

## Medical Therapeutics Derived from Leeches (Phy. Annelida; Cl. Hirudinea)

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Of all blood-feeding invertebrates, few are more notorious than leeches (Abdualkader et al. 2013). Members of the phylum Annelida and subclass Euhirudinea, leeches predominately inhabit aquatic environments (Hildebrandt and Lemke 2011; Abdualkader et al. 2013). Of the many different species of leech, those that target larger vertebrates have evolved in accordance with their environment to be voracious ectoparasites (Abdualkader et al. 2013). Indeed, leeches have evolved sensory systems that allow them to locate their prey by detecting their chemical signature (Elliot 1986). This is facilitated primarily through sensory organs found under the dorsal lip, which are necessary for detecting chemical species produced by the body surface, such as arginine and sodium chloride (Elliot 1986). After the host has been located, a leech attaches via a sucker, makes an incision on the outer surface using its three jaws, and draws blood, which it then gathers nutrients from (Hildebrandt and Lemke 2011).

It is this ability of leeches to suck blood that was first utilized by humans for medicinal purposes. The first documentation of this is in an Egyptian illustration dating back to approximately 1500 B.C. (Munshi et al. 2008). Some physicians of the 19<sup>th</sup> century hypothesized that the root cause of many illnesses, such as appendicitis and gastroenteritis, was pooling of blood in the affected tissues. Bloodletting through leeching was thought to be an ample treatment for these and other diseases (Adams 1998). There have been many species of leech that have been used to treat disease including *Hirudo nipponia*, *Hirudo orientalis*, and *Hirudinaria manillensis* (Abdualkader et al. 2013). However, the species that has been used

more than any other is the European *Hirudo medicinalis* (Abdualkader et al. 2013).

Despite their widespread use over the ages, it is only recently that researchers have begun to elucidate the true therapeutic functions and the molecular mechanisms by which leech isolates work (Hildebrandt and Lemke 2011). Perhaps the most notable advances are in our understanding of the ability of leeches to continue drawing blood from a victim without it clotting (Hildebrandt and Lemke 2011). When a blood vessel becomes damaged, or a leech pierces the skin and vasculature of an unsuspecting victim, a cascade is initiated in which the synthesis, activation and aggregation of coagulating factors occurs (Hildebrandt and Lemke 2011; Coughlin 2000). The protein prothrombin, an upstream precursor, is transformed into the enzyme thrombin (Coughlin 2000). Thrombin's primary function is turning the molecule fibrinogen into smaller fibrin subunits, which link with one another to produce a polymer (Coughlin 2000). In combination with cell fragments called platelets, the fibrin polymer forms the clot which causes bleeding to stop (Markwardt 1994).

In response, leeches have evolved mechanisms to prevent this coagulation response in order to take a blood meal (Hildebrandt and Lemke, 2011). In 1884, the anticoagulant nature of leech extracts was initially characterized by Haycraft (Markwardt 1994; Salzet et al. 2001). However, one of the main proteins responsible for the anticoagulant effect, hirudin, was not isolated and identified until the 1950s (Markwardt 1994). Initially hirudin's clinical use was limited as it was difficult to find enough of the *H. medicinalis* variety to cultivate the necessary quantities (Markwardt 1994). After the gene encoding hirudin was discovered, it was cloned into *E. coli*, which allowed for mass production of hirudin (Harvey et al. 1986; Markwardt 1994). Hirudin is a 65 amino acid long

protein that acts as a direct thrombin inhibitor (Markwardt 1994). By stopping thrombin's enzymatic function, hirudin can prevent a clot from forming (Markwardt 1994). In animal models, besides preventing clot formation, hirudin has been shown to significantly decrease the size of induced clots in the carotid artery and jugular veins. At certain doses, hirudin was able to completely prevent clots (Markwardt et al. 1989) While the formation of a clot (thrombosis) is important to prevent blood loss should one suffer a lesion, in many instances it can be deleterious (Coughlin 2000; Wadajkar et al. 2013). For instance, should a clot develop within the deep veins, it may dislodge from its place of origin, and travel through the circulatory system to the heart, resulting in a heart attack or a stroke (Wadajkar et al. 2013).

Of all anticoagulants used clinically to ameliorate the effects of clot formation, the most common is the compound heparin (Greinacher and Lubenow 2003). As opposed to hirudin, heparin inhibits thrombin indirectly by binding to and further activating the protein anti-thrombin, whose role is to inhibit thrombin's catalytic activity (Hirsh et al. 2001). Despite its widespread use, heparin administration is coupled with serious side effects. One of the most detrimental side effects is heparin-induced thrombocytopenia, which is a reduction in the number of platelets following heparin use (Greinacher and Lubenow 2003). Because of this, hirudin has been evaluated as an alternative treatment (Topol et al. 1994). It should be noted that while adverse reactions following hirudin use are rare, severe allergic reactions have been reported in 0.015% of patients (Greinacher and Warkentin 2008). Whether or not this is due specifically to hirudin administration is relatively unknown. Additionally, hirudin use can cause excessive bleeding which may be exacerbated by kidney dysfunction

(Greinacher and Warkentin 2008; Markwardt 1994). However, patients treated with hirudin have been shown to have excessive bleeding less often than those treated with heparin (Stone et al. 2008).

Myocardial infarction (heart attack) is responsible for nearly 16,000 deaths in Canada per year. It results when there is not enough blood flowing to the heart muscle, and as a result, the lack of oxygen brings about cell death (Heart and Stroke 2015; University of Maryland Medical Center 2014). One of the events that commonly precedes myocardial infarction is unstable angina (Falk 1985). Unstable angina is heart pain that results from a lack of blood flowing to the heart (Braunwald 1989; Maseri et al. 1999). While the exact mechanism of its occurrence is still being investigated, it is suggested by some that, when atherosclerotic plaque within a coronary artery breaks, the thrombin cascade is initiated, and a clot forms, narrowing the diameter of the blood vessels and restricting blood flow (Maseri et al. 1999). In a study by Topol et al. (1994), the effects of hirudin on patients with unstable angina pectoris were examined. In the study, patients were administered either heparin or hirudin. Following treatment, the mean coronary artery cross sectional area was found to increase more in patients treated with hirudin compared to the heparin control group. This indicates that hirudin was able to reduce clotting to a greater extent (Topol et al. 1994). Furthermore, there was a smaller incidence of myocardial infarction in those treated with hirudin compared to those treated with heparin (Topol et al. 1994).

In a study by Stone et al. (2008), patients with elevated ST-segments, an indicator of a blocked coronary artery, underwent Primary Percutaneous Coronary Intervention (Pierard 2007; Stone et al. 2008). Patients were either administered hirudin or heparin. It was found that patients administered hirudin experienced fewer

complications after treatment compared to the heparin group. This included less excessive bleeding as well as reduced thrombocytopenia. The mortality rate among the hirudin patients was also significantly lower than those treated with heparin (Stone et al. 2008).

Clots can also travel to the cerebral vasculature, resulting in a stroke (Wadajkar et al. 2013). Approximately 14,000 strokes in Canada each year are fatal (Heart and Stroke Foundation 2014). Hirudin has shown promise in reducing the severity of physiological injury and cognitive impairment in animal models of stroke (Karabiyikoglu et al. 2004). While hirudin has yet to be applied on a large scale clinically for stroke, in a study by Harrigan et al. (2004), intra-arterial hirudin injection was able to ameliorate thrombosis within a cerebral artery, allowing for a reduction in motor deficits caused by the blockage.

Coagulation has also been shown to play a role in the development of cancer (Hara et al. 1980). Cancer cells and platelets can bind together and anneal to the vasculature. This interaction can greatly contribute to the spread of cancer across the body particularly when platelets bind to cancer cells that are travelling freely in the blood stream (Li 2016). As a result, some leech extracts have been tested for anti-oncogenic properties (Gasic et al. 1983). In a study by Esumi et al. (1991) skin cancer cells were found to increase blood coagulation in vitro. When hirudin was administered clotting ceased. On this basis it has been proposed that this may be one avenue by which leech derivatives, particularly hirudin, could be used to clinically ameliorate metastasis (Esumi et al. 1991). Hirudin has also been shown to hinder aggregation of mesothelioma cells (Bastida et al. 1983). Mesothelioma is a variant of cancer that commonly originates and develops in the pleural membrane of the lung or the pericardium of the heart (Remon et al. 2015). This may be a result of coming into contact with asbestos (Remon et al. 2015).

The anticoagulant nature of leech derivatives is not exclusive to hirudin (Gasic et al. 1983). In a study by Gasic et al. (1983), leech saliva extract was found to dramatically decrease the size and prevalence of lung tumors in vivo. Another protein isolated from leech saliva, Lj-RGD<sub>3</sub>, has been shown to significantly reduce cell growth and blood vessel development (Wang et al. 2010). This plays an important role in the development of metastasis (Wang et al. 2010). In a later study by Jin et al. (2012), Lj-RGD<sub>3</sub> was also found to halt growth and invasiveness of drug resistant breast cancer cells.

Despite these promising findings, leech extracts and hirudin have not been tested in humans as cancer treatments. However, heparin, whose function is similar to that of hirudin, has been tested. For instance, in a study by Altinbas et al. (2004), patients with small cell lung cancer were either given chemotherapy in addition to heparin or only chemotherapy. Tumor progression was evaluated after 18 weeks of treatment. Cancer progression was found to be reduced in 69% of patients treated with chemotherapy and heparin compared to only 43 % of patients within the chemotherapy control group (Altinbas et al. 2004). The length of patient survival following treatment was also significantly higher in the heparin and chemotherapy group compared to those patients treated exclusively with chemotherapy (Altinbas et al. 2004). Therefore, it is possible that hirudin could be used to treat cancer in a similar way without the deleterious side effects accompanied by heparin use (Greinacher and Lubenow 2003).

To avoid being detected by the host while attaching and feeding, leeches may have evolved mechanisms to reduce sensation and subsequent pain (Hildebrandt and Lemke 2011). This is supported by the findings of Michalsen et al. (2003). In the study, patients with osteoarthritis of the knee received either the application

of diclofenac, an anti-inflammatory, or leeches to the knee (Michalsen et al. 2003). Their reported levels of pain were examined (Michalsen et al. 2003). Following treatment, patients in the leech therapy group had a greater reduction in knee pain compared to patients treated with diclofenac. The patients in the leech therapy group also reported having less joint stiffness and improved utility of the knee (Michalsen et al. 2003).

Some have suggested that derivatives of leech saliva may be a valuable intervention following neurodegeneration. Recent studies have documented the usefulness of compounds, such as eglin C, bdellastasin, and destabilase, in significantly increasing the rate of spinal neurite outgrowth in vitro. Another derivative, high-molecular-weight bdellin, was found to increase this outgrowth by 60 percent (Chalisova et al. 2001). Hirudin has also been evaluated in treating patients with mild to moderate progression of Alzheimer's disease (Li et al. 2012). In a recent investigation, patients treated with both Donepezil and hirudin showed cognitive decline to a significantly lesser extent compared to the Donepezil control group. A greater improvement in ability to perform daily activities was also noted compared to patients treated exclusively with Donepezil (Li et al. 2012).

While there have been many compounds isolated from the saliva of leeches, such as hirudin, continuing research has led to the discovery of many others (Hildebrandt and Lemke 2011). These molecules have shown tremendous efficacy in ameliorating a wide variety of ailments from myocardial infarction to cognitive decline (Topo et al. 1994; Li et al. 2012). Furthermore, although clinical trials have yet to commence, leech-derived substances have shown promise in cancer treatment (Esumi et al. 1991). Leech evolution tied to vertebrate hosts has allowed them to develop mechanisms to become efficient predators (Hildebrandt and Lemke 2011). While leeches may

predate and harm humans, the very biological mechanisms involved in this interaction has allowed humans to use the compounds involved for therapeutic purposes, and we can expect that, as research continues, even more pharmaceutically effective drugs derived from leeches will become available (Abdualkader et al. 2013).

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