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Review Article

Medicinal leech therapy—an overall perspective

Ali K. Sig[a,*], Mustafa Guney[b], Aylin Uskudar Guclu[b], Erkan Ozmen[c]

[a] Erzurum Public Health Laboratory, Public Health Institution of Turkey, Erzurum, Turkey
[b] Department of Medical Microbiology, Gulhane Medical Faculty, University of Health Sciences, Ankara, Turkey
[c] Department of Medical Microbiology, Medical Faculty, Ataturk University, Erzurum, Turkey

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ABSTRACT

Complementary medicine methods have a long history, but modern medicine has just recently focused on their possible modes of action. Medicinal leech therapy (MLT) or hirudotherapy, an old technique, has been studied by many researchers for possible effects on various diseases such as inflammatory diseases, osteoarthritis, and after different surgeries. *Hirudo medicinalis* has widest therapeutic usage among the leeches, but worldwide, many different species were tested and studied. Leeches secrete more than 20 identified bioactive substances such as antistasin, eglins, guamerin, hirudin, saratin, bdellins, complement, and carboxypeptidase inhibitors. They have analgesic, anti-inflammatory, platelet inhibitory, anticoagulant, and thrombin regulatory functions, as well as extracellular matrix degradative and antimicrobial effects, but with further studies, the spectrum of effects may widen. The technique is cheap, effective, easy to apply, and its modes of action have been elucidated for certain diseases. In conclusion, for treatment of some diseases, MLT is not an alternative, but is a complementary and/or integrative choice. MLT is a part of multidisciplinary treatments, and secretes various bioactive substances. These substances vary among species and different species should be evaluated for both treatment capability and their particular secreted molecules. There is huge potential for novel substances and these could be future therapeutics.

1. Introduction

Medicinal leech therapy (MLT) or hirudotherapy is a kind of complementary and integrative treatment method applied with blood-sucking leeches. One or more leeches are attached to the skin of problematic area and the purpose is to gain potential utilities of leech saliva that is secreted while the leeches are feeding. MLT has been used for centuries and the term leech was provided from the word “laece” (physician). The first recorded applications were observed in ancient Eygpt.[1,2] In addition, Chinese, Arabic, Anglo-Saxon, Ancient Greek, and Roman medical records have many references to MLT. In 17th century Europe, MLT reached its widest area of application.[1,3] Since the 1900s, attention of medical professionals has decreased, but in the last 30

* *Corresponding author*. Erzurum Halk Sagligi Laboratuvari, Muratpasa Mahallesi, Fatih Sultan Mehmet Bulvari, No. 23, PC: 25100 Yakutiye – Erzurum, Turkey.
E-mail address: dr_korhan@hotmail.com (A.K. Sig).
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years, MLT has become an important part of much scientific research.[1,4]

Leeches live in fresh water and are segmented, hermaphrodite, carnivorous worms. They are sensitive to vibrations on the water, touch, light, heat, sound, and various chemicals. They are multisegmented, including "brain parts", and each segment has different organs such as ganglions and testicles. Two sucker parts work for creeping and adherence; the anterior one has three jaws including many teeth. They generally bite warm parts of the host and suck its blood with rhythmic contractions.[3,5] Feeding usually takes almost 40 minutes and a leech digests 10–15 mL of blood per feeding. Digestion is achieved by many enzymes and mutual microorganisms such as *Aeromonas hydrophila* and *Pseudomonas hirudinia*.[6,7]

MLT was previously tested and is widely used after plastic, reconstructive, and microsurgical applications, in cardiovascular diseases, deep vein thrombosis, postphlebitic syndrome, complications of diabetes mellitus, tinnitus, acute and chronic otitis, and in reducing the pain of osteoarthritis.[4,8] There are more than 600 leech species, but *Hirudo medicinalis*, *Hirudo troctina, Hirudo nipponia, Hirudo quinquestriata, Poecilobdella granulosa, Hirudinaria javanica, Hirudinaria manillensis, Haementeria officinalis*, and *Macrobdella decora* are the most frequently applied worldwide.[3]

Many studies have found that leeches have various bioactive molecules in their secretions. More than 20 molecules and their modes of action have been identified, but there are many more awaiting exploration. These molecules have analgesic, anti-inflammatory, platelet inhibitory, anticoagulant, and thrombin regulatory functions, as well as extracellular matrix degradative and antimicrobial effects.[6,9–15] It is believed that with further studies, more indications may emerge due to recently elucidated effect mechanisms. In this article, we aim to gather information about MLT, provide an overall vision, and to take a broad look at modes of action.

1.1. *Leeches work with secreted proteins*

To date, many scientific studies have shed light on the effect mechanisms of leeches. Although more than 100 particular proteins with different molecular masses are observed in leech secretions, only a few have been identified that have a major active role.[16] The effect mechanisms are divided into six types to make them more understandable, but these mechanisms are closely related to each other and should be evaluated as a whole (Table 1). Following a leech bite, it has to establish a sucking pathway (extracellular matrix degradation); inhibit adhesion, aggregation, and coagulation (inhibition of platelet functions, and anticoagulant effect); increase blood flow; protect itself (antimicrobial activity); and avoid detection (analgesic and anti-inflammatory effects).

1.2. *Extracellular matrix degradation*

Following the bite, leeches immediately release hyaluronidase (27.5 kDa) and collagenase (100 kDa) enzymes to facilitate tissue penetration and spread of their bioactive molecules. These enzymes also support antimicrobial activity.[9,10,14]

Table 1 – Potential bioactive substances in leech secretions.

Modes of action	Substance
Analgesic and anti-inflammatory effect	Antistasin,[10,14,17] hirustasin,[10,14] ghilantens,[18,19] eglin C,[14] LDTI,[20] complement C1 inhibitor,[21] guamerin and piguamerin,[10,14] carboxypeptidase inhibitor,[14] bdellins and bdellastasin,[14,18]
Extracellular matrix degradation	Hyaluronidase and collagenase[9,10,14]
Increasing blood flow	Acetylcholine,[10,14] histamine-like molecules[7,10,14]
Inhibition of platelet function	Saratin,[9,10,14,22] calin,[9,10,14,23] apyrase,[9,10,14] decorsin[9,10,14,24,25]
Anticoagulant effect	Hirudin,[7,9,14,15] gelin,[9,14] factor Xa inhibitor,[9,10,14] destabilase,[9,14,26–28] new leech protein-1, whitide, and whitmanin[29]
Antimicrobial effect	**Destabilase,[9,14,26–28] chloromycetyn,[9,10,14] theromacin, theromyzin, and peptide B[30,31]**

LDTI, leech-derived tryptase inhibitor.

1.3. *Analgesic and anti-inflammatory effects*

It is believed that leeches exert analgesic and anti-inflammatory effects so as to avoid detection by the host while feeding.[15] Despite this, it has not been possible to isolate any analgesic molecule acting in this way from leech secretions until now. So, studies have focused on indirect mechanisms to achieve this goal. For example, some studies have indicated that some kininases and antistasin may inhibit the kinin–kallikrein mechanism, which is a major nociceptive route.[17] There is more information about the anti-inflammatory effects.

Antistasin was identified from *H. officinalis* (Mexican medicinal leech) and it serves as a potent factor Xa inhibitor and has an inhibitory effect on the kinin–kallikrein system.[17] Factor Xa is a prothrombin activator, and plays a critical role in the common pathway of the coagulation cascade.[32] The Kinin–kallikrein system is also connected to the coagulation cascade and has a major role in the inflammatory response.[33] Researchers claim that antistasin has both anticoagulant and anti-inflammatory effects, but current studies have often focused on the anticoagulant activity, which seems to be the predominant mechanism of action.[34] The Ghilantens were also found in secretions of *Haementeria ghilianii* (Amazonian Leech) and they show high structural homology with antistasin. There are only few data about their anticoagulant effects, and other possible functions are controversial due to lack of additional studies.[18,19]

Leech-derived tryptase inhibitor (LDTI) has three isoforms (a, b, and c) and acts by inhibiting proteolytic enzymes of mast cells. LDTI, a Kazal-type serine protease inhibitor, especially inhibits mast cell tryptase, but also trypsin and chymotrypsin.[20] Mast cell tryptases are serine proteases in cell granules and their release causes inflammatory reactions. These effects are strongly related to the kinin–kallikrein system, chemotaxis, leukocyte activation, vasoactive actions, and accordingly, pain-generating interactions. Their levels are

correlated with allergic and inflammatory diseases such as anaphylaxis, asthma, and arthritis.[35,36] LDTI is an inhibitor of mast cell tryptase, trypsin, chymotrypsin, thrombin, and plasmin, but inhibitory effects on factor Xa, plasma kallikrein, and neutrophil elastase are controversial.[37] Even with inhibition of mast cell tryptase, potential benefits of anti-inflammatory effects can be foreseen. However, recombinant LDTI has shown inconsistent actions in different studies, so it is hard to comment on actual clinical effects of LDTI.[37,38]

Eglin C is an inhibitor of human neutrophil elastase and cathepsin G.[14] These two enzymes are immune serine proteases in the chymotrypsin family that are stocked in azurophil granules of polymorphonuclear neutrophils and released as a part of the inflammatory response.[39,40] Inhibition by eglin C causes decreasing levels of free oxygen radicals in neutrophils and prevents tissue inflammation and destruction. In test models, eglin C was shown to be a potential therapeutic agent for shock and emphysema.[14] Further studies are needed to show other potential effects, but the molecule itself is promising. Other isolated eglins act in similar ways, resulting in anti-inflammatory effects. Another leukocyte elastase inhibitor is cysteine-rich guamerin, which was isolated from *H. nipponia* (Korean medicinal leech). From the same leech, piguamerin was also isolated and has an inhibitory effect on kallikrein and trypsin. As previously stated, hirustasin (Hirudo antistasin) is a serine protease inhibitor and acts as an inhibitor of kallikrein, trypsin, chymotrypsin, and cathepsin G. It was isolated from *H. medicinalis* (European medicinal leech) and *H. officinalis* (Mexican medicinal leech).[10,14] Separately, bdellins and bdellastasin were detected as trypsin, plasmin, and sperm acrozine inhibitors.[14,18] Human neutrophil elastase and cathepsin G have activating effects on factor X (prothrombin activator) and enhancing activity on factor XII and tissue factor, so, as a result, their inhibition by these substances may cause additional anticoagulant outcomes, but this area needs further study.[40,41]

Complement component C1 has a critical role in the classic pathway of the complement system.[42] In leech secretions, complement C1 inhibitor is a 60- to 70-kDa protein, but the effect mechanism is only partially known.[21] This protein might be just one part of the protein pool that inhibits the complement system in many ways. In addition, the original C1 inhibitor in humans suppresses factor XIIa, factor XIa, plasma kallikrein, and thrombin. This substance inhibits both the coagulation cascade and kinin–kallikrein system.[43] Currently, there are no data of similar effects of leech C1 inhibitor, but it is possible and needs further study.

The mechanism causing the inhibitory effect on carboxypeptidases (kininase 1) is contentious. The enzymes carboxypeptidase N and M participate in kinin degradation, resulting in agonism of B receptors, which causes a bradykinin-related inflammatory response.[33] Inhibition of carboxypeptidases by leech secretions should not affect bradykinin action via B_2 (constitutive) receptors, but may prevent B_1 (inducible) receptors. Although these two receptors basically work with similar mechanisms, it has been stated that B_1 receptors are related to chronic inflammation, whereas B_2 receptors are related to acute inflammation. Strong correlations have been found between B_1 and inflammatory diseases such as multiple sclerosis, asthma, and rheumatoid arthritis. However, studies have indicated that action of bradykinin is not limited to these receptors, so possible anti-inflammatory effects of carboxypeptidase inhibition are controversial and should be tested separately.[44]

1.4. *Increasing blood flow*

Leech feeding and therapeutic effects require increased blood flow. These are achieved mainly by histamine-like molecules that cause vasodilatation and arise via local vascular permeability.[7,10,14] Acetylcholine is also a component in leech secretions, causing endothelial muscle relaxation and vasodilatation.[10,14,45]

1.5. *Inhibition of platelet functions*

Destruction of the blood vessel wall for sucking blood causes activation of platelets and the coagulation cascade, which are fatal for the leech. For this reason, leech secretions contain many bioactive molecules to locally inhibit these actions.

In a normal host, wall destruction causes spread and release of collagen particles and they are targets of free vonWillebrand factor (vWF). This complex strongly binds to glycoprotein (GP) Ib on platelets as vWF works like a bridge. With this binding, upregulatory mechanisms occur, especially with the critical role of adenosine diphosphate (ADP), and via GpIIb–IIIa and fibrinogen, platelets bind to each other to make a plug and stop any bleeding. This reaction also starts another chain of releasing substances such as thromboxane A_2, platelet activation, and coagulation cascade.[42] In leech secretions, various molecules (saratin, calin, decorsin, and apyrase) react against different parts of this chain.[9,10,14]

Saratin, a 12-kDa protein, affects only the initial stage of platelet adhesion, and inhibits collagen–vWF reaction competitively. Some animal studies have indicated promising results with recombinant saratin molecule as a potential local therapeutic agent for antithrombotic therapies and atherosclerosis.[22] Other leech-secreted proteins, calin and leech antiplatelet protein, show the same action on platelet adhesion.[24] In contrast, decorsin, which is isolated from *Macrobdella decora* (American medicinal leech), is structurally similar to anticoagulant leech proteins hirudin and antistasin, but functionally it is an efficient GPIIb–IIIa inhibitor and acts potentially against platelet aggregation.[24,25]

As previously stated, ADP has a critical role in platelet aggregation by especially activating GPIIb–IIIa receptors and increasing affinity of platelets to vWF.[42] The enzyme apyrase converts ADP to adenosine monophosphate and blocks aggregation by indirectly inhibiting these receptor mechanisms. ADP also has strong relations with arachidonic acid, platelet-activating factor, and epinephrine activity, so additionally apyrase indirectly acts in an opposing way to these substances.[14] An additional molecule is also described that acts as an inhibitor of platelet-activating factor and thrombin-induced platelet aggregation by suppressing thromboxane production in platelets.[46–48]

The enzyme collagenase also destroys collagen particles, which initiates all these adhesion and aggregation reactions, and provides additional supportive action to the inhibitory effects.[46]

1.6. *Anticoagulant effect*

Coagulation during feeding is fatal for leeches, so anticoagulant effects are necessary.[15] The coagulation cascade is a chain reaction and bioactive molecules in leech secretions have effects at various points. Hirudin and gelin mainly work as thrombin inhibitors, factor Xa inhibitor breaks the chain reaction, and destabilase has a fibrinolytic effect.[9,14] Thrombin has a strong effect on platelet activation and ADP release and so these inhibitors may indirectly have a negative impact on platelet functions.[42]

Hirudin is a 7.1-kDa protein and irreversibly binds to thrombin, which causes consumption of active thrombin and results in antithrombin activity.[7,15] This substance is the most interesting one and was the subject of many studies. There is a strong consensus about it being a therapeutic alternative to heparin, since it has higher anticoagulant activity and fewer adverse effects.[15] Gelin is an eglin analog and a potent thrombin inhibitor. Gelin also shows inhibitory effects on chymotrypsin, cathepsin G, and neutrophil elastase.[14]

Factor Xa inhibitor breaks the coagulation cascade and has a direct anticoagulant effect. It has a critical role in MLT of osteoarthritis and rheumatoid arthritis.[9,10] In addition, as previously stated, antistasin directly inhibits factor Xa,[17] and ghilantens, LDTI, C1 inhibitor, and eglins have possible anticoagulant effects, potentially via direct and/or indirect inhibition of coagulation factors.[18–20,35,36,40,41,43]

Destabilase is an enzyme with glycosidase activity and shows both antibacterial and fibrinolytic actions.[26,27] This enzyme has various isoforms with different capabilities, and is extracted from different leech species.[28] Destabilase has a major degradative action on stabilized fibrin and it should also be evaluated as an anticoagulant agent.[26]

Recently, novel anticoagulant peptides from different leech species have been identified (new leech protein-1, whitide, and whitmanin). Many other peptides have also been isolated, but their function is unknown at present.[29]

1.7. *Antimicrobial effect*

To date, only two main molecules, destabilase and chloromycetin, have been shown to have antimicrobial activity.[9,10,14] As previously stated, destabilase has β-glycosidase activity, which directly disrupts β1–4 bonds that are important in the peptidoglycan layer in bacterial cell walls.[14,49] It is clear that this action is similar to that of lysozyme (muramidase) that is commonly found in human saliva and lachrymal fluid.[50] Other studies have shown that antimicrobial activity does not only depend on glycosidase enzymatic activity, but it also has nonenzymatic components.[27] Even the denaturated form destabilase shows a dose-dependent bacteriostatic effect on *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*.[51] Chloromycetin is a potent antibiotic found in leech secretions, but unfortunately the data are limited about this molecule.[9] Additionally, theromacin, theromyzin, and peptide B have been isolated as antimicrobial peptides.[30,31]

1.8. *Other possible actions*

Many *in vitro* studies have indicated the anticancer effects of leech saliva extracts. Since coagulation is related to metastasis and tumor progression, blocking the cascade can have an antitumor effect.[52] Hirudin has been studied in this regard, with promising results for metastasis, especially from mesothelioma. In addition, other anticoagulant derivates are claimed to have similar effects, as well as reducing cell growth and tumor angiogenesis.[15,29] The extracts have been found to induce apoptosis and cell differentiation and cause cell cycle arrest. The main mechanisms of action seem to depend on suppressing oncogenic gene expression and upregulating apoptotic chains.[29] Effects against cell degeneration have also been reported. Eglin C, bdellastasin, destabilase, bdellins, and hirudin are cytoprotective and exert positive stimulatory actions, especially on neurons, but these studies are only at the preliminary stage.[15]

Leech saliva extracts have also been studied for possible effects on cerebral ischemia–reperfusion injury. Although, as previously stated, leech saliva extracts induce apoptosis, these studies have indicated that saliva extracts have opposing actions by protecting cerebral cells from ischemia–reperfusion injury. Significant changes in superoxide dismutase, nitric oxide, and malondialdehyde levels, and expression of adhesion molecules have been detected on cerebral cells treated with with leech saliva extracts. Pteridines have been isolated as potential antianoxic substances, but it is clear that this activity cannot be related to only one substance.[29]

2. Conclusion

MLT has a long history but it is only recently that its effect mechanisms have started to be clarified. When a leech bites, hyaluronidase and collagenase allow access to the tissues and blood vessels; vasodilatation occurs by the action of histamine-like molecules; platelet functions, kinin activity, and the coagulation cascade are inhibited; and inflammatory reactions are suppressed. In addition, analgesic and antimicrobial effects are observed. Experiments on mice have shown a positive effect on wound/tissue repair.[6,9–14]

There is no consensus on the duration of application and number of simultaneously applied leeches. Medical professionals usually suggest a maximum of four or five leeches at the same time and a maximum duration of 6–8 hours, but these may vary due to physician's clinical evaluation. The total duration of MLT is another unclarified issue. Physicians should consider the bleeding period after application, which may cause excess blood loss. Clinical monitoring and laboratory tests (blood count) are strongly recommended.[7] The necessity for blood transfusion is related to the number of leeches applied, the duration of their application, patient conditions, and comorbidity.[7,53]

Joint diseases such as osteoarthritis and epicondylitis, extremity vein diseases, and flap surgery (skin grafting) are major indications for MLT. MLT is also useful for soft-tissue

and periorbital hematoma, purpura fulminans, macroglossia, penile replantation, postphlebitic syndrome, and ecchymosis. In addition, anticoagulants obtained from leeches are used for peripheral arterial occlusion and infectious myocarditis.[9–13,54,55] Their use in dentistry has also been tested.[56] MLT is not recommended when there is hemorrhagic diathesis, anticoagulant therapy, leukemia, bone narrow suppression, dialysis, cirrhosis, chemotherapy, radiotherapy, and cachexis.[7]

There are some potential complications with MLT. Allergies to leeches and their secretions should be considered.[57] Infection is a serious condition that shows wide variability from local infections to bacteremia. Antibiotic prophylaxis significantly reduces the risk of leech-borne infections. Infectious agents vary depending on etiology, leech species, application area, and patient condition, but by far, *Aeromonas* spp. is the most common.[7,53,58–63] Furthermore, leeches can be vectors for some viruses, fungi and parasites in animals,[64–67] but it seems that application of leeches to humans also has infection potential.[68,69] However, these complications are rare and the most common adverse effects are itching and bleeding on the application area. These adverse effects can be eliminated by small interventions. Orthostatic hypotension and vasovagal symptoms may occur especially in elderly patients. Regional lymphadenopathy has also been reported. MLT usually leaves a scar, therefore, patients should be informed about this, especially before application to particular body parts.[6,7,54,70] Since this therapy has a potential risk of blood-transmitted diseases, reuse of leeches is strictly forbidden.[71]

In conclusion, MLT is a valuable traditional technique with strong biochemical actions. Although modes of action and bioactive substances still await further exploration, their utility in certain medical conditions is obvious. Indications and potential complications should be evaluated, including antibiotic prophylaxis and application frequency, and dosage and delivery timing depend on the patient and physician's opinion. It must be noted that MLT is not a treatment method by itself, but it can be an important part of a multidisciplinary approach.

Conflicts of interest

All authors have no conflicts of interest to declare.

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