевые слова: биологически активные вещества, медицинская пиявка, гирудин, дестабилаза, оргелаза, антистазин, декорзин, калин, эглин.

Treatment with medicinal leech Hirudo medicinalis is one of the oldest methods. Some of the first mentions of this procedure are found in Hippocrates. Research has shown that the healing effect of the leech is largely due to the unique chemical composition of its saliva. This saliva, the secretion of special glands, contains more than a hundred useful components, including hormones, enzymes, vitamins and amino acids. Many supporters of traditional medicine and hydrotherapy claim that this method has its advantages, such as improved microcirculation, antithrombotic effects, anti-inflammatory properties and pain relief [8–11].

Medical leech (*Hirudo medicinalis*) – a species of ringworms from the subclass of leeches (Hirudinea), most often used in Europe for medical purposes (in America, Asia and Africa other species of leeches are more often used). A parasite feeding on human and animal blood, the useful

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properties of which are known to people since ancient times [3, 4]. In its wild form, the medicinal leech is almost ubiquitous in Europe, although its numbers have been reduced in many regions due to industrial fishing in the past, draining of marshes and water pollution [5, 7].

The medicinal leech has a rounded, dorsally flattened body with two suction cups at the posterior and anterior ends, with a mouth opening in the center of the anterior suction cup. The animal stalks its prey while in the water, attached to underwater plants or snags. For one feeding hungry leech weighing 1.5-2 g is able to suck up to 15 ml of blood at one time, increasing in this case 7-9 times in mass [6].

Sucked blood is stored in the stomach in a liquid state for months, not curdling, and live up to two years. To digest the absorbed blood and keep it in liquid form leech helps being in its intestines bacteria-symbionts Aeromonas hydrophila. They also help it to cope with foreign bacteria which can get into a stomach together with blood of the sick animal.

In Uzbekistan medicine leeches are used in live form in treatment of many diseases: varicose veins, haemorrhoids, wounds, trophic ulcers etc., in Europe and the USA — mainly in micro- and plastic surgery for removal of venous stasis in transplanted tissues. Extracts of medical leech and preparations on their basis, extract of salivary glands of medical leech are also used. In recent years recombinant preparations of leech proteins (hirudin, hirudostasin, bdellastasin, etc.) have been created and even attempts to construct an artificial leech have been made.

How does a leech heal? Using its three jaws, it bites through the patient's skin to a depth of 1.5 mm and sucks out 5 to 15 ml of blood. Approximately the same volume of blood flows from the bite site over the next few hours. In one session of hydrotherapy, 5-7 leeches are usually used.

The therapeutic effect of using leeches is threefold. Firstly, leeches have a mechanical effect as a method of therapeutic bloodletting. Thanks to the loss of a certain amount of blood, which is completely tolerated by patients, the regional blood flow is unloaded. This frees blood vessels, normalizes blood pressure and has a general beneficial effect on the body. Secondly, the use of leeches has a reflex effect comparable to the effects of acupuncture. This effect occurs because the leech, as already mentioned, bites the skin only at biologically active (acupuncture) points, which are approached by the largest number of nerve endings associated with various organs and systems of the body. Finally, thirdly, the biological effect of leeches is very important, caused by the body’s reaction to the secretion of the salivary glands, which the leech injects into the human blood. The secretion contains more than 100 biologically active components with healing effects.

Biologically active substances that make up the secretion of the leech’s salivary glands disinfect the leech’s blood and saliva. But these substances are also indispensable for humans: they improve some properties of the blood and affect blood flow and the walls of blood vessels. Of course, a leech by nature takes care of itself, and not of a person: it protects itself from pathogenic flora, improves blood quality, makes blood vessels easier to bite through and changes the nature of blood flow in order to consume more blood without much effort. All this is very important for a leech, but it also helps a sick person get rid of many problems.

According to the nature of the effect on the human body, the components of leech secretion are divided into three main groups. The former affect human immunity and pathogenic microflora, and, therefore, have anti-inflammatory, bacteriostatic and immunostimulating effects. The second group of enzymes, acting on the walls of blood vessels, has anti-atherosclerotic and anti-ischemic effects. Finally, enzymes of the third group, which influence the movement of blood and lymph, are useful to the patient due to their hypotensive and lymphatic effects.

The most important components of the leech salivary gland secretion extract include functional proteins that are unusual in their properties: hirudin, pseudohirudin, destabilase, orgelase, antistasin, decorsin, viburnum, eglin and some other compounds. At the same time, hirudin,

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destabilase and orgelase are considered first-order components, and antistasin, decorsin, viburnum and others are considered second-order.

The most important and absolutely irreplaceable substance for humans in the leech secretion is hirudin.

In 1884, a substance extracted from leeches that slows blood clotting was first isolated from leech extract by J. B. Haycraft and subsequently named hirudin. Before the discovery of heparin, extracts from the head part of leeches were widely used as an anticoagulant. In the 1940s, A. V. Kirsanov and M. N. Bystritskaya developed a preparation of raw hirudin. A method of fractionating the extract from the head part of the medicinal leech was used by F. Markwardt to isolate pure hirudin [5].

Hirudin, being a specific inhibitor of the thrombin enzyme, forms a strong non-covalent stoichiometric complex with thrombin. This enzyme has high specificity for thrombin and differs from other natural inhibitors of this enzyme, such as antithrombin III, heparin and α2-macroglobulin. Compared to a number of synthetic thrombin inhibitors, hirudin is considered an ideal inhibitor of this enzyme.

In addition to inhibiting thrombin activity, hirudin also slows down the reaction of thrombin activation of coagulation factors V, VIII and XIII. It prevents the release and aggregation of platelets, and also causes the dissociation of the thrombin complex with specific receptor proteins on platelets. Oxidation of disulfide bonds leads to loss of antithrombin activity of hirudin. Chemical modification of the free carboxyl groups in hirudin reduces its affinity for thrombin, indicating ionic interactions between the molecules during complexation of hirudin with thrombin [2].

When hirudin is isolated from whole medicinal leeches, it is accompanied by an inactive component from the bodies of leeches, called pseudohirudin. Unlike hirudin, which contains isoleucine at the N-terminus, pseudohirudin contains valine at the N-terminus.

The amino acid composition of pseudohirudin is somewhat different from hirudin. Hirudin is characterized by a higher content of aspartic and glutamic acids, lysine, isoleucine and tyrosine. The cysteine content in pseudohirudin is 3 times lower than in hirudin.

Scientifically known as a hirudinoid, pseudohirudin is a man-made analogue of hirudin, a thrombin inhibitor derived from leeches. Pseudohirudin has some similar properties, but it is synthetic and intended for use in medical applications. Like hirudin, pseudohirudin is a thrombin inhibitor. It forms a complex with thrombin, preventing blood clotting. Pseudohirudin, like hirudin, has high specificity for thrombin, making it an effective inhibitor of this enzyme. Pseudohirudin, like hirudin, is able to maintain antithrombotic properties, preventing the formation of blood clots. Studying pseudohirudin can also be used to better understand the molecular interactions between inhibitors and thrombin, which in turn could help in the development of new antithrombotic drugs [1].

Destabilase e-(g-Glu)-Lys isopeptidase was first discovered in the secretion of the salivary glands of Hirudo medicinalis in 1986. The enzyme carries out its fibrinolytic (thrombolytic) activity through the hydrolysis of isopeptide bonds formed during the stabilization of fibrin in the presence of blood coagulation factor XIII, causing an unconventional mechanism of fibrinolysis.

Destabilase is capable of forming aggregates, which, thanks to the lipid component, can change their spatial orientation. This is supported by the fact that the destabilase exhibits its properties (i.e., hydrolysis of isopeptide bonds) in both aqueous and organic solvents. Destabilase aggregates formed in solution acquire the properties of a micelle, capable of changing its spatial orientation, depending on the physicochemical properties of the solvent, exposing either the hydrophilic or hydrophobic parts of its structure.

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However, the antithrombotic potential of destabilase is difficult to explain solely by the blockade of platelet aggregation caused by a prostacyclin analogue, the lipid component of destabilase. When analyzing the effect of destabilase on blood coagulation parameters, it was shown that in its presence the thrombin time and the time of recalcification of blood plasma are significantly prolonged. It is natural to assume that such an effect is provided by hirudin and the blood plasma kallikrein inhibitor, which were found in destabilase preparations [2].

Destabilase is a fairly strong complex containing destabilase and prostaglandin components, hirudin and a plasma kallikrein inhibitor, which can be called the “destabilase complex”. The strength of this complex is evidenced by the fact that it cannot be destroyed by common methods of biochemistry. Naturally, the preventive antithrombotic effect of destabilase is due to both the blockade of the internal blood coagulation mechanism (inhibition of platelet adhesion and aggregation and the activity of plasma kallikrein) and the antithrombin activity of hirudin.

The penetration of the destabilase complex into the blood is carried out by two mechanisms: conventional transport through intercellular contacts (passive transfer) and transmembrane (active transfer) transport, i.e. through the cell membrane due to integration into the membrane structure. And this is possible for such a high-molecular complex only if it has the properties of a liposome. The ability of the destabilase complex to change its spatial orientation depending on the nature of the solvent is clearly demonstrated by analyzing the activities of the components of the complex during the transition from the aqueous phase to the organic phase and vice versa. In the aqueous phase, all components of the destabilase complex exhibit their activity, while in ethyl acetate only the activity of destabilase (amidase) and prostaglandin (blockade of platelet aggregation); When the complex is transferred back into the aqueous phase, all components exhibit their activity. Thus, the ability of destabilase to aggregate into micelles, as well as to bind hirudin and the kallikrein inhibitor, provides the destabilase complex with the properties and structure of a liposome. All hirudin and kallikrein inhibitor in blood plasma are in a bound state, i.e. in the composition of the liposome, and only in the bacterium-symbiont of leeches these substances are in a free state [6].

Thus, destabilase, which is a strong protein-lipid complex, has a high aggregation ability. As a result of aggregation of destabilase monomers, a micelle is formed that is capable of changing its spatial orientation depending on the nature of the solvent or contacting substrate, exposing either the hydrophilic or hydrophobic parts of its structure. As a result of contact with blood, the micellar structure of destabilase binds free hirudin and the kallikrein inhibitor of blood plasma, forming a liposome, which in aqueous solvents exhibits the activity of all components of DC (i.e. destabilase, an analogue of prostacyclin, hirudin and IC), while in organic solvents it demonstrates activity only of destabilase and prostacyclin analogue. The monomeric form of the liposome is the DC fraction with a MW of 25 kD [1].

Eglins were first identified in commercial hirudin preparations together with bdellins. Eglins are a group of polypeptides with a molecular weight of 6600 to 6800 Da. They have the ability to inhibit α-chymotrypsin, subtilisin and neutral proteases of human granulocytes, such as elastase and cathepsin G. Eglins form strong complexes with these proteases, and their dissociation constants are approximately (2-3) × 10-10 M [5].

Eglins have been successfully obtained in pure form, and their composition and physicochemical properties have been well studied. Eglin C, for example, has a primary structure of 70 amino acid residues. One of the features of these proteins is the absence of disulfide bonds and methionine, isoleucine and tryptophan residues. This highlights their unique structure among proteins, which may have important implications for their functionality and potential medical applications.

It is important to note that research into eglins and other components of leech saliva is ongoing, and scientists continue to explore their potential applications, including in medicine and biotechnology.

Conclusion

Thus, the biologically active substances of leeches exhibit an antithrombotic effect, blocking various parts of the internal blood coagulation mechanism and preventing the formation of blood clots. In addition, they have a thrombolytic effect, especially on old fibrin clots, with a possible effect on newly formed blood clots. A normotensive effect of medicinal leech saliva associated with low molecular weight substances of prostaglandin nature is of great importance. Leech saliva has a regenerative effect on damaged vessels, helping to restore the atrombogenic surface. It exhibits an antiatherogenic effect, interfering with lipid metabolism and reducing the level of cholesterol and triglycerides in the blood. An immunostimulating effect activating the compliment system and increasing the phagocytic activity of the blood, which contributes to the anti-inflammatory effect, has been also manifested.

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