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Short Communication

Complementary/Alternative Medicine Section

Snake Venom, an Unbeheld Drug for Nipah Virus? A Lead from Ayurveda

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ABSTRACT

Newly emerging viral fevers are affecting the people all over the world. Recently Nipah outbreak occurred in Kerala (May 2018) and claimed many deaths. Anti-viral drugs for these dangerous viruses have not been discovered yet. A major hurdle is the low incidence of discovery of efficient molecules with anti-viral property. In Ayurveda, a handful of formulations are explained for serious disorders especially fevers in which one of the constituent is snake venom. No researches have been conducted regarding this due to many ethical concerns and ignorance. Anyway, it is possible to draw a general outline of anti-viral activity of the snake venom in terms of available researches. Anti-viral property of snake venom is a known fact today based on researches done on dengue virus, parvo virus etc. Researches have shown that viruses have a strong affinity towards phospholipase 2 (PLA2), as most of these viruses use host machinery for infection and replication. However, a structural homology between snake PLA2 and human PLA2 has also been proved. Likewise, due to affinity, virus may attach to snake venom PLA2. Further, snake venom PLA2 can inactivate the lipid bilayer of virus causing partial exposure of virus RNA, making it unable to attach with the host cells. It is time to focus on snake venom research towards dreadful fevers taking the lead from Ayurveda.

Keywords: Antiviral property, Ashtanga samgraha, Fever

This short communication is in reference to the recent Nipah virus (NiV) outbreak in Kerala, India, which has claimed 15 deaths till May 31, 2018 [1]. Niv is a newly emerging zoonotic disease, first identified during an outbreak of the disease that took place in Kampung Sunghai Nipah in Malaysia during 1998. The natural host of the virus are the fruit bats of Pteropodidae family and Pteropus genus. Later pigs were identified as intermediate hosts. Fruit bats are thought to be the carriers of NiV worldwide. In Bangladesh in 2004, humans became infected with Niv as a result of consuming date palm sap that had been contaminated by fruit bats.

Antiviral drug discovery is the need of the century and it is indeed a complex process. Only about 90 drugs are discovered till date [2]. Targeted antiviral chemotherapy is a promising area with several complexities. With 60 years of strenuous research in antiviral chemotherapy, drugs for various viral diseases have been discovered. Despite this, antiviral drug development is in snail like pace as compared to antibiotics due to many hurdles in the field. Complexity in the structure of viruses, limitations with the experimental models, economical aspects are some of the major manacles in the path of antiviral drug research. Another important aspect is that the low incidence of discovery of efficient molecules [3]. Search for effective antiviral molecules plays a key role in this aspect.

NiV has an incubation period of 5-14 days. Niv infection in humans may cause asymptomatic infection or acute respiratory syndrome and even fatal encephalitis. The signs and symptoms may progress in to coma within 24-48 hours. Frequent convulsions or personality changes may occur in patients as long term sequelae [4]. Nipah virus is a paramyxovirus included in the Henipa virus genus. The Henipa virus family is highly pleomorphic, showing high variance in shape. It is 40-600 nm in diameter and the core consists of linear Ribonucle Protein (RNP), with negative sense single stranded RNA and three important proteins namely nucleocapsid proteins (N), phospho proteins (P) and large polymerase proteins (L). The N proteins are found in abundance and are necessary for the capsid structure. P-proteins assist RNA polymearse in transcription of RNA to mRNA further to antigenomic RNA. The core is enveloped by a layer of lipids and glycoproteins [5]. These glycoproteins are responsible for associating the viral and the host membranes. This highly specific glycoproteins bind to surface proteins Ephrin B2 and B3. Some other proteins are also found in the cytoplasm for regulating the process of transcription. Even though, complete structure of Nipah virus is not discovered yet, a hypothetical structure can be elicited from other viruses similar in structure and genome. The high virulence of the Niv is attributed to the P gene, which is monocystronic that code for a single protein. Intercellular transmission of interferons by the host cells is essential for building the immunity against a specific organism. Niv inhibits the interferon transmission and doubles the risk of virulence of the disease. It multiplies within the host cell with the help of specific proteins [6].

Complementary and alternative systems of medicine throughout the world can contribute much in this regard as they are aware of the unrealised potency of some of valuable herbs in Ayurveda, the traditional medicine of India. It explains a handful of medications for life threatening fevers that are uncontrollable with available management strategies. The key ingredient in all those medications is nothing but the snake venom. The modus operandi for the collection and administration of snake venom is elucidated in a special manner. An enraged snake is made to bite many times on a piece of fresh meat and this meat powder is administered in pinches depending on the severity of the disease and the strength of the patient, as per Ayurveda. In Ashtanga samgraha, one of the classical Ayurveda text book, one whole chapter (Uthara sthana-Chapter no. 48) is devoted to describe the practice of poisonous drugs as remedial medicines [7].

Counter poisons were assumed to be the last option in medicine for any dreadful disease. It consists of combinations of different origins of poisons such as metallic, herbal and snake venom along with some herbs. Snake venom compositions were indicated for chronic as well as intermittent fevers. These comprised of herbs like

Plumbago zeylanica, Pistacia integrima, Santalum alba, Symplocus racemosus and powdered meat impregnated with snake venom [8]. No researches have been undertaken in this field till date. It is high time to search for an anti-viral drug powerful enough to prevail over the newly emerging fevers like Nipah virus, Dengue virus, Ebola and many others. Nevertheless, some of the valid research findings on snake venom and dreadful fevers are used for correlation and explanation in this regard [9].

Anti-viral drugs may act through different mechanisms which include either direct virucidal activity or interfering the cell cycle like prevention of the attachment of virus to the cell membrane and inhibition of the replication process [10]. Drugs with virucidal property possess high cytotoxic potential which may create serious sequelae like frequent convulsions or personality changes. It has been proved that viruses independent of its genetic material or structure have glycoprotein expressions that mediate host cell entry. We should cleverly focus in to anti-viral drug development researches in terms of the other two pathways. Group of Henipa viruses, in which Nipah virus is also included, showed a different operational strategy for host entry independent from conventional ephrin B2 or B3, CD150 and Sialic acid. Surface proteins of host cells act as receptors for Nipah and Hendra viruses which are an exemption from other paramyxovirus [11]. The virus needs lipid hydrolyzing enzymes for the attachment and entry in to the host cells.

Phospholipase2 (PLA2) is a vital class of enzymes found in all forms of life and performs different metabolic functions. PLA2 is found in 15 sub-groups and 4 main groups. These consists of secreted s PLA2, cytosolic c PLA2, calcium dependent i PLA2 and platelet aggregating factor acetyl hydrolase Lp PLA2 [12]. Among different types of PLA2, secretory PLA2 are responsible for the process of hydrolysis of lipids. Altogether about 30 PLA2 are available in different forms. Consequently, all viruses show high affinity towards host PLA2 and lipid bilayer [13].

In human beings also, PLA2 (IIA) shows potential for hydrolysis of phospholipids. Since virus needs a host PLA2 for entering into the body, PLA2 forms effective target for antiviral drug discovery. It has been shown that PLA2 activity is essential for Parvo virus for infectivity. Likewise, Parvovirus and Human cytomegalo virus possess their own PLA2 for infectivity. Other viruses may use host machinery for the life cycle to get completed. Human sPLA2 are found to be effective against hydrolysis of HIV-1 virus. Human enzymes are challenging to obtain; hence we have to search for some other sources for sPLA2. Snake venom PLA2 (svPLA2) shows high similarity with human (PLA2 IIA) as proved through advanced studies like docking. Apart from other venoms, svPLA2 shares 44-99% amino acid identity in their primary structure which forms the basis for the high homology. Also, svPLA2 shows high similarity among different snake groups also. svPLA2s are found in all classes of snake venom and highest amounts are found in elapidae, viperidae and hydrophidae. Viperidae snakes shows 60% of svPLA2 and Bungarus species possess 71% of svPLA2. Hence irrespective of the venom protein composition, svPLA2 can act against viruses. There is high homology between the primary structure of snake and mammalian secretory PLA2 as per researches. As PLA2 is involved in various pathophysiology, snake PLA2 can be a suitable alternative for mammalian PLA2 [14].

Snake venom is composed of various simple as well as complex molecules such as glyco proteins, carbohydrates, proteins, enzymes and metallic aggregates. Numerous receptors found in snake venom proved effective pharmacological agents against life threatening conditions like cancer, myocardial ischemia and hematological disorders. Its virucidal activity is proved against various viruses Anti-viral activity of snake venom peptides is proven against Measles virus, Dengue virus and Senda virus [15]. Snake venom PLA2 is a powerful component that is responsible for anti-viral activity. Initially venom PLA2 interacts with the host cells and prevents the intra cellular release of virus capsid protein. Thus blocking the virus entry in to the host cell. Different snake venoms are similar in many ways especially structure and functions of PLA2. PLA2 derived from *Crotalus durissus* cleaves the Dengue virus envelops and causes protein destabilization. It leads to partial exposure of viral RNA and causes RNA deactivation; thereby making it unable to enter the cell. Some other pharmacological properties of venom are also being elicited. *Crotalus durissus terrificus* venom is found to be effective against Measles virus. When *Naja nigricollis* venom treated with Sendai virus infected RBC, it causes selective destruction of infected erythrocytes. Another enzyme from *Bothrops jararaca* venom, L-amino acid oxidases are effective against Dengue viruses. Immunokine is another potent component of *Naja siamensis* venom, that inhibit the spread of infection in HIV infected lymphocytes through receptors. Cobra-α-neuro toxin has proven its efficacy against Herpus Simplex Virus (HSV1) [16].

Researches revealed that Parvo virus capacitates the Ca2+ channel for entry into host. Similarly, cobra cardio toxin induces Ca2+ influx and may lead to cardiac ischemia like conditions. These prove that both have somewhat similar action on calcium channels [17]. These poisons can act in the body irrespective of the immune defense mechanism. Most toxic PLA2 is retrieved from *Russell's viper*, its, Lethal Dose (LD 50) corresponds to 0.1 mg/kg [18]. Venom from *Crotalus durissus* below 100 µg/mL does not exhibit any type of toxicity. Approximately, 63 µg of toxins enters a body per bite [19]. This gives an approximate idea about the dosage described in Ayurveda about snake venom. Poison impregnated meat powder is indicated in pinches to be administered in patients. Yet, it has to be proven with scientific researches. Homology with 30-40 amino acid residues of *Naja simensis* and *Bungarus multicinctus* neurotoxin long loop and 164-174 short segment HIV-1 has already been proved. Structural similarity of snake venom loop 2 and similar regions of Rabies virus glycoproteins are also being understood [20,21].

To conclude, drugs that inhibit the virus infectious cycle at any stage can be considered as a powerful anti-viral drug. Due to high affinity with the virus and host PLA2; structural analogy with the snake and mammalian PLA2, snake venom can be a candidate for the receptor competition with virus. Similar to the drug discovery of many other diseases, this crucial hint from ancient science of India can do better against fever epidemics for the welfare of the mankind.

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