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The Medicinal Leech Possesses All Mechanisms of Influence for Preventing Pathogenesis of SARS-CoV-2

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Abstract

Leeches, an unlikely allay in the fight against SARIS-CoV-2. Ten months after I contracted Covid-19, I had finally found a doctor who believed my story. Dr. Ivanovich Krashenyuk, founder of Acadamy of Hirudotherapy St. Petersburg Russia and around the world, has been teaching Systemic Leech Placement (SLP) to post-doctoral students for over 25 years and has dozens of articles published in medical journals. Without diminishing the importance of medical therapy, he states salivary cell secretions (SCS) of Medicinal Leech (ML) contain all the mechanisms of influence for preventing SARS-CoV-2 reducing mortality. Today, Dr. Krashenyuk's aim at treating post-covid syndrome encompasses application of Medicina Leech Therapy (MLT) in combination with principles of homeopathy and acupuncture. His proposed solution is based on his nearly 30 years of experience in treating children and elderly with multiple organ pathology presented of person surviving SARS-CoV-2 with ML treatment.

Keywords: SARS-CoV-2; Medicinal Leech; Systemic Leech Placement

Introduction

SARS-CoV-2's distinctive "spike" proteins help the virus infect its host by attaching on to healthy cells. A major study shows that the virus spike proteins (which behave very differently than those safely encoded by vaccines) also play a key role in the disease itself. In the study the researchers created a "pseudovirus" that was surrounded by SARS-CoV-2 classic crown of spike proteins, but did not contain any actual virus. Exposure to this pseudovirus resulted in damage to the lungs and arteries of an animal model – proving that the spike protein alone was enough to cause disease.

Tissue samples showed inflammation in endothelial cells lining the pulmonary artery walls. When the team replicated this process in the lab, exposing healthy endothelial cells (which line arteries), results demonstrated that the spike protein damaged the cells by binding ACE2. The binding alone disrupts ACE2 signaling to mitochondria which support cellular energy, and results in damage and fragmenting changing its shape [1].

SCS of ML contribute

Ficolins, and F5/8 type C domain and ficolins are involved in the recognition of bacterial cell wall components. The fibrinogen-like domain is present in proteins with affinity for erythrocytes, e.g., tachylectin-5A (TL5A). TL5A exhibits strong haemagglutinating and anibacterial activity in the presense of Ca+ ions. C domaine F5/8 type plays an important role in binding of various ligand molecules, including phospholipids and carbohydrates. Due to these features, DS-containing proteins are actively involved in cell adhesion, migration, and proliferation and activation of signaling cascades. Leech DS domain- containing proteins appear to act as lectins. As many known R- type lectins are involved in adhesion and trigger haemolysis, this molecule is of interest for further study [2]. SARS-CoV-2 viral entry into cell begins with fusion. This is primarily accomplished by molecular interaction between the virus's spike (S) protein and the host cell surface receptor, angiotensin- converting enzyme 2 (ACE2), although other host cell-associated receptors, such as, neuropilin 1 (NRP-1) and neuropilin 2 (NRP-2), and C-Type Lectin Receptors (CLRs) are recognized. Cleavage of the S protein by Proteases TMPRSS2 (transmembrane serine protease 2), furin, and cathepsin L, play a crucial role in infection, tropism, pathogenesis and clinical outcome [3]. Different cleavage sites targeted by different proteases are often associated with drastically different virulence and host cell tropism in various RNA viruses. For example, the low-pathogenicity forms of the H1N1 influenza virus has a cleavage site by trypsin-like proteases in contrast to the high-pathogenicity forms with a furin cleavage site cleaved by furin-like proteases. Trypsin-like proteases typically have a narrow tissue distribution in humans. For example, trypsin-like transmembrane serine protease 11D (gene name TMPRSS11D) is expressed only in the esophagus. Another member of the trypsin family, PRSS1, is expressed mainly in the pancreas. In contrast, furin-like proteases are ubiquitous. Thus, if a coronavirus needs to be cleaved TMPRSS11D or PRSS1, then its cellular entry is limited to the esophagus where TMPRSS11D is expressed or the pancreas where PRSS1 is expressed. However, if the virus gains a furin cleavage site, then this restriction is removed because FURIN is ubiquitous in human tissues, resulting in dramatic broadening of host cell tropism. For this reason, viruses with different cell tropism may accumulate tissue-specific genomic signatures. Although some success has been achieved in terms of the development of vaccines and antiviral drugs, specific treatment is still awaited and urgently required [4].

Literature Review

The Medicinal Leech contains wide spectrum of proteinase inhibitors, Proteinases, and Molecules of Adhesion that systematically work to target all receptors and proteases of SARS-CoV-2. TMPRSS2 is a 492 amino acid single-pass type II membrane protein. It contains a Serine protease domain of the S1 family, followed by a scavenger receptor cysteine-rich domain; an LDL receptor class A domain forms a binding site for calcium; a predicted transmembrane domaine. TMPRSS2 shares a common structural fold with conserved triad residues Ser441, His296 and Asp345 at the active site for catalytic activity [5]. TMPRSS2 shares 35% sequence identity with the transmembrane trypsin like serine protease hepsin [6]. Several drugs target this serine specific protease a1-antitrypsin the circulating protein inhibitor of neutrophil elastases [7]. It inhibits serine proteases by acting as a slow tight-binding inhibitor or suicide substrate.

Inhibitors that use this type of mechanism are called serpins. The reactive site, which lures the protease can be engineered to target specific proteases [8]. Knowledge about innate immune factors of the respiratory tract against SARS-CoV-2 is limited. Analysis of antiviral fractions, derived from bronchoalveolar lavage, revealed the presence of α1-antitrypsin (α1AT), a highly abundant circulating serine protease inhibitor. Furthermore, studies demonstrate that α1AT binds and inactivates the serine protease TMPRSS2, which enzymatically primes the SARS-CoV-2 spike protein for membrane fusion. Thus, the acute phase protein α1AT is an inhibitor of TMPRSS2 and SARS-CoV-2 entry, and may play an important role in the innate immune defense against the novel coronavirus. Findings suggest that repurposing of α1AT- containing drugs has prospects for the therapy of COVID-19. SCS of ML contribute: Eglin- like small cysteine-free proteins, a family of serine proteinase inhibitors. Eglins from leeches have inhibitory activity against neutrophil elastases and cathepsins G, Furin is a ubiquitously expressed 794-amino-acid Type-1-Transmembrane protein found in all vertebrates and many invertebrates. Its large luminal/extracellular region has an overall homology with the same regions of other members of Proprotein Convertase (PC) family which belongs to the Subtilisin Superfamily of serine endoproteases. Subtilisin Superfamily of serine proteases are composed of the subtilisin and chymotrypsin (including trypsin, thrombin, and elastase) superfamilies. These two superfamilies are evolutionarily distinct, yet the atoms that form the catalytic centre are in nearly identical positions. Furin's role is broad and ranges from homeostasis to alzheimers, cancer to Anthrax and Ebola fever. Furin's cleavage site requirements have been used to produce potent peptide- and protein-based inhibitors that block furin activity in vitro and in vivo. Perhaps the two most widely used furin inhibitors are the stoichiometric peptidyl inhibitor decanoyl–Arg–Val–Lys–Arg–CH2Cl (where Val is valine) and α1- antitrypsin Portland (α1-PDX), a bioengineered variant of α1-ANTITRYPSIN. Decanoyl–Arg–Val– Lys–Arg–CH2Cl inhibits all PCs with a low nanomolar Ki , although the alkylating properties of the reactive group limit the usefulness of this reagent. Nonetheless, in cell-culture studies, decanoyl–Arg–Val–Lys–Arg–CH2Cl blocks the processing of several furin substrates. The α1-PDX inhibitor was generated by mutating the reactive-site loop of α1-antitrypsin to contain the minimal consensus sequence for furin cleavage (–Arg–Ile–Pro–Arg–) (where Ile is isoleucine and Pro is proline), and it is highly selective for furin in vitro (Ki=600 pM), although at higher concentrations it will also inhibit other PCs. In biochemical, cellular and animal studies, α1-PDX has been used to block furin activity and to prevent the production of pathogenic viruses, bacterial toxin activation, and cancer metastasis [9]. The SCS of ML mainly contribute tryptase inhibitors and Eglins. Cathepsin L belongs to a family of proteases that are responsible for recycling cellular proteins inside of the lysosomes. These proteases are comprised of serine, aspartate, and cysteine peptidases and exhibit endo or exopeptidase activities. In humans cathepsins have a role in various physiological processes, such as apoptosis, antigen processing extracellular matrix remodeling and MHC class II immune responses. Elastolytic cysteine proteases are mobilized to the cell surface of macrophages and other cells under inflammatory conditions, which lead to accelerated collagen and elastin degradation, exacerbating inflammation and tissue damage. (8) SCS of ML contribute: Cystatins, specific Cathepsin L inhibitors. C-Type Lectin Family: C – Type: Calcium dependent lectins are a family of lectins which share structural homology in their high affinity carbohydrate-recognition domains. Lectins expressed on dendritic cells and macrophages interact with different glycans, often expressed on pathogen-derived glycans. These proteins function as adhesion and signaling receptors in many pathways, including homeostasis and innate immunity, and are crucial in inflammatory responses and leukocyte and platelet trafficking. Activation of CLT's occurs through glycan binding. CTLs include collectins, selectins, endocytic receptors, and proteoglycans, some of which are secreted and others are transmembrane proteins. They often oligomerize, which increases their avidity for multivalent ligands. CTLs differ significantly in the types of glycans that they recognize with high affinity [10]. Members of the mannose receptor family of R-type lectins are also in the C-type lectin family and represent a unique group of lectins with more than one type of lectin domain. However, although the mannose receptor and Endo180 can bind to sugar ligands, it is not clear whether any of the R- or C-type lectin domains in either phospholipase A2 receptor or DEC-205 can bind to sugars. SCS of ML contribute molecules: vWFA domain and R-type lectin. Neuropilin-1 (NRP-1), a member of a family of signaling proteins, was shown to serve as an entry factor and potentiate SARS Coronavirus 2 (SARS-CoV-2) infectivity in vitro. This cell surface receptor with its disseminated expression is important in angiogenesis, tumor progression, viral entry, axonal guidance, and immune function. Upon infection, the SARS-CoV-2 Spike (S) protein is cleaved by host cell protease, furin, into S1 and S2 polypeptides, thereby exposing the CendR motif in S1. This motif is named for the "C-end terminal rule," which is the requirement for the presence of a cationic amino acid, usually arginine, at the carboxyl terminus of the ligand, resulting in an RXXR configuration. The CendR binding pocket lies within the b1 domain of NRP-Daly and colleagues recently showed that the CendR motif in SARS-CoV-2 S1 protein binds to NRP-1 and potentiates virus infectivity [11].

Discussion

Angiotensin converting enzyme 2 (ACE-2), is an integral membrane protein and a zinc metalloprotease of the ACE family. Angiotensin-converting enzyme 2 (ACE2) is an aminopeptidase that converts Angiotensin (Ang) II into Ang 1-7. It is well-known that Ang II, acting on AT1 receptors, exerts powerful vasoconstrictor, pro-fibrotic, and pro-inflammatory effects. Moreover, SARS-CoV-2 disrupts the ACE/ACE2 physiological balance and activates the Ang II/AT1R pathways, leading to severe complications of the disease. Studies using different models of lung injury showed that the down-regulation of ACE2 receptors triggers important inflammatory lesions in the respiratory tree (alveolar wall thickening, edema, infiltrates of inflammatory cells, bleeding) which appear to be mediated by angiotensin II [12].

Cytokine Storm (CS) is a response characterized by overactivated inflammatory, innate immune response, and impaired protective, adaptive immune response. The CS is characterized by hyper production of an array of pro-inflammatory cytokines and is closely associated with poor prognosis [13]. Cytokine storms on cardiac and vascular endothelium may facilitate the onset of coagulopathies, thereby increasing the probability for organ ischemia and for multiple pulmonary and cardiovascular complications.

The virus downregulates ACE2, exacerbating the pro-inflammatory milieu of high ACE/ACE2 ratio (Figure 1). (22)SCS of ML contain: Metalloproteases-M13 One of their most important functions is the activation of biologically active peptides, particularly peptides involved in the regulation of blood pressure (angiotensin and bradykinin). Figure 1

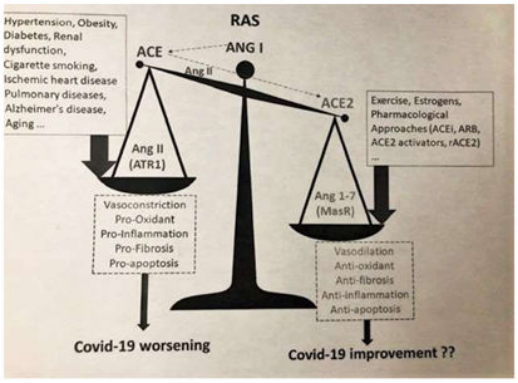

Figure 1: Proteinase inhibitors, proteinases, and molecules of adhesion all contribute to vasodilation, anti-oxidant, anti-fibrosis, anti-inflammation, anti-apoptosis.

Proteinase Inhibitors, Proteinases, and Molecules of Adhesion all contribute to Vasodilation, Anti-oxidant, Anti-fibrosis, Anti-inflammation, Anti-apoptosis 128 lb caucasion female, age 56, history of Multiple Sclerosis (MS) X5 years, Tysabri 300mg infusion every 28 days X5 years. January 23, 2020 - History: difficulty breathing/dry cough/night sweat/head and jaw pain X1 day. - no history of asthma, negative chest-x-ray/negative flu, Viral upper respiratory infection. Etiology unknown. Prescribed meds: Albuterol Inhaler, Codein Cough suppressant, Tussigon perles [14]. After 3 days of difficulty breathing - without improvement - I applied ML to chest. Results were immediate - within 5 minutes- ability to feel air exchange upon taking in a breath; and 12 hours later – breathing and cough issues completely gone. It was a remarkable improvement after just one a leech treatment. Three months later, serum antibodies revealed no antibodies present. Hirudo medicinalis, Hirudo orientalis, and Hirudo verbana contain many proteins homologous to those associated with blood feeding and homeostasis with synergistic properties (H.medicinalis, H. orientalis, H. verbena). A Combined analysis of transcriptome and proteomic data reveal Salivary Cell Secretion (SCS) have similar composition. Proteomic datasets obtained by different sample preparation methods and mass spectrometry techniques were combined to create a final list of the identified proteins for each medicinal leech species. The sample preparation method is critical to the resultant repertoire of the identified proteins because the SCS consists of both low- and high- molecular weight components and contains proteinase inhibitors, glycoprotein complexes, and lipids. The latter may form complexes with proteins. Genes encoding SCS anticoagulants and blood meal related proteins did not show the differential expression between salivary and muscle cells. Molecular samples of Saratin, eglin C, bdellins, hirustasin, destabilase, metallocarboxypeptidase inhibitor, apyrase, and Angiotensin Converting Enzyme (ACE) by the real time Polymerase Chain Reaction (PCR) of additional, independent tissue- specific cDNA libraries constructed for salivary cells and muscles were examined. The real-time PCR results for hirudin and destabilase also confirmed indicating that genes encoding anticoagulants and blood meal related proteins are involved not only in the blood feeding, but contribute to other, yet unknown physiological functions. The genome assembly was validated by the National Center for Biotechnology Information (NCBI). It was checked for adaptors, primers, gaps, and low-complexity regions. The genome assembly was approved, and the accession numbers MPNW00000000 and Bio Project PRNJNA257563 were assigned. The genome annotation performed in the study may serve as a blueprint for future experimentation on the medicinal leech as a model organism and provides a database of sequences encoding the unique bioactive leech proteins for use in developing novel pharmacological compounds [15].

Proteinase Inhibitors

Antistatin

Proteins of this family are commonly found in blood sucking leeches and play a role in the inhibition of blood coagulation. Their main targets are serine proteases participating in haemostasis, such as the factor Xa, kallikrein, plasmin, and thrombin [16].

CAP/CRISP

The cysteine-rich secretory protein/antigen 5/ pathogenesis- related 1 protein (CAP) superfamily includes numerous protein families, particularly Cysteine-Rich Secretory Protein (CRISP). (16 Cystatins: Small protein inhibitors of cysteine proteases (cathepsins B, H, C, L, S). Cathepsin L, H The identified sequence show similarity to protein sequences from the haematophagous parasitic nematode Ancylostoma caninum (hookworm), such as the potassium channel blocker AcK1 and the possible platelet aggregation inhibitor HPI, as well as to the snake toxins triflin and natrin. A cystatin sequence only in the proteome of H. verbana Among the differentially expressed genes, identified sequences with new “Cys-rich” motif. This group of proteins is characterized by the presence of a signal peptide and two cysteine patterns [17].

Eglin-like

Small cysteine-free proteins, a family of serine proteinase inhibitors. Eglins from leeches have inhibitory activity against neutrophil elastases and cathepsins G and also to participate in the protection of the crop contents from untimely proteolysis. Note: Sequences identified in the present study have low homology to the classical eglin from leech [18].

PAN domain

This domain is present in numerous proteins, including the blood proteins plasminogen and coagulation factor XI. The PAN/apple domain of plasma prekallikrein is known to mediate its binding to high-molecular weight kininogen, and the PAN/apple domain of the factor XI binds to the factors XIIa and IX, platelets, kininogen, and heparin.

Alpha-2-macroglobulin

Multifunctional a2M is involved in the inhibition of a broad range of proteases (serine, cysteine, aspartic, and metalloproteases), interacts with cytokines, and hormones, and plays a role in zinc and copper. This protein exhibits affinity for collagens I, III, and IV and thereby inhibits collagen-mediated platelet adhesion [19].

Proteases

Metalloproteases

Metalloproteasesof the M12,M13, and M28 family are the major enzymatic components of the SCS. The M12B (ADAM/reprolysin)

peptidases are a large family of disintegrin-like metalloproteinases that have a broad range of functions and are involved in many physiological processes. In haemostasis, secreted proteases of the M12 family can participate in the inhibition of platelet adhesion and clot softening due to degradation of fibrinogen. These proteins exhibit metal-dependent proteolytic activity against extracellular matrix proteins (gelatine, fibrinogen, fibronectin), thereby affecting the regulation of inflammation and immune responses. In mammals, proteases of the M13 family are involved in the formation and development of the cardiovascular system and in the regulation of neuropeptides in the central nervous system. One of their most important functions is the activation of biologically active peptides, particularly peptides involved in the regulation of blood pressure (angiotensin and bradykinin) [20]. The identified sequences of M28 family exopeptidases belong to the Q-type carboxypeptidases, also known as lysosomal dipeptidases or Plasma Glutamate Carboxypeptidase (PGCP). These peptidases were shown to be involved in the regulation of the metabolism of secreted peptides in the blood plasma and the central nervous system in mammals. In addition, secretions contain carboxypeptidase inhibitors, presumably preventing untimely digestion of blood meal by other peptidases.

Superoxide dismutase (SODC)

This family of metalloproteins is mainly typical of eukaryotes and is involved in free radical in·activation reducing oxidative process and appears to exhibit an antibacterial effect along with other proteins of the innate immune system. During feeding and digestion SODC appears to prevent unwanted blood oxidation during feeding and digestion.

Carbonic anhydrase

Main enzyme in bicarbonate buffer system involved in tissue regulation of pH values in blood, digestive tract, and other tissue. It appears to cause a local increase in acidosis at the bite site, decreasing the activity of blood coagulation factors [21].

Hyaluronidase

This family includes heparinases related to connective tissue. These enzymes catalyse the hydrolysis of hyaluronic acid, resulting in the loss of structural integrity of the extracellular matrix and thereby facilitating the penetration of anticoagulants and other active molecules deeper into the tissue. The low molecular-weight heparin produced cleavage by heparinase suppresses and inhibits blood coagulation.

Apyrase

These nucleotidases are involved in the enzymatic degradation of ATP and ADP to AMP. Ampyrase and 5'-nucleases are well characterized components of anticoagulation because they remove ADP, an important inducer of platelet aggregation at sites of tissue injury.

Adenosine/AMP deaminase

Catallyses the hydrolic deamination of adenosine to form iosine. ADA is thought to play an important role in the removal of adenosine because of its involvement in pain perception process.

Molecules Involved In Adhesion

Ficolin

Ficolin are a component of the innate immune system and trigger a lectin-dependent pathway of complement activation. In invertebrates, ficolins are involved in the recognition of bacterial cell wall components. The fibrinogen-like domain is present in proteins with affinity for erythrocytes, e.g., tachylectin-5A (TL5A). TL5A exhibits strong haemagglutinating and anibacterial activity in the presense of Ca+ ions.

F5/8 type C domain

A number of identified sequences contain one or several Discoidin Motifs (DS) known as F5/8 type C domain. This domain is present in numerous transmembrane and extracellular proteins, e.g., neuropilins, neurexin IV, and discoidin domain receptor proteins, and in proteins involved in haemostasis, such as coagulation factor V and VIII. DS domain plays an important role in binding of various ligand molecules, including phospholipids and carbohydrates. Due to these features, DS- containing proteins are actively involved in cell adhesion, migration, and proliferation and activation of signaling cascades. Leech DS domain- containing proteins appear to act as lectins with high affinity to galactose and may be components of the innate immune system of the leech. In addition they can bind to collagen or phosphatidylserine on the surface of platelets and the endothelium and thus by competitive inhibition, impair interactions between haemostatic factors.

Low-Density Lipoprotein Receptor (LDLR)

This family is important component of blood plasma and is involved in recognition and endocytosis of low-density lipoproteins in mammalian blood. In contrast to known homologous proteins, these receptors are secretory rather than membrane proteins, and they contain four LDLR class A (cysteine-rich) repeats. It is considered that this protein may be utilized by the leech for the scavenging and transportation of cholesterol-rich lipoprotein complexes.

R-type lectin

Proteins that contain the ricin-type beta-trefoil lectin domain have been found in prokaryotes and eukaryotes. In animals, R-type lectins exhibit diverse activities. They are present in scavenger receptors (mannose, fucose, collagen receptors), N-acetylgalactosaminyltransferases, haemolytic toxins and apoptosis-inducing cytotoxins. Previously, similar sequences were identified in leech transcriptomes; however, the author assumed that this molecule has a mitochondrial localization. As many known R-type lectins are involved in adhesion and trigger haemolysis, this molecule is of interest for further study. The R-type lectins are members of a superfamily of proteins, all of which contain a Carbohydrate-Recognition Domain (CRD) that is structurally similar to the CRD in ricin. Ricin was the first lectin discovered and it is the prototypical lectin in this category. R-type lectins are present in plants, animals, and bacteria, and the plant lectins often contain a separate subunit that is a potent toxin.

vWFA domain

This domain is present in various plasma proteins: complement factors, integrins, and collagens VI, VII, XII, AND XIV. One protein identified in the leech proteome is a secreted protein that consists of four copies of the vWFA domain. The sequence contains several putative recognition sites: the Metal Ion-Dependent Adhesion Site (MIDAS), the integrin-collagen binding site, and the glycoprotein Ib (GpIb) binding site. According to Blast X analysis, this domain is homologous to type VI collagen. Considering the domain organization of the protein and the presence of glycoprotein and collagen binding sites, one of the putative mechanisms of action involves binding to the surface of the endothelium or platelets, thereby preventing their interactions with collagen. This binding underlies the competitive inhibition during haemostasis (platelet scavenging).

Leech Derived Tryptase Inhibitor (LDTI)

Leech Derived Tryptase Inhibitor (LDTI) obtained from an extract of medical leeches. Tryptase is the main component of the secretory cytoplasmic granules of mast cells and leads to the destruction of extracellular matrix proteins. The important role of tryptase in allergic and inflammatory reactions is known. As with many of the compounds already described, recombinant LDTI has been created. Bdellins - are a group of polypeptides with a small molecular weight, among which Bdellins A with a molecular weight of 7 kDa are distinguished (bdelastazine with a molecular weight of 6.3 kDa is most studied in this group) and Bdellins B with a molecular weight of 5 kDa. Numerous forms of bdellins A and B were isolated by equilibrium chromatography; Both are potent inhibitors of trypsin, plasmin, and acrosin sperm. They do not block the activity of chymotrypsin, tissue and plasma kallikreins, subtilisin. They were first discovered in 1969. A recombinant form of bdelastazine was obtained.

Hirustazin

Hirustazin belongs to the same family of antistasin serine protease inhibitors. Isolated in 1994 from extracts of medical leeches. The molecular weight of hirustazin is 5.9 kDa. It inhibits tissue kallikrein (but not plasma), trypsin, chymotrypsin and cathepsin G neutrophils. The ability of hirustazin to block tissue kallikrein is a very important property, since the latter catalyzes the release of highly active kin ins. Kin ins through specific receptors on target cells modulate a wide range of biological activities, including those involved in maintaining normal blood pressure. Hirustazin is also obtained in recombinant form [1,2]

LCI (Leech Carboxypeptidase Inhibitor)

LCI (Leech Carboxypeptidase Inhibitor) is a carboxypeptidase A inhibitor. It was isolated in 1998 and has two isoforms with molecular weights of 7 .3 and 7 .2 kOa. It is steady in a wide range of pH and temperatures. Since this inhibitor is part of the secretion of the salivary glands of a medical leech, it can be assumed that it can block the hydrolysis of kin ins by metalloproteinases at the site of biting of the leech of the skin, thereby enhancing the kinin induced increase in blood flow. Created recombinant LCI [1,2].

Eglins

Eglins are low molecular weight proteins from medical leech extracts with molecular weights of 8.073 and 8.099 kDa ("b" and "c" forms, respectively). They were first described in 1977 by U. Seemuller., et al. Inhibit the activity of alpha-chymotrypsin, mast cell chymase, subtilisin and neutrophil proteinases, elastase and cathepsin G. They have high resistance to denaturation and heating. The inhibitory spectrum of eglin "C" allows us to consider it one of the most important anti-inflammatory agents. Primarily contained in the walls of the intestines, though its been discovered in the secretion of it salivary glands.

Destabllase

Destabilase-lysozyme has unique bactericidal and antimicrobial effects towards gram-positive and gram-negative microorganisms (zavalova LL. et al 2000 my book pg 74). Anticoagulation/ Thrombolytic effects/anti-inflammatory, anticoagulatory, effects. Has the unique ability to monomerize 0-dimer at the expense of splitting epsilon. Neurotrophic factors - associated with the presence of destabilase-m, bdelatazine and bdellin-B. (1,2) Following a leech bite, it has to establish a sucking pathway (extracellular matrix degradation) Hyaluronidase (27.5 kDa) and collagenase (100 kDa) enzymes are secreted to facilitate tissue penetration and spread of their bioactive molecules. These enzymes also support antimicrobial activity. 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. Acetylcholine is also a component in leech secretions, causing endothelial muscle relaxation and vasodilatation. [19] Destruction of the blood vessel wall for sucking blood causes activation of platelets and the coagulation cascade, which are fatal for the leech; and therefore, leech secretions contain many bioactive molecules to locally inhibit these actions. In a normal host, wall destruction causes spread and release of collagen particles sparking free Von Willebrand Factor (vWF). This complex strongly binds to Glyco Protein (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 to stop any bleeding. As a result another chain of releasing substances such as thromboxane A2, platelet activation, and coagulation cascade occur. In leech secretions, various molecules (saratin, calin, decorsin, and apyrase) react against different parts of this chain. 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. Other leech- secreted proteins, calin and leech antiplatelet protein, show the same action on platelet adhesion. 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. As mentioned previously, ADP has a critical role in platelet aggregation by especially activating GPIIb–IIIa receptors and increasing affinity of platelets to vWF. The SCS of ML 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. 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. The enzyme collagenase also destroys collagen

particles, which initiates all these adhesion and aggregation reactions, and provides additional supportive action to the inhibitory effects. Because coagulation during feeding is fatal for leeches, the multitude effects of anticoagulation are necessary. Also, molecules of Hirudin and gelin mainly work as thrombin inhibitors, factor Xa inhibitor – creating a break in the cascade, and destabilase provides a fibrinolytic effect. In addition these inhibitors may indirectly have a negative impact on platelet functions. Hirudin is a 7.1- kDa protein and irreversibly binds to thrombin, which causes consumption of active thrombin and results in antithrombin activity. 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. Gelin is an eglin analog with inhibitory effects on especially thrombin, chymotrypsin, cathepsin G, and neutrophil elastase. 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. In addition, as previously stated, antistasin directly inhibits factor Xa. Ghilantens, LDTI, C1 inhibitor, and eglins have possible anticoagulant effects, potentially via direct and/or indirect inhibition of coagulation factors. Leech-derived tryptase inhibitor (LDTI) has three isoforms (a, b, and c) and acts by inhibiting proteolytic enzymes of mast cells (Table 1). LDTI, a Kazal-type serine protease inhibitor, especially inhibits mast cell tryptase, in addition to trypsin and chymotrypsin. 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. 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. Eglin C is an inhibitor of human neutrophil elastase and cathepsin G. 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. Once released, 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. 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). Separately, bdellins and bdellastasin were detected as trypsin, plasmin, and sperm acrozine inhibitors. 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, an area that needs further study. Complement component C1 has a critical role in the classic pathway of the complement system. Complement C1 inhibitor is a 60- to 70-kDa protein in SCS, and mechanism is only partially known. This protein may be 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 SCS of ML inhibits both the coagulation cascade and kinin–kallikrein system. 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. Inhibition of carboxypeptidases from SCS should not affect bradykinin action via B2 (constitutive) receptors, but may prevent B1 (inducible) receptors. Although these two receptors basically work with similar mechanisms, it has been stated that B1 receptors are related to chronic inflammation, whereas B2 receptors are related to acute inflammation. Strong correlations have been found between B1 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. (19)(21) Destabilase is an enzyme with glycosidase activity and shows both antibacterial and fibrinolytic actions. This enzyme has various isoforms with different capabilities, and is extracted from different leech species. Destabilase has a major degradative action on stabilized fibrin and it should also be evaluated as an anticoagulant agent. To date, only two main molecules, destabilase and chloromycetin, have been shown to have antimicrobial activity. As previously stated, destabilase has β-glycosidase activity, which directly disrupts β1–4 bonds that are important in the peptidoglycan layer in bacterial cellwalls. This action is similar to that of lysozyme (muramidase) that is commonly found in human saliva and lachrymal fluid. Other studies have shown that antimicrobial activity does not only depend on glycosidase enzymatic activity, but it also has nonenzymatic components. For example, the denaturated form destabilase shows a dose-dependent bacteriostatic effect on Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. Chloromycetin is a potent antibiotic found in certain leech secretions, but unfortunately the data are limited about this molecule. Additionally, theromacin, theromyzin, and peptide B have been isolated as antimicrobial peptides. In vitro studies have indicated the anticancer effects of leech SCS in relation to anticoagulation since coagulation is related to metastasis and tumor progression; and blocking the cascade can have an antitumor effect. 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. SCS 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. 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. 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 SCS have opposing actions (anti-apoptosis) 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 SCS. Pteridines have been isolated as potential antitoxin substances, but it is clear that this activity cannot be related to only one substance. Another interesting aspect, available

studies indicate that there are barriers to microbial gut colonization of ML at various levels that the naturally occurring leech symbionts can overcome but many other bacterial species cannot. Other bacterial components may be able to colonize the leech intestine under certain conditions if the leech is weakened due to starvation, or ingested blood is not fresh. Medicinal Leech SCS species specific Numbers corresponds to species: H. verbena, H. medicinalis, H. orientalis

Molecular mass, daltons			
Protein	**Literature data**	**CM-10 chip**	**golden chip**
Tryptase inhibitor	4340	-	4719 (1)
	4481.18	-	4736 (2)
	4719.4	-	4739 (3)
	4737.45	-	-
Bdellin B	4380	4832 (2)	-
Hirustasin	5738.741	5735 (1)	-
	5866.587	5747 (2)	-
Bdellastasn (bdellin A)	6332.6	6341 (1)	-
	6334.2	-	-
Eglins b and c	8073	8078 (1)	8066 (3)

Table 1: Molecular mass, daltons protein literature datacm-10 chip golden chip.

Taken into account is that species often cross-breed and offspring develop unique coloration. "This raises the tantalizing prospect of three times the number of anticoagulants, and three times as many biomedically important developments in areas like protease inhibitors," said Mark Siddall of the American Museum of Natural History.

His genetic research revealed that commercially available medicinal leeches used around the world in biomedical research and postoperative care have been misclassified for centuries. Until now, the leeches were assumed to be the species H.medicinalis, but new research reveals they are actually a closely related but genetically distinct species, H. verbena.

The study also shows that wild European medicinal leeches are at least three distinct species, not one. His results appeared in the April 10, 2007 online version of the journal proceeding of the Royal Society B.

Conclusion

In conclusion, MLT is a valuable traditional technique with strong biochemical actions. Findings confirmed that genes encoding anticoagulants and blood meal related proteins are involved not only in the blood feeding, but contribute to other synergistic physiological functions warranting further investigations. Unfortunately, modern clinicians do not support the practice of MLT, but many do believe the use of leeches in certain, very specific situations has potential to save lives and limbs.

MLT is not recommended when there is hemorrhagic diathesis, anticoagulant therapy, leukemia, bone marrow suppression, dialysis, cirrhosis, chemotherapy, radiotherapy, and cachexis. I have not received fees for consulting or research. I am not employed by a related company or hold stocks or shares in a company which might be affected by the publication this paper.

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