A Systematic Overview of the Medicinal Importance of Sanguivorous Leeches

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Abstract

Leeches are a class of segmented invertebrates, known for their blood-feeding habits and used in phlebotomy to treat various ailments since antiquity. In Europe, medicinal leeches have recently been rediscovered and are used by maxillofacial and other microsurgeons to aid salvage of compromised venous engorged tissue and amputations, such as digits, ears, and nasal tips. Because of their important salivary components, blood-sucking (sanguivorous) leeches, such as *Hirudo medicinalis* and related species, have engendered great interest from pharmaceutical companies searching for anticoagulants to prevent blood clotting during microsurgeries. Scientific research reveals that the beneficial effects of leeching, in addition to decongestion, include injection of a cocktail of several medicinally useful bioactive molecules present in their saliva. Owing to its therapeutic potential, the research is continuing as many new salivary compounds are being isolated and synthesized.

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Introduction

Leeches are sanguivorous or blood-sucking annelid worms with ability to extend or contract their bodies. Table 1 describes leech taxonomy. Since antiquity, they have been known for their blood-feeding habits and use in the art of phlebotomy or bloodletting. Although this practice fell into disrepute by the end of the 19th century, it always occupied an important place in the Unani system of medicine to treat various ailments. Most leeches are permanent or temporary external parasites, attaching themselves to the host and sucking blood. Sucked blood is stored in lateral diverticula of the crop and, as the blood passes down the pharynx, it is mixed with a glandular secretion that prevents its coagulation. At a single meal a leech ingests several times its own weight of blood that may suffice for several months.[1,2] Recent scientific research on salivary components has restored its lost reputation.

Hirudo medicinalis is the most frequently used species of leech (Figure 1) that is not native to the Indian subcontinent. In India the species used traditionally for therapeutic purposes is *Hirudinaria granulosa*. Besides these, *Macrobdella decora* (American medicinal leech), *Hirudo michaelseni*, *Hirudo nipponia*, *Hirudo verbena*, and *Hirudo orientalis*[5-9] are also being used for therapeutic purposes (Figure 2).

Bioactive Ingredients of the Leech Body and Saliva

As mentioned previously, scientific research reveals the beneficial effect of leeching occurs via blood decongestion and injection of a cocktail of several medicinally useful bioactive molecules present in their saliva.[10-12]

Hirudin

In 1950, Fritz Marquardt of Germany isolated a protein from *Hirudo medicinalis* that he termed hirudin.[13-15] He also demonstrated its thrombin inhibitor effect. As a heparin-like substance, it is the most potent known natural inhibitor of thrombin. Due to its high affinity for thrombin, hirudin inhibits almost all the physiological actions of thrombin. It does not cross-react *in vitro* with antibodies from patients with heparin-induced thrombocytopenia. In fact, its administration has exhibited no side effects, including effects on platelets. A thrombin inhibitor similar to hirudin, known as bufrudin, has been isolated from the leech *Hirudo manillensis*, which differs in its structural and

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Table 1. Current Leech Taxonomy[3,4]

Class	Hirudinia		
Subclass	Euhirudinea		
Order	Rhyncobdellida	Arhyncobdellida	
Suborder		Erpobdelliformes	Hirudiniformes
Family	Glossiphoniidae	Erpobdellidae	Haemadipsidae
	Piscicolidae	Salifidae	Haemopidae
	Ozobranchidae		Cylicobdellidae
			Hirudinidae*

*Medicinal leeches fall under the family Hirudinidae.[4]

immunological properties.[15] Hirudins belonging to different leech species, Hirudo and Hirudinaria, are closely similar molecules and probably evolved from a common ancestral gene.[16-18] Application of hirudin or derivatives may be indicated for prophylaxis and treatment of postoperative venous thrombosis, especially in cardiac surgery; enhancement of fibrinolytic therapy and/or angioplasty to prevent reocclusion; and plastic surgery. Hirudin may be a useful alternative anticoagulant, particularly in patients sensitized to heparin or in patients with hereditary or acquired antithrombin III deficiency.[19] Due to its great demand, various recombinant systems have been developed, using bacteria, yeasts, and higher eukaryotes to obtain significant quantities of biologically active hirudin.[20]

Recombinant hirudins are currently undergoing clinical trials in deep venous thrombosis and acute coronary syndromes and as an adjuvant to thrombolysis in myocardial infarction.[21,22,23]

Among all cardiovascular diseases, thrombosis highlights the limitations of existing antithrombotic drugs.[24] Strokes and heart attacks are usually caused by a thrombus occlusion of cerebral or coronary arteries, respectively.[25] Since the common antithrombotic agents used in therapy (heparin, aspirin, coumarin derivatives) do not always have the desired effectiveness or cause complications,[26-30] hirudin and its analogs show promise as novel therapeutic agents[24,31,32] and are excreted in unchanged form in the urine.[33]

Hyaluronidase

Hyaluronidase is a spreading or diffusing substance that modifies the permeability of connective tissue through the hydrolysis of endoglucoronidic linkages of hyaluronic acid – a polysaccharide found in the intercellular ground substance of connective tissue.[34] The leech enzyme,

Figure 1. *Hirudo medicinalis*

Figure 2. Leeches Being Used Medicinally

although a P-glucuronidase, does not act on chondroitin or chondroitin sulfates A and C, which contain glucuronic acid but differ in the amino sugar from hyaluronate. Hyaluronidase from the leech, therefore, is the most specific enzyme known for identification of hyaluronic acid.[35] It reduces the viscosity and renders the tissues more readily permeable to injected fluids,[36,37] increasing the speed of absorption. This promotes resorption of excess fluids and extravasated blood in the tissues and increases the effectiveness of local anesthesia. Hence, hyaluronidase from leech saliva helps increase the spread of all salivary secretions. Currently, it is being examined in *ex vivo* studies for drug delivery through human skin.[38] It has also been investigated as an additive to chemotherapeutic drugs for augmentation of the anticancer effect.[39-42]

Calin

Calin has a rapid (1-10 min.) effect on collagen that is reflected in its ability to suppress collagen-induced platelet aggregation as well as adhesion of platelets to collagen coated micro-carrier beads. Besides inhibition of the direct platelet collagen interaction,[43] calin also interferes with von-Willebrand factor collagen binding,[44] which is believed to be one of the initiative events for thrombus formation at sites of damaged endothelium. Interference with this mechanism may provide an antithrombotic potential. A pharmacological preparation of calin was tested *in vitro* and *in vivo* for this activity in a thrombosis model in hamsters. Calin specifically and dose dependently (IC_{50} 6.5 to 13 μg/mL) inhibited platelet aggregation induced by collagen.[45]

Destabilase

Destabilase possesses glycosidase activity. Destabilase lysozyme is the first invertebrate lysozyme with combined enzymatic and non-enzymatic antibacterial action,[46] and it also dissolves blood clots. Thrombolysis occurs by the selective hydrolysis of isopeptide bonds of stabilized fibrin. Under intravenous injection, partially purified destabilase preparations exhibited thrombolytic properties, and thrombi formed in the rat were lysed by 75- and 100 percent in 67 and 137 hrs after intravenous injection of destabilase, respectively.[47,48] Thrombolytic therapy with streptokinase and tissue plasminogen activator is often complicated by repeated thrombi, which are not seen with leech therapy given for thrombophlebitis.[49-51]

Apyrase

Apyrase (adenosine 5'- diphosphate diphosphohydrolase) is a nonspecific inhibitor of platelet aggregation by virtue of its action on adenosine 5' diphosphate, arachidonic acid, platelet-activating factor (PAF), and epinephrine. Two apyrases (isoenzymes) have been isolated from the saliva of medicinal leeches.[52,53]

Eglin

Eglins (elastase-cathepsin G leech inhibitors) are small proteins present in *Hirudo medicinalis* that have strong inhibitory activity against chymotrypsin and subtilisin-like serine proteinases acting on non-cationic substrates.[54,55] One leech contains approximately 20 μg of eglin.[54] Eglin-like serine proteinase inhibitors have been isolated from the leeches *Hirudo medicinalis*[54,56] and *Hirudinaria manillensis*.[17] It is an inhibitor of alpha-chymotrypsin, subtilisin, chymosin, granulocyte proteinases, elastase, and cathepsin G. Eglin c is a potential therapeutic agent for the treatment of diseases associated with inflammation and has been proven effective for the treatment of shock and

emphysema in experimental models. Eglin c is well tolerated, with no significant effects on the cardiovascular and central nervous systems, basic metabolism, clotting, fibrinolysis, or complement. The main safety concern, however, relates to the allergenic potential of the inhibitor.[18,54,57-60]

Considering the role played by elastase in the process of leucocyte infiltration and accumulation in inflamed microvessels, eglin c could be used to prevent neutrophil infiltration (adhesion, penetration, and migration) into inflamed vessels and neutrophil-mediated injury to the microvascular endothelium. Eglin c significantly attenuates these processes *in vivo*, suggesting this serine proteinase inhibitor may be useful in controlling pulmonary septic shock.[59-67]

Bdellins

Bdellins – inhibitors of trypsin, plasmin, and sperm acrosin – were first discovered in 1969. A similar proteinase inhibitor, bdellin B-3, was isolated from extracts of *Hirudo medicinalis*. Bdellins were named after the Greek word for leech.[54,68,69] Bdellins, especially bdellin A, could be used as a plasmin inhibitor to control bleeding. Administered systemically, they are rapidly excreted into the urine.[54]

Decorsin

Decorsin is a protein isolated from American medicinal leech *Macrobdella decora*. It acts as an antagonist of platelet glycoprotein II b-III a and is a potent inhibitor of platelet aggregation.[70]

Hirustasin

Hirustasin (*Hirudo antistasin*) belongs to a class of serine protease inhibitors characterized by a well-conserved pattern of cystine residues.[71] It was first isolated from the salivary glands of the Mexican leech, *Haementeria officinalis*, but has recently been prepared from *Hirudo medicinalis*.[75] A protein similar to antistasin has been isolated from the giant Amazonian leech, *Haementeria ghilianii,* and was named ghilanten.[72] Hirustasin binds specifically to tissue kallikrein. Antistasin and ghilanten are potent specific inhibitors of the blood coagulation Factor Xa. In this respect, selective Factor Xa inhibition by recombinant antistasin: (1) prevents vascular graft thrombosis in baboons and rabbits, (2) accelerates reperfusion and prevents re-occlusion in a canine model of femoral arterial thrombosis, (3) reduces restenosis after balloon angioplasty of atherosclerotic femoral arteries in rabbits, and (4) affects the mitosis of cultured aortic smooth muscle cells.[72,73,75]

Antistasin also possesses marked antimetastatic properties. Metastatic tumor spread is correlated to abnormal blood coagulation, possibly due to direct activation of Factor X by tumor cells. Moreover, since the deposition of fibrin provides tumor cells with a protective covering, impermeable to the immune system, the effect of antistasin on tumor spread might be exerted via Factor Xa inhibition.[72-75]

Guamerin

A new human leukocyte elastase inhibitor has been extracted and purified from a Korean native leech, *Hirudo nipponia*. The complete amino acid sequence of guamerin reveals a cysteine-rich polypeptide of 57 amino acid residues that shows no similarity to any known elastase inhibitors, but has 51-percent sequence homology with hirustasin.[6]

Piguamerin

Piguamerin is a serine protease inhibitor of plasma kallikrein that has been screened and purified from the Korean leech, *Hirudo nipponia*. The peptide potently inhibits plasma and tissue kallikrein and trypsin.[76]

Gelin

Gelin is a potent thrombin inhibitor analogous to eglin, and is isolated from the saliva of the *Hirudinaria manillensis*, a leech belonging to the same family as *Hirudo medicinalis*. Like eglin, gelin inhibits elastase, cathepsin G, and chymotrypsin, but has little or no activity on plasmin, thrombin, pepsin, or trypsin.[76]

gamma-Glutamyl Transpeptidase

gamma-Glutamyl transpeptidase compound has been isolated from salivary gland secretion of *Hirudo medicinalis*. The properties of this enzyme are similar to those of bovine gamma-glutamyl transpeptidase.[77]

Platelet Activating Factor Antagonist (PAFA) and an Ornithine-Rich Peptide

PAFA has been isolated from lyophilized dilute leech saliva. It is identified as a phosphoglyceride, which may prove to be an important compound for the treatment of thromboembolic disorders and inflammation. In addition, two specific Factor Xa inhibitors from diluted leech saliva have been isolated. Although PAFA is a more effective antithrombotic agent than heparin, its effect on bleeding time in experimental animals did not differ from that of heparin.[78]

Other Leech Saliva Constituents

Besides the above-mentioned bioactive ingredients, leech saliva contains acetylcholine, histamine-like vasodilators that prolong bleeding time.[33] It also contains enzymes that reduce scar tissue and adhesions. Two types of fibrinases and a collagenase that are also present reduce the density of scar tissue and help reduce fibroblast formation in hypertrophic scars and keloids.[79]

During 1984-1989, it was experimentally proven that intravenous application of a compound of medicinal leech salivary glands to rats with experimentally-induced atherosclerosis reduced atherosclerosis in abdominal and lung arteries.

The proteases and protease inhibitors of saliva have been studied on the growth of neuritis of sensory neurons in chick embryos (10-11 days old). Destabilase and high molecular weight bdellin B (0.01, 0.02, 0.05, and 0.1 ng/mL) had neurite-stimulating effects on day three of spinal ganglia cultivation.[80]

Both kininase and kinin-like activity have been detected in leech saliva and other leech extracts. An analgesic effect was observed with the unheated extract but not with leech saliva after intranasal administration to rats. Anticoagulant complex isolated from lyophilized medicinal leeches exerted pronounced antithrombotic, thrombolytic, and hypotensive effects in experimental animals after intravenous injection and showed antithrombotic activity after oral administration in combination with hirudin.[81] Recently, a pharmacological preparation containing leech salivary gland secretion as an active component was evaluated clinically and found to have a potent arterial antithrombotic effect.[82]

Summary and Outlook

Despite the historical variations in leech therapy, the art of leech therapy remains useful to modern medicine. The spectrum of pharmacological activities of leech saliva is vast. Using recombinant DNA technology, scientists are exploring the potential of other therapeutically active compounds. Pharmaceutical companies are seeking to expand their repertoire of leech salivary components as anticoagulants. Identification of the neurite-stimulating activity of the salivary components provides new therapeutic agents for the treatment of neurodegenerative diseases.

Further search for biologically active compounds from the saliva of other sanguivorous leech species, including *Hirudinaria granulosa* (Indian medicinal leech) or *Hirudinaria manillensis*, is warranted. More medicinal compounds will undoubtedly be discovered in the future. In addition, the therapeutic concentration of these compounds is yet to be determined.

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