Protease Inhibitors in Potential Drug Development for Leishmaniasis

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Leishmaniasis is a deadly protozoan parasitic disease affecting millions of people worldwide. The treatment strategy of Leishmania infection depends exclusively on chemotherapy till date. But the treatment of the disease is greatly hampered due to high cost, toxicity of the available drugs and more importantly emergence of drug resistance. Hence the potential new drugs are highly needed to combat this disease. The first and foremost step of the drug discovery process is to search and select the putative target in a specific biological pathway in the parasite that should be either unambiguously absent in the host or considerably different from the host homolog. Importantly, Leishmania genome sequences enrich our knowledge about Leishmania and simultaneously reinforce us to identify the ideal drug targets that distinctly exist in the parasite as well as to develop the effective drugs for leishmaniasis. Though the leishmanial research has significantly progressed during the past two decades, the identification of suitable drug targets or development of effective drugs to combat leishmaniasis is far from satisfactory. Enzymatic systems of Leishmania metabolic and biochemical pathways are essential for their survival and infection. Concurrently, it is noteworthy that Leishmania proteases, especially the cysteine proteases, metalloproteases and serine proteases have been extensively investigated and found to be indispensable for the survival of the parasites and disease pathogenesis. Herein, we have discussed the importance of few enzymes, particularly the Leishmania proteases and their inhibitors as promising candidates for potential development of anti-leishmanial drugs.

Keywords: Leishmania, Leishmaniasis, Proteases, Biochemical pathways, Drug targets, Inhibitors, Chemotherapy

Introduction

Leishmaniasis, caused by the genus Leishmania still remains great concern for the public health worldwide. The disease prevails in 88 countries in the five continents — Africa, Asia, Europe, North and South America, affecting around 350 million people, where about two million new cases occur annually with major impact on the poorest. Leishmania parasite exhibits a digenetic life cycle: flagellated promastigotes, which reside at the midgut of the female sandfly and during blood meal, enter into the mammalian host and differentiate into non-flagellated amastigotes and then multiply inside the parasitophorous vacuoles of macrophages until lysis of the host cells.

Depending upon numerous factors, such as the infecting species, host, vectors and environment, the disease has four clinical manifestations: cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis (MCL), visceral leishmaniasis (VL) and post-kala-azar dermal leishmaniasis (PKDL). Among these, VL is the most lethal form of the disease, if untreated. Though VL is endemic in about 88 countries, 90% cases mainly occur in only 6 countries: Bangladesh, Brazil, India, Nepal, Sudan and Ethiopia. Near about 200 million people on the Indian subcontinent are at risk for VL with approximately 420,000 annual cases occurring mainly in poor rural communities. From 1987 through 2011, a total of 7, 60, 432 kala-azar or
VL cases occurred in 52 districts of four main states (Bihar, West Bengal, Jharkhand and Uttar Pradesh) of India, among them highly endemic state is Bihar, where more than 70–80% kala-azar cases occur in each year.3

Leishmaniasis is considered as the second most life-threatening parasitic disease after malaria according to WHO.3 Though extensive studies have progressed to tackle leishmaniasis, there is still no effective vaccine or drugs available for this disease.6 As poor people are mostly affected by leishmaniasis worldwide, researchers, pharmaceutical industries and funding agencies of developed countries have little interest in the drug development process of this disease. The existing chemotherapy of leishmaniasis encounters lack of safe and effective drugs and more importantly the manifestation of widespread drug resistance strain of the parasite. Moreover, rising incidence of human immunodeficiency virus (HIV) with Leishmania co-infection is now of major concern.7 So, there is an emerging need for the development of a new and inexpensive anti-leishmanial drug with high efficacy.8,9 Hence, non-conventional drugs and new drug targets are being frequently investigated for leishmaniasis.

Several investigators have focused on the roles of Leishmania virulence factors in host-parasite interaction and their suitability as potential drug targets.10-15 Consequently, the search for active compounds is rapidly progressing to identify the ideal drug to treat the parasitic diseases effectively.16 Availability of the complete genome sequence of various Leishmania species like L. major, L. infantum and L. braziliensis enhances our knowledge about the biochemical and physiological differences between the parasite and host.17,18 Furthermore, the analysis of complete genome sequence helps to compare the parasite genome with the human genome sequence and to identify genes unique to the parasite towards selecting and new potential drug targets. This review presents an overview of the potential target proteins, especially the Leishmania proteases as drug targets and protease inhibitors as prophylactics against leishmaniasis.

Potential drug targets in leishmaniasis

The preliminary step of the drug discovery process is to search and select the drug target that is essential in certain biological pathway. Theoretically, to identify a drug target in a pathogen, there should be no ambiguity that the putative target is either explicitly absent in the host or different to a large extent from the host homolog, so that it can be employed as a drug target. Eventually, a protein which is indispensable for the survival of the microbe or vital for controlling a particular signaling pathway is frequently targeted. The traditional approach is to find a new crucial protein as a potential target is done experimentally by using gene knock-out strategies in that organism. Moreover, it is vital to consider the life cycle stage, where the gene is highly expressed.

The most important targets are the enzymes, because of their role in regulating specific biochemical and metabolic pathways. There should be major structural and functional differences of the target enzymes from mammalian system for their distinct inhibition of the target sites. So, the specific inhibitors can be designed or identified in nature that binds to the active sites of the enzymes and cause enzyme inhibition and loss of cell viability. Lastly, it is highly important that the selected target should be assayable, so that molecules can be screened by employing specific assay system that is economical.19 Furthermore, it is more useful and effective to target more than one enzyme of a metabolic pathway. Though Leishmania parasites are eukaryotes, there are significant differences in their cell organization from mammalian cells and thus, it is possible to find out the targets that are unique to the parasite. Before discussing the proteases, it is important to mention some enzymatic systems, which are believed to be superior targets in Leishmania (Table 1).

Like all other parasites, Leishmania parasites require metabolic pathways for their survival and virulence.20 On the basis of recent genomic and biochemical analysis of the metabolic potential of Leishmania as well as analysis of the virulence phenotypes of metabolic mutants, many metabolic processes have been identified to be essential for intracellular survival and pathogenesis.5,21 Many enzymes of Leishmania, which are essential for some biochemical pathways, such as sterol biosynthesis, glycolysis, purine salvage, glycosyl phosphatidylinositols biosynthesis, glyoxalase and trypanothione system, folate biosynthesis and special enzymes, such as protein kinases, tropoisomerases, metacaspases and more importantly proteases guarantee Leishmania survival and proliferation to maintain infection9,21,22. These enzymes are exclusively considered as good targets in Leishmania.
The sterol biosynthetic pathway is considered to be an attractive drug target in *Leishmania* as ergosterol and other 24-methyle sterols that are absent from mammalian cells, are required for parasites growth and viability. Glycolysis is the most important energy source of *Leishmania* parasites. Due to large phylogenetic distance of the glycolytic enzymes of *Leishmania* with mammalian hosts, leishmanial glycolytic intermediate enzymes could be potential drug targets.

*Leishmania* have to utilize purine from host by purine salvage system when they are unable to synthesize purine nucleotides. *Leishmania* use the most important enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) in purine salvage pathway. Hence, this enzyme has been targeted due to its difference in substrate specificity with the host enzyme.

Allopurinol which is phosphorylated by this enzyme and incorporated into nucleic acid causes selective death of the parasite. Allopurinol is found to be effective against cutaneous and visceral leishmaniasis and its effect is augmented when added in combination with other anti-leishmanial drugs.

Polyamines like putresine, spermidine and spermine, which not only have important role in parasite growth and differentiation, but also attenuate lipid peroxidation and make the parasite compatible with the environment for survival from vector to host. Hence, the development of *Leishmania*-specific inhibitor to stop polyamine biosynthesis and transportation may be quite useful for novel anti-leishmanial therapies.

Glycosylphosphatidylinositol (GPI) glycolipids of *Leishmania* parasites act as membrane anchor for many cell surface glycoproteins.
glycoproteins protect the parasite from the alternate complement pathway and external hydrolases. Selective inhibition of glucosylphosphatidylinositol biosynthetic enzymes has been suggested to be very effective against parasites. These enzymes are other target molecules for development of anti-leishmanial agents.

*Leishmania* parasite has evolved a unique defense mechanism against respiratory burst activities of macrophages. *Leishmania* trypanothione metabolism is required to counteract mammalian antioxidant glutathione metabolism. In *Leishmania*, it has been reported that trypanothione \([T(SH)_2]\), a dithiol is capable to reduce NO and Fe (II) into a harmless stable dinitrosyl iron complex with 600-times more affinity than the mammalian glutathione reductase (GR) system and thus protects the parasite against lethal NO molecules. Thus, trypanothione reductase (TR) and enzymes of trypanothione metabolism appear to be attractive drug targets for anti-leishmanial drug design, without affecting the host GR as *Leishmania* TR and mammalian GR exhibit differences in structure and catalysis.

Trypanothione is a co-factor of protein disulfide isomerase (PDI). *Leishmania major* PDI (LmPDI) has been identified as a potential virulence factor. *L. major* parasites deficient in LmPDI become non-virulent in experimental leishmaniasis. Thus, LmPDI is also considered as a suitable target for anti-leishmanial chemotherapy. Consequently, bacitracin has been found to inhibit both LmPDI isomerase and reductase activities and lead to the arrest of parasite multiplication in the infected macrophages *in vitro*. Hence, LmPDI could be an important target for the anti-leishmanial therapy.

Dihydrofolate reductase (DHFR), a key enzyme for production of thymidine of *Leishmania*, which is important for DNA biosynthesis, has been found to be structurally different from the human enzyme. Interestingly, *Leishmania* parasites are unable to survive in animals lacking dihydrofolate reductase-thymidylate synthase (DHFR-TS), which is required for the conversion of dihydrofolate from methylene tetrahydrofolate and thymidine. However, to confirm DHFR as an authentic drug target requires further investigations.

The glyoxalase system is vital for detoxification of the cell by removing toxic and mutagenic intermediates such as methylglyoxal, a by-product of glycolysis. Unlike mammalian hosts, *Leishmania* glyoxalase exclusively uses trypanothione as the substrate. Therefore, additional biochemical and genetic investigations are highly desired as the glyoxalase pathway would be a possible target for drug design.

Protein kinases which are essential in cell signaling and cell cycle control are essential enzymes to be investigated as potential therapeutic targets in many parasites, including *Leishmania*. The cyclin-dependent kinases (CDKs) play a crucial role in cell division. CDK-related kinase 3 (CRK3) encodes cdc2-related protein kinase which is active in the G2/M phase of the *Leishmania* cell cycle. *Leishmania* CDKs are essential for promastigotes. CRK3 inhibitor impairs growth and replication of *Leishmania* amastigotes within the host macrophages, thus potentiating CDKs as suitable drug target. Owing to the involvement of *L. donovani* glycogen synthase kinase 3 (LdGSK3) in cell cycle control and apoptosis, LdGSK3 in combination with CRK3 can be exploited as potential target.

Furthermore, mitogen-activated protein kinases (MAPKs) play vital role in the regulation of transcription, proliferation and differentiation of cells in response to the external stimuli and fifteen such MAPKs have been so far reported in *L. mexicana*. But, except LmxMPK (*L. mexicana* mitogen protein kinase) others are not quite effective. The null mutants of LmxMPK are still able to infect macrophages and differentiate into amastigotes, but they are unable to proliferate inside the parasitivorous vacuoles and thus LmxMPK inhibitors could have some effect on amastigote survival. Hence, these kinases might be suitable target for therapeutic immunomodulation.

Topoisomerases are now being considered as the major targets in leishmaniasis like cancer and bacterial chemotherapy. DNA topoisomerases are ubiquitous enzymes that play essential roles in DNA replication, transcription, recombination and repair. *Leishmania* topoisomerases I and II have been aimed for chemotherapeutic applications due to their discrete biological properties in the parasite. Subsequently, a number of topoisomerase inhibitors like camptothecin, dihydrobetulinic acid (DHBA), 3, 39-dindolyl methane (DIM) derivatives and recently niranthin have been found as promising anti-leishmanial agents. But, still the structural analysis of these enzymes is required to develop anti-topoisomerase agents against drug resistant *Leishmania* parasites.
Metacaspases which are believed to induce programmed cell death in trypanosomatids are another kind of target molecules in Leishmania. Leishmania metacaspases are expressed in the promastigotes and amastigotes that have been found to be essential for the appropriate division of the nucleus and kinetoplast. It has also been reported that the overexpression of metacaspases makes Leishmania parasites sensitive to H2O2-induced programmed cell death. Hence, this can be used as an efficient anti-leishmanial agent that will be able to induce the expression of metacaspases in Leishmania. Moreover, metacaspases can also be directly targeted, as they are essential for the chromosomal segregation as well as parasite survival.

Overall, being unique and distinct from the mammalian host, a number of proteins and enzymes of different metabolic and biochemical pathways are being extensively explored as targets in the anti-leishmanial drug discovery and at the same time implication of the defined and specific compounds available will be a prerequisite for the development of potential anti-leishmanial therapeutics.

Protease inhibitors as antileishmanial therapeutic agents

Proteases, a large group of ubiquitous enzymes involved in many physiological functions, have been identified as potential targets for drugs. There are certain diseases, such as numerous infectious and various inflammatory diseases where protease inhibitors are being investigated as effective drugs. Indeed, increased proteolysis has been shown to emphasize various pathological processes and, therefore, proteases have been emerged as important therapeutic targets. Successful protease inhibitors used in the therapeutic intervention of many diseases include treatment of hypertension by angiotensin converting enzyme (ACE) inhibitors, treatment of progression of AIDS by HIV aspartyl protease inhibitors and treatment of multiple myeloma by protease inhibitors.

A potential strategy for the treatment of diseases caused by parasites is the design of compounds which selectively inhibit enzymes that are pivotal for survival of the parasite within the host and are part of the biochemical pathways unique to the parasite. Parasite proteases are attractive target enzymes because of their roles in replication, metabolism, survival and pathogenesis. Specific protease inhibitors can be used to regulate protease activity within cells or in the organisms. In addition, the biochemical properties and the biological functions of proteases can be investigated by employing specific inhibitors. Besides, many protease inhibitors are able to block the inversion process of many parasites, including Phytoponas serpens, Plasmodium falciparum, Toxoplasma gondii, Trypanosoma cruzi, Trypanosoma brucei and Schistosomiasis mansoni.

Importance of proteases during Leishmania infection has greatly advanced our knowledge and underscores the use of specific protease inhibitors as anti-leishmanial agents (Table 2). Demonstration of low toxicity of protease inhibitors at the concentrations used in vitro or in vivo may be due to several factors. First, parasite takes up and accumulates the inhibitor as compared to the host cell organelles more efficiently. Parasites have no redundancy of protease activity like host cells. It is noteworthy that even the inhibition of one or more host proteases may lead to little phenotypic effect. Lastly, the level of proteases within host cells is considerably much higher (milli molar) than that in parasites. Hence, proper understandings of proteolytic events that lead to parasite pathogenesis strengthen the use of protease inhibitors as new drug candidates for leishmaniasis therapeutic purposes. Moreover, proteases should be the excellent choice of targets for drug development because small molecules can readily interact with a well-defined active site of the target protease and thus many classes of small molecule protease inhibitors are now being searched or developed.

Leishmania introduce a number of virulence factors to maintain their infection by activating survival mechanisms. Among them, proteases are crucial for their life cycle and pathogenesis. To guarantee their survival and proliferation for maintenance of the infection within the host, Leishmania proteases play essential roles, such as modulation of host immune system, invasion and penetration of host tissues, parasite dissemination, and acquisition of essential nutrients. Consequently, these criteria of the proteases make them to be potential targets for anti-leishmanial therapy. On the basis of proteases function and genetic or chemical knock-out studies, several proteases have been substantiated as potential targets in protozoan parasites, including Leishmania.
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infection by *Leishmania*12,13,15,93,94. But, serine proteases and their inhibitors have been relatively less explored as far as leishmaniasis is concerned93.

*Leishmania* cysteine proteases play key roles in multiple processes such as modulation of the host immune response, degradation of various host proteins, parasitic autophagy, differentiation from promastigotes to amastigotes and cellular invasion to modify host responses to its own advantage15,94. On the other hand, *Leishmania* strains deficient in cysteine proteases and parasites treated with specific cysteine protease inhibitors show immensely reduce virulence and infectivity95-99. Thus, it is believed that inhibition of *Leishmania* cysteine proteases will be an ideal therapeutic option for combating *Leishmania* infection16. Inhibitors that would effectively target both types of cysteine proteases in *Leishmania* would be the ideal drug leads. On the basis of gene knock-out studies, it has been found out that 80% inhibition of *Leishmania* cysteine proteases appear enough to prevent *Leishmania* replication101. However, inhibition of a single type of cysteine protease is not sufficient to do so. At least two of the three cysteine protease gene families (cpa, cbb, cpc) are to be eliminated to absolutely block the parasite invasion or replication in host cells as well as lesion development in vivo100.

In addition, the natural cysteine protease inhibitor, cystatin has been found to be very promising anti-leishmanial agent, as it induces protective response in experimental visceral leishmaniasis101-103. Moreover, leishmanial inhibitor of cysteine peptidases (ICP) is thought to protect the parasite against the hydrolytic environment of the sandfly gut and/or the parasitophorous vacuole of host macrophages104,105. The natural biflavonoids compounds show anti-leishmanial activity and antiproteolytic activity against recombinant cysteine protease B106. It is appreciable that the use of cysteine protease inhibitors at the desired concentrations displays no toxicity towards host cells100. Besides, it has also been demonstrated that *Leishmania* cathepsin-like cysteine protease which is particularly expressed in amastigotes is vital for host macrophages infection and intracellular survival of the parasites96.

Based on cathepsin tertiary structures, cathepsins specific inhibitors (CA-074 for cathepsin B, CLIK-148 and -195 for cathepsin L, CLIK-60 for cathepsin S) have been developed107. Cathepsin B inhibitor CA-074 suppresses the response to *Leishmania major* antigen and induces Th1 type immune response, thereby downregulates the production of IgE and IgG1. But, cathepsin S inhibitor CLIK-60 suppresses the Th-1 type immune response107. Recently, some cathepsin B inhibitory compounds have been found as good anti-leishmanial agents108-110. A total twenty-seven calpain-like proteins have been found in *L. major* and some of which are assumed to encode large functional proteins111. It is also assumed that *Leishmania* calpains might be involved in cytoskeleton remodeling during differentiation. Moreover, reduction of promastigotes growth and induction of parasites death by a potent calpain inhibitor, MDL28170 has been demonstrated112. Overall, leishmanial cysteine proteases are believed to be possible targets in *Leishmania* for therapeutic application to treat leishmaniasis.

*Leishmania* metallopeptase GP63, mainly located on the surface of the promastigote, has been suggested to be essential to evade and survive from the complement-mediated lysis prior to its internalization by macrophages as it actively cleaves the complement component C3b to its inactive form C3bi113. GP63 also cleaves the extracellular matrix proteins of the host cells and thus contributes to parasites migration113,114. It inhibits the natural killer cell function and enables *Leishmania* to escape from antimicrobial killing. In addition, *Leishmania* GP63 is able to activate the host protein tyrosine phosphatases (PTPs), thereby dephosphorylate and inactivate several key signaling kinases in biochemical cascades and thereby making the host cells unresponsive facilitating the establishment of parasite infection15,116. Interestingly, the overexpression of GP63 enhances *Leishmania* infection and thus favors their survival; alternatively, GP63-deficient *Leishmania* shows reduced infection115. Furthermore, *Leishmania* deficient in GP63 are unable to activate host PTP to undermine the host cell signaling and therefore lose their ability to establish and maintain the infection115.

Hence, it can be obviously conjectured that potent and specific inhibitors of GP63 could be able to favor the functional activation of the host microbicidal functions and thus would gain the accessibility of future anti-leishmanial therapeutics14. Though the biological importance and structure of GP63 is well known, this protease has been relatively less attempted in drug-discovery research programs117. Previously, it has been demonstrated that higher
affinity metalloproteases inhibitors have the potential for treatment of parasitic diseases. However, the activation of host macrophages by cytokines leads to increase MARCKS (myristoylated alanine-rich C kinase substrate)-related protein (MRP) expression, but during infection with Leishmania, MRP expression was depleted. Recently attempts have been made to design peptide inhibitors based on MARCKS, one of the target proteins of this protease. Interestingly, it has been observed that these inhibitors significantly reduce Leishmania infectivity.

Another metalloprotease MP-Ld has recently been identified in L. donovani and found to play major role in the parasite development. Moreover, L. donovani dipeptidylcarboxypeptidase (LdDCP), an angiotensin converting enzyme (ACE)-related metallopeptidase has also been identified and characterized as a putative drug target for the anti-leishmanial chemotherapy. Thus, specific metalloproteases inhibitors could inhibit Leishmania proteolytic activity and prevent the progression of leishmaniasis. However, some recent reports have demonstrated that uncontrolled host matrix metalloproteinases (MMPs) activities are associated with Leishmania pathogenesis and lead to parasites dissemination and delay wound healing. Thus, in this context, it is highly important to develop metalloprotease inhibitors which can inhibit both the parasite and host proteases to protect the host from this parasite.

Recently, HIV and Leishmania co-infection has emerged as of great concern due to the worldwide rapid dispersion. Consequently, the use of HIV protease inhibitors (HIV-Pis) to control HIV and Leishmania co-infection has been of great interest. Despite protease inhibitors are being extensively used to treat HIV and Leishmania co-infected patients, the expression of CPB and gp63 are induced by the HIV-Pis. Hence, it demands further investigations to utilize the HIV-Pis for the treatment of HIV/Leishmania co-infected patients.

Many investigators have attempted to use the serine protease inhibitors in parasitic diseases to establish the serine proteases as potential drug targets. As specific protease inhibitor can regulate the serine protease activity within the cells or in the organisms. These inhibitors are valuable tools to explore the biochemical properties and the biological functions of the proteases as well. Moreover, specific serine protease inhibitors are able to block invasion of many parasites, including Plasmodium falciparum, Babesia gondii, Toxoplasma gondii, Perkinsus marinus and Schistosomiasis mansoni. Previously, it has been reported that pentamidine and suramin show trypanocidal activity by inhibiting the cytosolic serine protease oligopeptidase B, a putative virulence factor in trypanosome.

Although the serine proteases play important role in the virulence of Toxoplasma and Plasmodium, the role of serine proteases in Leishmania still remains unresolved. However, serine proteases have also been implicated to play crucial roles in invasion process and deactivating the macrophage during the initial interaction between the host and the parasite. Leishmania oligopeptidase B (OPB) is important to infect host macrophages, as it regulates enolase levels and facilitates the parasite entry into macrophages. Another serine protease, subtilisin has been demonstrated as essential Leishmania virulence factor as it maintains redox homeostasis by detoxifying reactive oxygen intermediates. Additionally, subtilisin-deficient Leishmania has been found to be incapable to differentiate from promastigote to amastigote that is important for Leishmania virulence.

Serine proteases from L. amazonensis increase their susceptibility to infection by directly activating TH2 type immune response. Importantly, L. donovani extracellular serine protease (pSP) is highly expressed during metacyclogenesis and that makes it important candidate as a member in host-parasite interaction. Recently, pSP has been found to be protective vaccine candidate in experimental visceral leishmaniasis by regulating host matrix metalloproteinase-9 profile. Moreover, L. donovani intracellular serine protease has been found to be associated in invasion process, as it downregulates the phagocytic activity of macrophages.

Apart from cysteine proteases, serine proteases have also been currently identified as therapeutic targets in Leishmania. The classical serine protease inhibitors—aprotinin, TPCK (N-tosyl-l-lysylchloromethylketone) and benzamidine and a Kunitz-type inhibitor (ShPI-I) obtained from sea anemone Stichodactyla helianthus have been found to inhibit Leishmania growth and induce tremendous morphological alterations in the parasite, suggesting that serine proteases could be potential drug targets. Specific doses of these inhibitors can cause considerable
morphological alteration in the flagellar pocket region with bleb formation that coats the flagellar pocket. These observations indicate that serine protease inhibitors act in the flagellar pocket region. These inhibitors also induce the formation of autophagic vacuoles and kill Leishmania. Moreover, L. amazonensis promastigotes antigens (LaAg) pretreated with irreversible serine protease inhibitors have invalidated their disease-promoting effects and that corroborates the exploitation of serine proteases inhibitors in the development of anti-leishmanial drugs.\(^4\)

Furthermore, L. major genes encode ecotin-like inhibitors of serine peptidases (ISPs)\(^5\) and it is believed that ISPs of L. major influence the early stages of infection of the mammalian host by targeting host serine peptidases.\(^6\) Subsequently, gene deletion study has demonstrated that ISPs prevent the activation of TLR4 by neutrophil elastase (NE) during the host-parasite interaction to uphold parasite survival and growth.\(^7\) Although, a few recent reports have shown that serine proteases are essential virulence factor for the parasite, but extensive studies are still needed to elucidate their biochemical function in Leishmania physiology as well as to develop suitable serine protease inhibitor as a novel anti-leishmanial agent.\(^8\) Recently, it has been hypothesized that the structure-based drug design could be attained through three-dimensional model.\(^9\) Recent reviews have also described that prolyl oligopeptidase and oligopeptidase B would be of commendable choice for drug design against the parasitic diseases, including the Chagas disease, leishmaniasis and African trypanosomiasis.\(^10\)

Overall, proteases in Leishmania species play central role in diverse processes, such as the parasite differentiation, cell cycle progression, nutrition acquisition, parasites migration, immune evasion and modulation of host cells signaling.\(^11\) Hence, it will be invaluable to develop suitable protease inhibitors for the effective treatment of leishmaniasis. In the long run, the developments of both synthetic and natural protease inhibitors targeting particular protease(s) will offer new therapeutic interventions for the treatment of leishmaniasis. This will also further advance our understanding of physiological significance of the proteases in disease pathogenesis.

**Perspectives**

Over the last few decades, tremendous efforts have been put forth to develop effective drugs for the therapy of parasitic diseases, including leishmaniasis. The new strategy of developing target-based drug rather than the haphazard screening of compounds provides better insight towards a problem within the vicinity of therapeutics. Some proteins or enzymes which are critical for metabolic or biochemical pathways have been identified as suitable drug targets. However, Leishmania proteases are the most important virulence factors as they are vital for parasites physiology as well as pathogenesis of leishmaniasis. Hence, they may be attractive targets for drug development. Nevertheless, further studies are needed to develop novel drugs targeting one or multitude of proteases against leishmaniasis to overcome rising resistance to current drug therapies. In addition, some inhibitors have already been experimentally employed against leishmaniasis and they have yielded positive results. By utilizing the combination of advanced technology and information science, the structure and function based natural or synthetic inhibitors can be exploited to successfully discover/design an efficacious drug for the treatment of leishmaniasis and other debilitating parasitic diseases.

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