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## Haemostasis

# The role of the leech in medical therapeutics

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Leeching is considered by many to be a discredited medical relic of the past. This view is not justified, since leeches still play an important part in modern medicine, as in microsurgery and in the treatment of patients with post-phlebitic syndrome. Hirudin, the potent thrombin inhibitor of leech saliva, has been cloned and is used in the treatment of cardiological and hematological disorders. In our search for other antihemostatic factors in *Hirudo medicinalis* saliva, we found inhibitors of platelet aggregation induced by thrombin, collagen, adenosine 5′-diphosphate, epinephrine, platelet-activating factor and arachidonic acid. We purified apyrase (adenosine 5′-triphosphate diphosphohydrolase), which is a non-specific inhibitor of platelet aggregation by virtue of its action on adenosine 5′-diphosphate. We isolated and characterized the platelet-activating factor antagonist and also identified and recovered an inhibitor of coagulation factor Xa from leech saliva. This report summarizes our findings and those of other investigators, as well as the experience of one of us (A.E.) in leech therapy.

In an era when many folk remedies are being rediscovered and the use of homeopathic and natural drugs is increasingly popular, it is not surprising to witness a revival of the use of leeches in medical practice. In the ancient world, leeches were used as one of several methods of bloodletting. The concept of removing vitiated blood in an attempt to restore health was a fundamental one, and instruments such as lancets, scarifiers and bleeding cups were in common use among apothecaries and surgeons. Tools for venesection were unearthed from archeological excavations of the Stone Age.[1] Leeches were used for bloodletting and were applied to congested or inflamed parts of the body in conditions of engorged hemorrhoids, swollen testicles, laryngitis, prolapsed rectum and inflamed vulva. Compared to venesection, the use of leeches was considered a less painful procedure, in which a limited amount of blood could be removed.

Nicander of Colophon (200–130 BC) was probably the first medical practitioner to use leeches for therapeutic purposes, and leeching was described in a work of the celebrated 2nd-century physician, Galen. Early Chinese writings from the first century AD describe the use of leeches, a practice which is also mentioned in ancient Sanskrit, Persian and Arabic literature. The Old Testament mentions the leech in Proverbs 3.15, although not in connection with healing. The widely held view that leeches were important in therapy during the Middle Ages is probably incorrect. The word 'leech' is actually the Anglo-Saxon term for physician, and the 'leech books' of that era contained hardly any reference to the animal. On the whole, it looks as if

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the creature acquired the name from the doctor and not the other way around.[2,3]

In the 18th and 19th centuries, the popularity of leeching was at its height in Europe and leeches became a major item of international trade. It was calculated that Parisian hospitals used approximately 5–6 million leeches between 1829 and 1836 and that 84 150 kg of blood were removed annually from treated patients.[1] There is documentation testifying that 97 300 leeches were used in St Bartholomew's Hospital in London in 1832.[1] The demand for *H. medicinalis* in Europe was so great that the natural leech population became almost extinct and a $500 premium was offered in the USA to those who could breed leeches.[1] Leeches were used for practically every disease, from various local aches and inflammatory processes to nephritis, laryngitis, eye disorders, brain congestion and even obesity and mental illnesses. Stokes recommended the use of 20–40 leeches for gastritis and Behrend reported a beneficial effect of 6–10 leeches in scarlet fever.[1] The Russian novelist Gogol was subjected to leeching in his nostrils[4] and Napoleon was treated with leeches for his hemorrhoids.

Leeches are segmented, hermaphroditic worms of the phylum Annelida. They are equipped with two suckers. Both suckers are used for clinging and crawling. The smaller anterior sucker houses the mouth. The mouth leads into the buccal cavity which houses three jaws each bearing a row of many denticles or 'teeth'. Leeches live in freshwater ponds and swim to their target within 25° of a wave source. They usually bite into a warm area of the skin and suck the host's blood by means of rhythmic contractions into the crop, where it is stored until digested. Strong muscles move the jaws back and forth, cutting rapidly and painlessly into the dermis. The lowered sensitivity to pain from a leech bite has been demonstrated by the tail-flick test and has been related to two complementary activities of leech saliva, each of which reduces kinin-like activity in the host's blood. These are (a) the inhibition of plasma kallikrein, determined by the inhibition of kininogenase activity and (b) kininase activity.[5] Our earlier results on the heat–pain threshold point in the same direction but are inconclusive.[6] A leech bite usually leaves a distinctive scar made by the three jaws, which is reminiscent of the Mercedes-Benz emblem. As it sucks, the leech secretes its saliva, rich in active materials, both into the wound and into the aspirated blood and thus maintains a continuous flow of blood. These materials include hyaluronidase, collagenase, coagulation and platelet aggregation inhibitors. *Aeromonas hydrophila*, the symbiotic bacterium in the intestine of *H. medicinalis*, plays a dominant role in the digestion of the blood. In one meal, which lasts approximately 20–40 min, the leech removes about 5–15 ml of blood, which amounts to approximately ten times leech body-weight. This is regarded as one of the largest meals taken by any living creature relative to its own size. Satiated leeches are much less active and tend to hide for periods of 12–18 months, during which they digest their meal and do not bite.[7] The neuronal co-ordination of leech movements and behavior is the subject of intensive research by neurobiologists.[8] Interestingly, the array of neurotransmitters in leech neurons and ganglia is similar to that found in mammalian brains.[8,9]

Several species of leeches have been used in medicine, the most common being *Hirudo medicinalis*, which is still collected from rivers and ponds in eastern Europe but is chiefly being bred in controlled environments. Other species that have been used are: *H. troctina* in North Africa, *H. nipponia* in Japan, *H. quinquestriata* in Australia, *Poecilobdella granulosa*, *Hirudinaria javanica* and *Hirudinaria manillensis* in south-east Asia, *Haementeria officinalis* in Mexico and *Macrobdella decora* in the USA. There are about 650 leech species, but only a few suck blood by piercing mammalian skin. *Limnatis nilotica*, the most widespread leech species in Egypt, Israel and Lebanon,[10,11] rarely bites human skin and feeds on the softer mucous membranes of the mouth, pharynx and larynx. This may be the reason why the Hebrews did not use leeches for medical purposes, although they practiced venesection. The Talmud (compiled between the 3rd and 6th centuries AD) warns against the danger of leeches when drinking water from rivers or lakes.[12] *Limnatis nilotica* was first described by Savigny, who accompanied Napoleon's army to Egypt. Both soldiers and horses were tormented by these leeches, as were British troops in Egypt during World War I.[2]

## ANTICOAGULANTS IN LEECH SALIVA (TABLE 1)

In the wake of quackery and with the advent of modern medicine, leeching fell into disrepute. However, limited interest in the medicinal use of leeches still persisted into the 20th century, mainly because it was already known that extracts of leech heads contain a powerful anticoagulant. Leeches were used for the treatment of post-operative thrombosis and even coronary thrombosis.[1] Hirudin, the principal anticoagulant in leech saliva, was discovered by Haycraft in 1884 and employed in blood transfusion in 1915.[1] More than 70 years elapsed from the discovery of hirudin until Markwardt isolated it and identified it as a polypeptide, a highly potent antiprotease with a strict

**Table 1** Anticoagulants and related inhibitor[1] from leeches

| Substance | Activity | Leech species | References |
|---|---|---|---|
| Hirudin | Inhibits thrombin | *Hirudo medicinalis* | 13–24 |
| Hirudin variants | Inhibit thrombin | *Hirudinaria manillensis* | 28–31 |
| Thrombin inhibitor (not named) | Inhibits thrombin | *Poecilobdella granulosa* | 25 |
| Thrombin inhibitor (not named) | Inhibits thrombin | *Limnatis nilotica* | this review |
| Haemadin | Inhibits thrombin | *Haemadipsa sylvestris* | 26 |
| Theromin | Inhibits thrombin | *Theromyzon tessulatum* | 27 |
| Plasma kallikrein inhibitor | Inhibits plasma kallikrein | *Hirudo medicinalis* | 5 |
| Destabilase | Liquefies cross-linked fibrin, antithrombotic in rats | *Hirudo medicinalis* | 32, 33 |
| Antistasin | Inhibits Factor Xa, antimetastatic | *Haementaria officinalis* | 34–41 |
| Factor Xa inhibitor (not named) | Inhibits Factor Xa | *Hirudo medicinalis* | 42–46 |
| Factor Xa inhibitor (not named) | Inhibits Factor Xa | *Limnatis nilotica* | this review |
| Ghilanten | Inhibits Factor Xa, antimetastatic | *Haementeria ghilianii* | 48, 48a |
| Hementin | Degrades fibrinogen and fibrin<br>Inhibits tumor spread and metastasis | *Haementeria ghilianii* | 49, 50–52 |
| Hementerin | Plasminogen activator | *Haementaria depressa* | 54 |
| Hirustasin[1] | Inhibits cathepsin G, tissue kallikrein, trypsin and chymotrypsin | *Hirudo medicinalis* | 53 |

specificity for thrombin.[13] Hirudin has since been the subject of extensive research,[14–21] is now produced by recombinant DNA technology[22–24] and is administered to patients undergoing coronary angioplasty, in the treatment of deep venous thrombosis and as a substitute for heparin in patients developing heparin-induced thrombocytopenia.

Interest in leeches and other hematophagous animals is growing, as attempts are being made to discover new biological compounds with therapeutic potential. We found potent thrombin inhibitors in *Poecilobdella granulosa* (formerly named *Hirudinaria granulosa*)[25] and in *Limnatis nilotica* (unpublished). Haemadin, a thrombin inhibitor of about 5 kDa, has been isolated from the Indian leech *Haemadipsa sylvestris* and has been sequenced and cloned.[26] Two variants of theromin, specific thrombin inhibitors of molecular weight 14 and 9 kDa respectively, were isolated from the leech *Theromyzon tessulatum*. The 14 kDa variant, which has also been cloned, possesses 127 amino acids.[27] Hirudin variants from *Hirudinaria manillensis* have been described in depth.[28–31]

Leeches also affect coagulation by other mechanisms: the saliva of *Hirudo medicinalis* was found to inhibit plasma kallikrein,[5] and to contain destabilase, which liquefies cross-linked fibrin and is antithrombotic in rats.[32] Destabilase converted the DD fragment of cross-linked fibrin into the D monomer.[33] Antistasin, a 119-amino acid two-domain protein isolated from the salivary glands of the Mexican proboscis-feeding leech *Haementeria officinalis* is a potent inhibitor of coagulation Factor Xa(FXa). This protein was also found to exert an antimetastatic effect in animal tumor models.[34–41] An FXa inhibitor was identified in the saliva of *Hirudo medicinalis*.[42] This salivary inhibitor and a novel FXa inhibitor from an *H. medicinalis* complementary deoxyribonucleic acid (cDNA) library were recently cloned by BioTechnology General Ltd (Rehovot, Israel).[43–45] The novel FXa inhibitor was shown to be an effective antithrombotic agent in animal models of arterial and venous thrombosis. This inhibitor significantly shortened the time to thrombolysis induced by tissue plasminogen activator compared to hirudin or heparin, in an experimental arterial thrombosis model in rabbits.[46] Compared to heparin and aspirin, r-Antistasin accelerated recombinant tissue plasminogen activator-induced reperfusion in canine thrombosis models.[47] We also detected an FXa inhibitor in the crop contents of *Limnatis nilotica* (unpublished). Another FXa inhibitor, a close homolog of antistasin, was isolated from the Amazon leech *Haementaria ghilianii*.[48,48a] This leech also secretes hementin, which degrades fibrinogen and fibrin[49,50] and disaggregates platelets by breaking the fibrinogen link between them.[51] Salivary gland extracts of *H. ghilianii* have been shown to inhibit tumor-spread and metastasis in experimental animals.[52] An antistasin-type, one-domain polypeptide from *Hirudo medicinalis*, hirustasin, has been isolated and characterized. It inhibits cathepsin G, tissue kallikrein, trypsin and chymotrypsin, but does not prolong either prothrombin time or partial thromboplastin time, nor does it inhibit FXa.[53] Fibrinolytic compounds were also found in leeches. Hementerin from *Hirudo depressa* possesses a plasminogen activator activity whose mechanism resembles that of streptokinase.[54]

Anticoagulants are found in the salivary glands of other blood-sucking animals. The 60-residue anticoagulant peptide (TAP) of the soft tick *Ornithodorus moubata* is a potent FXa inhibitor and is the subject of intensive research.[39,55–57] The black fly *Simulium vittatum* possesses an FXa inhibitor,[58] the tsetse fly *Glossina morsitans* has a thrombin inhibitor[59] and the human body louse *Pediculus humanus humanus* has both a thrombin inhibitor and an FXa inhibitor.[60] The tick *Dermacentor andersoni* bears anticoagulants directed against Factors V and VII[61] and the bug *Rhodnius prolixus* secretes anticoagulants directed against Factor VIII.[62] A FXa inhibitor has been identified and partially purified from adult *Ancylostoma* hookworms,[63] and plasminogen activators have been isolated and characterized from the saliva of the only hematophagous mammal, the vampire bat.[64] Bacteria are also a potential source of antihemostatic agents: ecotin, from the periplasm of *Escherichia coli*, is a potent inhibitor of FXa and FXIIa.[65]

## INHIBITORS OF PLATELET AGGREGATION (TABLE 2)

Sustained bleeding from a leech bite suggested that leech saliva may contain inhibitors of hemostasis besides coagulation inhibitors. We decided to look for inhibitors of platelet aggregation. Our method of collecting dilute leech saliva (DLS) was based on the finding of Galun and Kindler that leeches may be stimulated to suck a solution of arginine in saline through a membrane.[66] The weight gain is equivalent to a meal of whole blood. We squeeze the satiated leeches from back to front, immediately after they have fed on the stimulant solution.[25,67] We used only clear, colorless fluid that was found to be free of blood components. It possesses the same enzyme, enzyme inhibitor and antiaggregant activities that we found in salivary gland extracts.[6,25,67] It was shown to inhibit platelet aggregation induced by thrombin, collagen, adenosine 5′-diphosphate (ADP), epinephrine, platelet activating factor [1-O-alkyl-2-acetyl-sn-3-glycerophosphorylcholine (PAF)] and arachidonic acid.[6,68] As expected, hirudin was shown to inhibit platelet aggregation induced by thrombin but did not inhibit the activity of any other aggregating agent.

To further characterize the active substances that inhibit platelet aggregation, we lyophilized large quantities of DLS and subjected them to sizing chromatography on a Bio-Gel P-2 column (Bio-Rad Laboratories, Hercules, CA, USA).[67] Four major fractions were obtained, differing in specificity towards platelet aggregation agonists. The first fraction – the protein fraction – was eluted in the void volume (Mr> 2 kDa) and it inhibited aggregation induced by thrombin, ADP and collagen. The first two effects are due to hirudin and apyrase respectively. The protein fraction also inhibited the effect on platelets of epinephrine and arachidonic acid. The remaining three fractions were of low molecular weight (Mr< 2 kDa). The second fraction – FrII – inhibited PAF- and thrombin-induced thromboxane $A_2$ formation with consequent inhibition of platelet aggregation. It had no effect on other agonists. The third fraction inhibited aggregation induced by ADP, epinephrine and arachidonic acid, and the fourth fraction only inhibited the effect of arachidonic acid. We also showed that platelet aggregation induced by collagen, epinephrine and ADP was inhibited by the dilute saliva and salivary gland extracts of *Poecilobdella granulosa*[6] and the crop contents of the Nile leech, *Limnatis nilotica* (unpublished results). Baskova et al also found an inhibitory effect on ADP-induced platelet aggregation by the low-molecular-weight fraction of *H. medicinalis* saliva. The inhibition was found to be due to the presence of the prostanoid 6-keto-$PGF_{1a}$, which enhances platelet cyclic adenosine 3′-5′-phosphate (cAMP) production.[69–71]

The protein fraction of DLS was found to contain apyrase, a substance which hydrolyzes adenosine 5′-triphosphate (ATP) and ADP to adenosine 5′-monophosphate (AMP) and strongly inhibits ADP-induced platelet aggregation. Shochat-Gelbart, in our group, purified and characterized leech salivary

**Table 2** Inhibitors of platelet aggregation from leeches

| Substance | Aggregating agents | Leech species | References |
|---|---|---|---|
| Calin | Collagen | *Hirudo medicinalis* | 73(75 |
| Leech antiplatelet protein | Collagen | *Haementeria officinalis* | 76, 77 |
| Hirudin | Thrombin | *Hirudo medicinalis* | 25 |
| Apyrase | ADP | *Hirudo medicinalis* | 25, this review |
| Low molecular weight Fraction II | PAF, thrombin | *Hirudo medicinalis* | 67, 68 |
| Low molecular weight Fraction III | ADP, epinephrine, arachidonic acid | *Hirudo medicinalis* | 67, 68 |
| Low molecular weight fraction | ADP, epinephrine | *Hirudo medicinalis* | 69, 70 |
| Decorsin | General inhibitor (binds glycoprotein IIb(IIIa) | *Macrobdella decora* | 91 |
| Ornatins | General inhibitors (bind glycoprotein IIb(IIIa) | *Placobdella ornata* | 92, 93 |

apyrase (manuscript in preparation). Two forms, > 400 kDa and 56 kDa, were obtained by sizing chromatography. Apyrase is not a specific inhibitor, since different agonists release endogenous ADP from platelets before aggregation. It is by far the most commonly occurring platelet aggregation inhibitor in the saliva and salivary gland extracts of blood-sucking arthropods, such as ticks, bugs and mosquitoes.[72]

We also identified a collagenase in the protein fraction which hydrolyses Type I collagen from calf skin, producing the collagen degradation pattern characteristic of mammalian type collagenase on sodium dodecyl sulphate (SDS) gels.[25] Collagenase is the only proteinase we detected in leech saliva.[25] Calin, a protein of approximately 65 kDa found in *H. medicinalis* saliva, binds specifically to collagen and inhibits collagen-induced platelet aggregation and adhesion and collagen-mediated thrombin formation.[73,74] Calin also inhibits the binding of collagen to von Willebrand factor.[75] A leech antiplatelet protein of approximately 16 kDa that specifically blocks collagen-induced platelet aggregation and adhesion has been isolated from *Haementeria officinalis* salivary glands and characterized as well.[76,77] Here, too, the results indicate that the protein also inhibits collagen binding to von Willebrand factor.[78] Collagen-induced platelet aggregation is also inhibited by the salivary secretion of the bug *Rhodnius prolixus*.[79] Other inhibitors of collagen-induced platelet aggregation and adhesion are moubatin (17 kDa) from the tick *Ornithodorus moubata*,[80,81] pallipidin (18 kDa) from the assassin bug *Triatoma pallipidensis*[82] and a 50-kDa protein from the venom of the snake *Bothrops atrox*.[82]

We found *H. medicinalis* saliva to contain hyaluronidase (spreading factor) and eglin.[25] Hyaluronidase has been prepared from leech extracts[84,85] and was found to occur in leech salivary cells.[86] On the other hand, eglin from the leech was reported not to occur in leech salivary cells.[87] Eglin inhibits granulocytic elastase, cathepsin G, subtilisin and chymotrypsin.[87,88] As mentioned, Fraction II strongly inhibited PAF-induced platelet aggregation.[67,68] PAF is a naturally occurring phosphoglyceride released into the blood on immunogenic challenge from neutrophils, basophils and macrophages, and from platelets in response to specific stimuli from platelets. It is a most powerful platelet-aggregating agent. PAF also induces chemotaxis and aggregation of neutrophils, hypotension, smooth muscle contraction and bronchoconstriction, and is a potent mediator of inflammation.[89,90] Fraction II also inhibited thrombin-induced platelet aggregation, although thrombin coagulant activity was not inhibited.[67,68] Fraction II inhibited formyl Met-Leu-Pro- and ionophore A23187-induced superoxide formation in human neutrophils, which may be due to the inhibition of PAF generated within these cells.[67] This property of Fraction II suggests anti-inflammatory activity.

We recently isolated the PAF antagonist from DLS by liquid–liquid extraction followed by high-pressure liquid chromatography (HPLC) and reverse phase HPLC (Orevi et al, manuscript in preparation). One peak obtained competitively inhibited only PAF-induced platelet aggregation, with an inhibitor concentration at 50% inhibition ($IC_{50}$) of 0.37 mM.

Thrombin antagonist activity was also observed in some batches. The antagonist is amphipathic, being soluble both in organic solvents and in water. Phospholipases reduce its activity, particularly phospholipases C and D, suggesting that it is a phosphoglyceride. It is the only PAF antagonist from an animal source described hitherto. Other naturally occurring inhibitors of PAF-induced aggregation are gingkolides (terpenes) and lignans from Chinese and Brazilian plants, and gliotoxines from certain fungi and micro-organisms.[90]

Researchers at Genentech Inc. (South San Francisco, CA, USA) have isolated potent antagonists of the platelet fibrinogen receptor, glycoprotein IIb-IIIa (GPIIb-IIIa) from North American leeches. These are decorsin from *Macrobdella decora*[91] and ornatins from *Placobdella ornata*.[91,93] They are proteins of 39 to 52 amino acids containing the sequence Arg-Gly-Asp (RGD), the proposed recognition site of many adhesion proteins. They inhibit ADP-induced aggregation with $IC_{50}$ values in the 0.1 mM–0.5 mM range. They are similar to the family of RGD peptides found in snake venoms, which inhibit platelet aggregation by binding to the GPIIb-IIIa receptor.[94] Decorsin and hirudin possess a similar three-dimensional structure, although the binding epitopes are different. Amino-acid sequences suggest that ornatin and antistasin share a similar structure with them.[95]

## LEECH THERAPY

Haycraft's seminal discovery of hirudin was followed a century later by the discovery of other antihemostatic agents in leech saliva: platelet-aggregation inhibitors, coagulation inhibitors and destabilase. It is doubtful, however, whether the known antihemostatic armamentarium of *H. medicinalis* saliva can account for the prolonged bleeding observed after a leech feed. Munro et al[96] examined the blood oozing from a leech bite. During the first minute after the leech had dropped off, whole blood clotting time, thrombin clotting time, and prothrombin time were elevated, and ADP- and thrombin-induced aggregation were inhibited. Coagulation parameters and aggregation reverted to normal within 15 min. In our work, we found that hirudin and the

PAF antagonist were washed away in the blood oozing from a leech bite in the course of 20 min, and that activated partial thromboplastin time, which was elevated at first, reverted to normal (Rigbi et al manuscript in preparation). Nonetheless, bleeding continued for hours. It has been suggested that vasodilation in the vicinity of a leech bite[97,98] and calin[73] may contribute to this phenomenon. Notwithstanding the important role of known antihemostatic agents, most of which are unavailable commercially, the prolonged bleeding from a leech wound, i.e. delayed clot formation, makes a strong case for leech therapy in situations such as those mentioned here. Indeed, the use of leeches in both the lay and medical communities is growing.

## USE OF LEECHES IN PLASTIC SURGERY

The medicinal leech is used to salvage tissue with compromised circulation. Leeches were applied to skin flaps, or replanted fingers or auricles,[99–108] in which arterial revascularizations were performed but vein repairs were limited. Leeches are usually applied once or twice daily to the engorged organ. Hyaluronidase and collagenase 'open up' the tissue, and capillary circulation is maintained by evacuation of blood and injection of anticoagulants, antiaggregating agents and vasodilating substances. Leech therapy is repeated daily for 4–5 days until venous capillary return is established across the wound by angiogenesis.[99,101,107,108] We used leeches in treating more than 50 patients recovering from plastic surgery. The leeches were obtained from an authorized leech farm (Ricarimpex, Eysines, France). In order to avoid the transfer of blood pathogens, the leeches were discarded immediately after use by immersion in an alcohol solution. Most patients had congested skin flaps, one 19-year-old girl had a reimplantation of an ear that had been amputated after a car accident, and several patients underwent reimplantation of partially amputated fingers. Patients were not accepted if they had a tendency to hemorrhage, severe anemia or were taking anticoagulants or platelet-inhibitor drugs (aspirin). Leeches were applied daily for 4 days, and continued oozing from the leech bite was allowed. Excellent results were obtained in most of the skin flaps and the amputated ear. There were no side-effects such as fever, drop in hemoglobin or local infection. It should be noted that leech therapy may sometimes be complicated by *Aeromonas hydrophila* infection.[108] This Gram-negative bacterium is a normal enteric organism of the leech. Effective antibiotics for *Aeromonas* are tetracycline, chloramphenicol, trimethoprin sulfamethoxasole, cefoxitin, ciproflaxacin, aminoglycosides and third-generation cephalosporins.[108] All of our patients were given one of these antibiotics.

## POST-PHLEBITIC SYNDROME

About 1–1.5% of patients suffering from deep venous thrombosis in the legs develop post-phlebitic syndrome, a complication that occurs as a consequence of venous valve destruction. This complication is manifested by swelling, induration and pigmentation around the ankle and the lower third of the leg, as well as by ulceration that usually occurs in the region of the medial malleolus.

With the aim of decongesting the affected limb, restoring skin microcirculation and introducing the antihemostatic, vasodilatatory and anti-inflammatory salivary agents, we started to use leeches on patients with post-phlebitic syndrome.[106] This ancient treatment was used on 40 patients. Between 7 and 12 leeches were applied to the affected limb every 3–4 weeks in the course of 1–25 sessions and the results were impressive. Twenty-one patients reported diminished leg pain and described the legs as feeling 'less heavy'. Improved mobility, with a greater ability to walk for long distances, was reported by 28 patients. The effect of leech therapy was almost immediate and persisted for about three weeks. Twelve patients came regularly once a month and received between 6 and 25 treatments. The patients had received other treatments prior to leech therapy, including various vasodilators and pentoxifyllin. All of them described leech therapy as being superior to the other modalities. The above subjective effects were accompanied by objective beneficial effects in 16 patients. Leech therapy improved skin circulation, and skin color turned from dark purple to light red. Healing of chronic skin ulcers was observed in seven patients, and diminished circumference of the swollen leg (by 1–2 cm) was observed in five patients. All of the patients received prophylactic tetracycline (2 g/day) therapy for two days. None developed fever or infection at the skin bites. No systemic bleeding or drop in hemoglobin was noted even in patients who received more than 20 treatments. It usually takes about 20 min until the leech is engorged, whereupon it drops from the bitten site and side-effects include itching and scarring of the bite wound. The bites are then cleaned with an antiseptic and covered with dry gauze and an elastic bandage to avoid excessive bleeding. We are currently continuing this study, which has been approved by the Human Experimentation Committee of Tel-Aviv Sourasky Medical Center and the Ministry of Health. The willingness of the patients to attend

regularly once a month for leech application is a testimony to the beneficial results of this procedure.

We may conclude that the medical lesson to be learned from the leech is that successful antithrombotic therapy should aim at inhibiting both platelet aggregation and blood coagulation and should include more than one drug. The discovery of potent anticoagulants, antiaggregants and anti-inflammatory agents in leech saliva points to the potential for further development of drugs for use singly or in combination. Both known and unknown antihemostatic factors and processes appear to be involved in prolonged bleeding from a leech wound. This phenomenon of delayed clotting creates a place for leech therapy in modern medical practice.

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