Review Article

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Mechanisms of virus resistance and antiviral activity of snake venoms

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Abstract: Viruses depend on cell metabolism for their own propagation. The need to foster an intimate relationship with the host has resulted in the development of various strategies designed to help virus escape from the defense mechanisms present in the host. Over millions of years, the unremitting battle between pathogens and their hosts has led to changes in evolution of the immune system. Snake venoms are biological resources that have antiviral activity, hence substances of significant pharmacological value. The biodiversity in Brazil with respect to snakes is one of the richest on the planet; nevertheless, studies on the antiviral activity of venom from Brazilian snakes are scarce. The antiviral properties of snake venom appear as new promising therapeutic alternative against the defense mechanisms developed by viruses. In the current study, scientific papers published in recent years on the antiviral activity of venom from various species of snakes were reviewed. The objective of this review is to discuss the mechanisms of resistance developed by viruses and the components of snake venoms that present antiviral activity, particularly, enzymes, amino acids, peptides and proteins.

Key words: snake venom, viruses, antiviral agents.

INTRODUCTION

Viruses are obligate intracellular parasites that depend on cell metabolism for their own propagation. This intimate virus-cell relationship led viruses to develop numerous survival strategies aimed at protecting themselves from the host defense system (1-4).

One of the criteria for a successful viral infection is the ability to remain inside the host cell for the time required to allow replication of the viral genomic nucleic acid, envelopment and the production of new infectious virus progeny. The first barrier to be overcome is the host innate immune response (5).

The association between virus persistence and disease in the host may lead to a long term relationship that does not result in any chronic virus-induced symptoms or in a fatal disease (6). Persistent viral infections constitute an example of immune evasion and, consequently, a successful relationship (7).

Antiviral therapy has changed the natural history of numerous viral infections, delaying progression of the disease, improving the quality of life of infected individuals and reducing the frequency of hospitalization (8-10). Nevertheless, one of the principal problems today is therapeutic failure, including antiviral drug resistance (11, 12).

The rich biodiversity in Brazil is an important source of wealth, since the country is home to at least 14% of the world's species, giving it an enormous advantage over other countries with poorer biodiversity, principally in a century in which biotechnology is expected to play such an important role in the global economy (13).

Snake venoms are composed of a mixture of proteins and peptides (90-95%), but also include free amino acids, nucleotides, lipids, carbohydrates and metalloproteinase enzymes (5%) (14-19). Although snakebites may be deadly, snake venoms comprise a biological resource that contains components of significant therapeutic value. Snake venoms have been shown to exert beneficial effects in the treatment of certain diseases, including drug resistant human immunodeficiency virus (HIV) infection (20).

MECHANISMS OF VIRAL RESISTANCE

In response to selective pressure, viruses react differently according to the type of nucleic acid that forms their genome. The majority of RNA-genome viruses use all available means to diversify their structures, which can function as antigenic determinants of immune response. Viral evasion may occur through genetic diversification. In this process, the viral genome undergoes gradual events of mutation such as point mutation, as reflected in nucleotide substitution; insertions and deletions; generation of protein domains or *de novo* genes; gene duplication followed by functional divergence; fusion; gene loss and translocation; recombination or rearrangement between related viruses; and gene capture from the host cell or from another virus (21).

Gene capture function by viruses comprises an adaptation mechanism to the host, and is not exclusive to DNA-genome viruses such as the poxviruses (with their cytoplasmic life cycle) and herpes viruses (21). Herpes simplex virus (HSV) is an example of a DNA virus that probably evolved over a long period of time in parallel with the host immune system (22).

Host gene capture is a process found in many viral lineages during their evolution. Many viruses have, for example, DNA or RNA polymerase and helicase, functions that were probably required at early stages in viral evolution and that presumably had been captured (21).

RNA viruses have the highest mutation rates in nature, around 10^{-3} to 10^{-5} per nucleotide per replication cycle (23, 24). This means that a wrong nucleotide may be incorporated in each 1,000 or 100,000 bases copied. The molecular basis of the variability in RNA viruses is the result of the absence of the overseeing activities of the RNA polymerase and viral reverse transcriptase enzymes. Lack of this activity results in errors in the incorporation of nucleotides, and the rate of errors in DNA polymerases is 10^{-8} substitutions/site/replication cycle. In the case of HIV-1, its genome, which has approximately 10,000 base pairs, acquires an average of one substitution of nucleotide in each replication cycle (25). Considering that on average thousands of new viral particles are produced each day, it is possible to imagine the immense diversity generated throughout the long course of the infection (26). Although the majority of the mutations produced during the viral replication cycle exert a negative effect on the capacity of the virus to adapt, mutants containing advantageous mutations replicate more effectively than the genomes containing deleterious mutations, creating a selective balance. A balance such as this leads to the generation of a viral population composed of a high number of single, albeit related genomes (27, 28).

Other factors that affect the evolution of RNA virus are that they have a short generation time but are capable of constituting immense quantities of viral populations (29, 30). All variables combined contribute towards the existence of rapid progression in RNA viral populations. This continuous generation of genetic diversity provides RNA viruses with immediate escape from the immune activity of the host.

Cytomegalovirus (CMV), a member of the Herpesviridae family, has also developed mechanisms to escape the host immune system. It has a distinctive characteristic in its capacity for latency and can be reactivated under different circumstances. In an immunocompetent individual, most viruses are destroyed by CMV-specific cytotoxic T cells and the infection progresses asymptomatically (31, 32).

CMV regulates the cell surface expression of HLA class I (HLA-I) molecules, which protects the CMV-infected cells from being recognized by the cytotoxic T cells, and, consequently, constitute an escape route for CMV (33).

Viruses that cause severe diseases and result in the death of the victim within a short period of time would be compromising their own future existence. Therefore, to ensure their successful survival, some viruses have developed other strategies to adapt to the human species. The mechanism consists of causing low mortality rates in victims while provoking a dormant

infection. This is the strategy used by the human herpesvirus family, particularly by the varicella zoster virus, which can remain inactive in the nerve pathways for many years (34, 35).

RNA viruses that replicate in the cytoplasm cannot reach host genes directly; therefore, they have had to develop other immune escape strategies. The "erroneous replication" escape strategies used by RNA viruses end up overloading the host immune system by continuously creating new viral antigen determinants (6).

One consequence of the complexity of viral populations is that these populations are able to respond quickly and effectively to changes in the environment in which they replicate, since they offer a wide spectrum of mutants on which natural selection may act. When changes occur in the environment, for example, as a result of the administration of antiviral drugs, the presence of one or more mutants better adapted to replication in this new environment ensures that the population resulting from these mutants increases its ability and likelihood to survive. The selective pressure constantly exerted by the immune system results in the adaptation of the virus to the new target cells and in the maintenance of a persistent infection (25).

Based on the type of nucleic acid in their genome and the site of replication, viruses with a DNA genome that replicate in the cell nucleus may, during their process of evolution, be at an advantage in being able to capture genes from the host and then mimic, block and/or, in some other way, regulate the key to the cell processes. One of the strategies developed by viruses to escape the action of the immune system is a process referred to as molecular piracy, in which viruses codify their own viral version of cell proteins. Molecular piracy facilitates viral escape from the immune pressure of the host. During the process of viral piracy, viruses often integrate parts of the host genome into their own genome or create their own genes, which are similar to those of the host (1-4).

In addition to errors in incorporating nucleotides, a process known as homologous recombination also contributes to the genetic variability of viruses such as HIV-1. During reverse transcription, the reverse transcriptase enzyme may jump from one RNA strand to another, producing a viral DNA strand that contains segments of the two initial RNA (36). Nevertheless, to generate substantial diversity, during recombination the two RNA strands in a viral particle must be separated from each other, i.e. heterozygous viral genomes must already have been produced. Therefore, the formation of heterozygous genomes depends on a single host cell, at some moment, have been simultaneously infected by two variants of the virus (36).

In recent years, although considerable progress has been made in understanding viral biology and numerous clinical trials have been conducted with various candidate antiviral drugs, many obstacles remain to be overcome, including antigen diversity, the high frequency of viral mutations, the destruction of the immune system cells after infection and imperfect animal models, among others (37). Moreover, the pharmaceutical companies are not interested in the development of new drugs based on this antiviral active biomolecules derived from the snake venom. Several studies have attempted to evaluate alternative therapeutic strategies (38). The antiviral activity of snake venoms represents a new and promising therapeutic alternative against the resistance mechanisms developed by viruses.

SNAKE VENOMS WITH ANTIVIRAL PROPERTIES

The biomolecules that constitute snake venoms have great therapeutic potential (15, 16). Recently published studies report the existence of snake venom fractions with an antiviral effect; however, few studies have been conducted to evaluate Brazilian snake venoms (39-49).

A new study employed non-cytotoxic venom fractions from *Crotalus durissus terrificus* (Cdt) and identified the effect of this snake venom against the measles virus. At concentrations below 100 μg/mL, the Cdt venom showed no cell cytotoxicity. Replication of the measles virus was inhibited in Vero cells when the venom was added either prior to or during cell infection by the virus. The concentrations that successfully inhibited replication of the measles virus were 0.1 μg/mL and 100 μg/mL, respectively (41).

Another study that demonstrated the potential therapeutic value of venoms was developed using *Naja nigricollis* venom in human erythrocytes infected with the Sendai virus. This research found that cells infected by the Sendai virus

were ten times more susceptible to lysis when exposed to two of the five venoms tested. Four cytotoxins isolated from *Naja nigrocollis* snake venom also showed that cells infected by the virus were ten times more susceptible to the cytotoxic action of the venom when compared to normal cells. Therefore, the study showed the clinical importance of the selective destruction of cells infected with the Sendai virus by the venom (39).

L-amino acid oxidases (LAAO), flavoenzymes obtained from the venom of *Bothrops jararaca*, were found to show antiviral activity against the dengue virus. In a recent study, in cells infected with the dengue type 3 virus (DENV-3) and treated with LAAO isolated from the venom of *Bothrops jararaca*, there was a reduction in viral load compared to cells infected with the same virus and not treated with enzymes from the venom (48).

Immunokine, an oxidized derivative of the α-toxin extracted from *Naja siamensis* snake venom has shown to inhibit the infection of lymphocytes by HIV and feline immunodeficiency virus (FIV) through the chemokine receptors CCR5 and CXCR4 (47). A group of investigators in Asia reported a remarkable similarity between the 164-174 sequence of the short segment of gp120 of HIV-1 and 30-40 amino acid residues of the long-chain neurotoxins in the venom of snakes such as *Naja siamensis* and *Bungarus multicinctus* (50, 51). Therefore, both are able to compete for the same receptor or HIV binding site.

Metalloproteinase inhibitors present in snake venom may prevent the production of new viruses by inhibiting protease enzymes (52, 53). Protease inhibitors commonly block the protease enzyme and prevent the cell from producing new viral particles.

LAAO, obtained from the venom of the Asian snake *Trimeresurus stejnegeri*, was purified and cloned. At concentrations that had little effect on cell viability, LAAO was able to inhibit the infection and replication of HIV-1 in a dose-dependent manner. This was the first report of potential antiviral activity shown by LAAO extracted from snake venom (42). LAAO, present in the venom of *Crotalus atrox*, *Pseudechis australis* and *Trimeresurus stejnegeri* inhibit the infection and replication of HIV through the p24 antigen in a dose-dependent manner (42, 54). The presence of the p24 antigen is directly related to viral load (55). As well as binding to the cell membrane protein, hydrogen peroxide (H_2O_2), an oxygen free radical, is able to inhibit infection and the replication of HIV, further improving its antiviral effect. In contrast, catalases use H_2O_2 as a substrate, degrading it and thus reducing its antiviral effect (42). LAAO is also an important component of the venom of the snake *Calloselasma rhodostoma*, found in southeastern Asia, which induces cell apoptosis and exerts antibacterial and anti-HIV effects (56).

Some studies conducted with the venoms of various snakes such as *Crotalus adamanteus*, *Oxyuranus microlepidotus*, *Bungarus candidus*, *Hydrophis cyanocinctus*, *Naja naja*, *Notechis aterater*, *Naja sumatrana* and *Naja kaouthia* have demonstrated an anti-HIV effect (44-46).

Another example of the anti-HIV effect of biomolecules extracted from venoms is phospholipase A_2 (PLA_2). This phospholipase has been associated with a variety of biological effects such as avoiding the intracellular release of the virus capsid protein through an unknown mechanism, suggesting that this substance block the entry of the virus into the cells prior to and regardless of the use of a co-receptor. The antiviral activity appears to involve a specific interaction between PLA_2 and the host cells.

Venom PLA_2 protects the primary leukocytes in human blood from the replication of HIV-1 variants (52, 53). PLA_2, found in the venom of many snakes, has been shown to block entry of the virus into the cells prior to the virus uncoating process by preventing the intracellular release of proteins from the viral capsid (57).

A study developed with 12 synthetic peptides derived from PLA_2, contained in venom, showed an anti-HIV effect. The p3bv peptide prevents HIV-1 from binding to the T cells, since it binds to the CXCR4 receptor (40). Therefore, the viral particles are unable to bind to cells and after a certain period of time they become unviable since they are unable to multiply.

In a study conducted by Villarrubia *et al.* (43), crotoxin – a phospholipase isolated from the venom of *Crotalus durissus terrificus* (PLA_2-Cdt) – showed *in vitro* activity against HIV. The anti-HIV effect of PLA_2-Cdt (inhibition of gp24) is probably the result of its capacity to destabilize some binding receptors (cell-surface heparans).

Chemokines and their derivatives form a class of HIV inhibitors that show promise as potential

therapeutic agents (57). These substances are able to compete with the glycoprotein (gp120) from the viral envelope to bind with the receptor (58-59). Furthermore, various low-molecular-weight compounds that bind to CXCR4 and inhibit HIV-1 replication have been identified (40). The ability of peptides and small molecules to block the entry of HIV through binding with CXCR4 has already been described (60-62)

FINAL COMMENTS

The development and use of antiviral drugs for the control of infectious diseases has produced immediate clinical benefits. Nevertheless, the efficacy of the mechanisms disappears with the onset of viral resistance. Under these conditions, since the available possibilities are limited and the alternatives for effective and prolonged treatment are finite, new therapeutic strategies must be found to guarantee a long lasting therapeutic effect (63).

The increasing rates of viral resistance must be minimized by implementing effective means of infection control, rational use of antivirals and the identification of new natural molecules that have a potent effect against such agents (64).

The pharmacological effects of snake venom are complex and currently little is known about; therefore, great interest has been generated in evaluating their fractions and components. A few studies have been conducted on the antiviral activity of snake venoms. Due to the rich biodiversity in Brazil, it is reasonable to suppose that antiviral effects may be present in the venom of some Brazilian species of snakes. Thus, further research is required to determine the exact mechanism of their antiviral effect.

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