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Case Report

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

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Leeches, an unlikely ally in the fight against SARS-CoV-2. Ten months after I contracted Covid-19, I had finally found a doctor who believed my story. Dr. lvanovich Krashenyuk, founder of Academy 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 secretions of Medicinal Leech (ML) contain all the mechanisms of influence for preventing SARS-CoV-2, reducing mortality [1]. Today, Dr. Krashenyuk's aim at treating post-covid syndrome encompasses application of Medicinal 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 [2]. He specializes in Cerebral palsy and attributes the discovery of neurotrophic factor as one element in the treatment of nervous system diseases to include Parkinson's disease, multiple sclerosis, Alzheimer's, symptoms of traumatic brain dysfunction, to name a few [3]. The case study presented involved treatment with Hirudo medicinalis and Hirudo verbana (H. medecinalis, and H. verbana) at the start of the SARS-CoV- 2 Pandemic January 20th, 2020.

"Salivary cell secretions can be classified according to function and possible roles in hemostasis respectively Proteinase Inhibitors, Proteases, and Molecules involved in adhesion… Their genome assembly was validated by the National Center for Biotechnology Information (NCBI)…Results were 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" [4]. This study identified 189 proteins of H. medicinalis, 86 proteins of H. orientalis, 344 proteins of H. verbana [4].

By nature, following a leech bite, molecules are released from the saliva of the leech in order to breakdown tissue, create vasodilation, provide muscle relaxation, and ultimately increase blood flow to bite site during ingestion of blood without added interference of clots that normally results from insult to tissue, triggering von Willebrand factor [5]. In addition, leech salivary secretions (LSS) provide antimicrobials ensuring its survival with every feeding preventing inflammatory response while ingesting blood. Research sponsored by the Swiss National Funds the National Science Foundation (grant # MCB – 0334267) and the Sandoz Foundation; experimentations demonstrate leeches' ability to survive exposure to virulent pathogens [6]. These unique synergistic molecules have allowed their continued existence for thousands of years. "The oldest literary source" that describes reference to a leech can be found in the Old Testament (15th-5th Centuries B.C) [7].

Entry routs for Sars-CoV-2 begins with Binding and attachment of the spike protein and ends with fusion/ transmission/ replication of virus [8]. Pharmaceutical interventions are aimed at preventing cleavage of S protein [9]. Targeting enzymes such as transmembrane protease serine S1 member 2 (TMPRSS2), Furin, Cathepsin B and L and receptor sites such as ACE2 and neuropilin 1 & 2 (NRP-1, NRP- 2) to name a few, are of major interest due to a highly mutating capabilities [10]. Noteworthy of mentioning, Sars-CoV-2 is considered a Class 1 Fusion glycoprotein. Class 1 Fusion glycoproteins contain hemagglutinin molecules (HA) on the surface of the virus molecule similar to influenza extending from the surface of the virus and binding and mediating viral entry to host cell [11]. Hemagglutinin causes red blood cells to agglutinate or clump together blocking mall blood vessels throughout the body, depriving tissue of oxygen and nutrients [12].

"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 pseudo virus 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 [9].

• "TMPRSS2 is a 492 amino acid single-pass type II membrane protein involved in physiological and pathological processes. It contains a Serine protease domain of the S1 family, followed by a scavenger receptor cysteine-rich domain; TMPRSS2 shares a common structural fold with conserved triad residues Ser441, His296 and Asp345 at the active site for catalytic activity" [13]. TMPRSS2 shares 35% sequence identity with the transmembrane trypsin like serine protease hepsin. Serine proteases are known for triggering complement activation, blood coagulation, inflammation and fibrinolysis [14].

LSS Contain
R-type Lectins involved in adhesion and trigger haemolysis [4]. Bdellins inhibit trypsin, plasmin, and acrosin sperm [1, 2, 3].

Antistasin Inhibits tissue kallikrein, trypsin, chymotrypsin and cathepsin G neutrophils [4]. Leech Derived Tryptase inhibitor – inhibits mast cell tryptase in addition to trypsin and chymotrypsin [1, 2, 3].

Destabilase lysozyme has unique bactericidal and antimicrobial effects towards gram positive and gram negative microorganisms. In addition Anticoagulation/Thrombolytic effect/anti- inflammatory, anticoagulatory effects [1, 2, 3].

• "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 which 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 center are in nearly identical positions" [14]. "Furin's role is broad and 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" [10].

LSS Contain
Eglins inhibit alpha chymotrypsin, mast cell chymase, subtilisin, neutrophil proteinases, Elastase, cathepsin G [1, 2, 3].

Factor X breaks down coagulation cascade (direct anticoagulant) [5].

Antistasin Inhibits tissue kallikrein, trypsin, chymotrypsin and cathepsin G neutrophils [1, 2, 3]. Main target are serine proteases related to factor Xa, kallikrein, plasmin, and thrombin [4].

Leech Derived Tryptase inhibitor – inhibits mast cell tryptase in addition to trypsin and Chymotrypsin [1, 2, 3].

Destabilase lysozyme has unique bactericidal and antimicrobial effects towards gram positive and gram negative microorganisms, and anticoagulation/thrombolytic /anti-inflammatory properties [1, 2, 3].

Bdellins inhibit trypsin, plasmin, and acrosin sperm [1, 2, 3].

• "Cathepsin B/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" [16].

LSS Contain
Cystatins - inhibitors of cysteine proteases cathepsins B, H, C, L, S [4].

Eglins inhibit alpha chymotrypsin, mast cell chymase, subtilisin, neutrophil proteinases, Elastase, cathepsin G [1, 2, 3].

Antistasin Inhibits tissue kallikrein, trypsin, chymotrypsin and cathepsin G, neutrophils [1, 2, 3]. Main target are serine proteases related to factor Xa, kallikrein, plasmin, and thrombin [4]. Leech Derived Tryptase inhibitor – inhibits mast cell tryptase in addition to trypsin and Chymotrypsin [1, 2, 3].

Destabilase lysozyme has unique bactericidal and antimicrobial effects towards gram positive and gram negative microorganisms. In addition Anticoagulation/Thrombolytic effect/anti- inflammatory, anticoagulatory effects [1, 2, 3].

• "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 (Sequence dependency C- end rule peptide) 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-1. B1/B2 domains are coagulation factor domain Daly and colleagues recently showed that the CendR motif in SARS-CoV-2 S1 protein binds to NRP- 1 and potentiates virus infectivity" [17].

LSS Contain
"Molecules involved in adhesion - F5/8 type C domain molecules. This domain is present in numerous transmembrane and extracellular proteins, e.g., neuropilins, neurexin IV, and

discoidin domain receptor proteins, an in proteins involved in hemostasis such as coagulation factor V/VIII. DS domain plays an important role in binding of various ligand molecules, including phospholipids and carbohydrates. Due to these features DS containing signaling cascades. Leech DS domain- containing proteins appear to act as lectins with high affinity to galactose and may be component of the innate immune system of leech" [4].

• "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 (Angiotensin I) 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" [18]. "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 [19]. Cytokine storm (CS) is a response characterized by overactivated inflammatory, innate immune response, and impaired protective, adaptive immune response potentially facilitating a Cytokine storm triggering coagulation cascade [20].

LSS Contains

"Metalloproteases- M12, M13, M28 are part of a family of disintegrin-like metalloproteinases that have broad range of fuctions – Vladislav V. Babenko, et.al, July 2020M12 family that 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" [4].

"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)" [4].

"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" [4].

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" [4].

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" [4].

According to Professor Vorobyev P.A, lead professor Moscow Scientific Therapeutic Society, analysis of brain tissue from deceased Covid-19 patients, demonstrated classical signs of hemorrhagic stroke with unusual mechanism of damage to brain tissue -diapedesis. Diapedesis refers to the passage of blood cells through the intact walls of the capillaries, typically accompanying inflammation... In addition, suspected lesions may affect serotonin system, leading to depression [3]. In his observations he concludes that Post Covid Syndrome includes chronic microvascular disease with a predominant lesion of the nervous system (brain, autonomic and peripheral systems) and skin [3].

Coagulation and Inflammation follow their respective pathways but are intertwined as evidenced by numerous studies. Results show coagulation factors get triggered under excessive inflammation [21]. Proteinase Inhibitors can help prevent such responses and can be found in the salivary secretions of respective leech.

December 9th, 2021 - According to Dr. David Agus - medical contributor to CBS news, and co-chair of the Global Health Care Security Consortium, on an interview with Stephen Colbert…he discusses Delta and Omicron differences related to the Pfieser and Moderna vaccines/boosters. He stated that getting a booster of the vaccine -more of it- boosts ones immune system by 40 fold in order to take on the various entry avenues of everchanging mutating virus. There are 40 different changes or mutations that allow it easier access to our cells in regards to Omecron . Dr Agus announced that a Proteinase Inhibitor pill, under FDA review (due to be released), would prevent hospitalizations [22].

Case Study

128 lb Caucasian 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.

Urgent Care Findings: negative chest-x-ray/negative flu swab

Diagnosis

Viral upper respiratory infection. Etiology unknown. Prescribed meds: Albuterol Inhaler, Codeine Cough suppressant, Tussigon perles.

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.

Genus

Hirudo

Species

H. verbana/H. medicinalis Amt: #20 total Age of leeches: 3 months old

According to Mark Siddall of the American Museum of Natural History, the potential for "three times number of anticoagulants may exist than previously expected because leeches are known to "crossbreed" as exemplified by their different designs and colors.

In 2007 he revealed startling discovery through his research. He discovered " that commercially available medicinal leeches used around the world in biomedical research and postoperative care have been misclassified for centuries; and until now, the leeches were assumed to be the species H.medicinalis, but were actually a closely related but genetically distinct species, H. verbana" [23].

Chart from article titled: Proteins and Peptides of the Salivary Gland Secretions of Medicinal leech, Hirudo verbana, and Hirudo orientalis (I.P. Baskova, et al, 2007) shows respective protein comparison in Literature, CM-10 chip and golden chip. Numbers corresponds to species: (1) H. verbana (2) H. medicinalis (3) H. orientalis [24].

Protein	Molecular mass, daltons		
	Literature data	CM-10 chip	golden chip
Tryptase inhibitor [10]	4340 4481.18 4719.4 4737.45		4719 (1) 4736 (2) 4739 (3)
Bdellin B [11]	4380	4832 (2)	
Hirustasin [12]	5738.741 5866.5869	5735 (1) 5747 (2)	
Bdellastasn (bdellin A) [13]	6332.6 6334.2	6341 (1)	
Eglins b and c [14]	8073 8099	8078 (1) 8068 (2) 8090 (1)	8066 (3) 8070 (2, 3)
Destabilase-Lysozyme [15]	12677.6 12724.1 12749.7 12784.4 12803.4 12839.7 12938.2	14780 (3) 1782 (3) 12836 (2) 12850 (2)	12779 (3) 12780 (3) 12812 (1)

(Source: I.P. Baskova, et. al, p. 8, Oct 2007)

Proteinase inhibitors by name: bdellins (inhibitors of trypsin, plasmin, and acrosin), hirustasin (inhibitor of tissue kallikrein, trypsin, alpha-chymotrypsin, and granulocyte cathepsin G), tryptase inhibitor, eglins (inhibitors of alpha-chymotrypsin, subtilisin, and chymasin and the granulocyte proteinases elastase and cathepsin G), inhibitor of factor Xa, hirudin (thrombin inhibitor), inhibitor of carboxypeptidase, and inhibitor of complement component C1s [25].

List of Proteinase Inhibitors from Dr. Krashenyuk's article titled: Coronavirus COVID-19 - Theoretical and Practical Substantiations for Reducing Mortality from Complications [1, 2, 3].

• 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 [1, 2].

• 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 by H Fritz., et al. in 1969. A recombinant form of bdelastazine was obtained [1, 2].

• 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) 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 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 [1, 2].

• Destabllase - destabilase-lysozyme has unique bactericidal and antimicrobial effects towards gram-positive and gram-negative microorganisms. 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].

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" [4].

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 [26]. MLT is not recommended when there is hemorrhagic diathesis, anticoagulant therapy, leukemia, bone marrow suppression, dialysis, cirrhosis, chemotherapy, radiotherapy, and cachexis [5].

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 [27, 28].

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