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REVIEW ARTICLE

Protease Inhibitors as *Ad-hoc* Antibiotics

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Abstract:

Background:

Proteases are important enzymes that can degrade proteins and are found in animals, plants, bacteria, fungi and viruses. The action of proteases can be controlled by Protease Inhibitors (PIs), chemical or proteinaceous in nature that can block the active site of protease. Since the step catalyzed by proteases may play important role in life cycle of microbes, hindering the action of proteases by PIs may act as therapeutic intervention for microbial infection.

Material and Methods:

A thorough study was performed and wide range of literature was surveyed to confirm our results of PIs showing antibacterial activity.

Results:

PIs have shown to be effective drugs against bacterial pathogens, pathogenic viruses- Human Immunodeficiency Virus (HIV), Herpes virus, Hepatitis Virus. PIs have recently been investigated for controlling protozoan parasites. Clinical value of proteases and their inhibitors has been studied in *Helicobacter pylori* which is the etiologic agent of gastritis.

Conclusion:

This review is intended to highlight the role of PIs in the Battle against Microbial Pathogens.

Keywords: Antibacterial, Anti-fungal, Anti-protozoal, Antimicrobial, Hepatitis C Virus, Herpes Virus, Human Immunodeficiency Virus, Protease Inhibitor.

INTRODUCTION

Proteases are protein digestive enzymes, also known by the terms as peptidase or proteinase which are prevalent in all plants, animals and most of the microorganisms [1]. They play a crucial role in many biological processes like birth, life, aging, and death; hence performing a pivotal role in survival and maintenance of the organism [2 - 10]. They constitute about 2% of the human genome and 1-5% of genomes of the infectious organism [11]. The proteolytic events catalyzed by these enzymes serve as mediators of signal initiation, transmission and termination of the cellular events such as inflammatory response, cellular apoptosis, coagulation of blood and hormone processing pathways [12]. Proteases play a prime role in regulation of the life of insects, agricultural pests, farm animal health, plants and marine food sources [11]. They possess the potential to contribute to our economy by improving plant and animal health through intensified growth and by treating and preventing parasite infections, crop protection through advanced herbicides and pesticides, and increased or speedy production of food resources [10].

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The conduct of proteases in spite of many advantages has to be closely regulated and controlled to avoid the excess activity of these enzymes, as it may possibly damage its host organism. This task of controlling is carried out by PIs [13]. As the name implies, the PIs aptly inhibit the operation of the indigenous proteases. They are supposed to be specific in nature as they target and block only the proteases and other proteins stay unaffected. Also, the studies have proved that the use of Protein PIs (PPIs) is more advantageous over chemical PIs, as PPIs are safer and more specific in their targeted action. PPIs are considered to be a good contender for oral administration, with lesser consequences [14].

In recent past, the microbial pathogens have developed resistance to currently available antibiotics which has elicited the interest of researchers to isolate and understand the mode of action of antimicrobial proteins (peptides) [15]. Also, due to adverse side effects, limited clinical efficacy and the emergence of drug-resistant viral strains, there has been a great advancement in the development of new antimicrobial agents lately which show activity against new resistant strains [16]. Therefore, PIs from plants represent strong candidature for the development of antimicrobial drugs due to their potent inhibition of the growth of a wide range of pathogenic bacteria. We will discuss in this review, some of the important general features of PIs, specifically highlighting new advances and the specific challenges associated with their role as antibacterial, antifungal, antiprotozoal and antiviral agents.

PIs as Antibacterial Agent

PIs play a defensive role in plants and protect them from phytophagous insects and microbes [1]. PIs act against pathogens by inhibiting proteases present in insect guts or secreted by microorganisms which cause decrease in the availability of amino acids which are required for their growth and development [17]. For PIs to be used as antimicrobial agents, the most important factor is safety. Hence, several PIs of bacterial or plant origin which are nontoxic have been purified and are available commercially to treat protease induced peri-anal dermatitis [18]. PIs may contribute to host defense against infection through numerous mechanisms, *i.e.* they may protect opsonins and their receptors against proteolysis or control proteolytic processing of antimicrobial peptide precursors or show direct antimicrobial activity [19]. Various *in vitro* studies prove that PIs form pores or channels in the cell membrane and the cell die due to the out-flux of cellular contents [20]. Hence, they inhibit the activity of proteases produced by the pathogens which interfere in the development of bacteria and hence cause death.

Recent studies have found that a neutrophil elastase inhibitor, secretory leukocyte protease inhibitor (SLPI) is proved to possess antibacterial and anti-inflammatory properties [21]. Indeed, SLPIs and elafin show good antimicrobial potential against broad-spectrum microbes [22]. Epithelial cells produce antimicrobial polypeptides that defend the mucosa against infection caused by pathogens. The epithelium produces large amounts of these protease inhibitors which have been shown to be active against Gram-positive and Gram-negative bacteria in lungs [19]. By targeting the factors causing proteolytic virulence, PIs decrease the bacterial malignity. Recently the study is being continued for the development of a protein which shows anti-virulence potential against Anthrax lethal factor (LF), a toxin secreted by *B. anthracis*. The inhibition of virulence has recently been established as an antimicrobial strategy [23 - 25]. The limited efficacy of antibiotics increases the demand for the development of such anti-virulence treatment related to this organism.

PIs as Antifungal Agent

One of the most problematic illnesses worldwide is mycoses. The people with weak immunity or with an immuno-compromised immune system are mostly the sufferers of this disease. Due to the use of chemicals since a long time, many strains of fungi have become mutant as a change in their structure and physiological mechanism has taken place. Therefore, the demand and development of novel antifungal drugs has become inevitable due to the loss of effectiveness and increased side effects of conventional drugs [26]. To mention, *Candida albicans* is found to be persistent in immune suppressed patients and is still the main fungal pathogen isolated in hospitals. *C. albicans* demonstrates pathogenicity by modifying host defense mechanisms that secondarily initiate transformations in the fungal behavior. Also, as the fungal cell is almost similar to human cells, targeted drug development is a very difficult task [27, 28].

The antifungal role has been reported for trypsin inhibitors during fungal cell wall development by interfering with the chitin biosynthetic process by inhibiting the proteolytic activation of the chitin synthase zymogen [29 - 31].These trypsin inhibitors belong to the Bowman Birk family and are usually accumulated only in developing seeds [32]. It is, therefore, possible that PIs act synergistically with other defense proteins expressed in plants contributing to limiting invasion by pathogenic fungi.

The pathogenesis of *C. albicans* is complex and the variation in different virulence results due to the various stages of infection. Some virulence factors, like the Secreted Aspartic Proteases (SAPs) cause infection in different stages and the blockage at any step may cause loss of virulence of the pathogen which could help in the treatment of disease. Therefore, SAPs have become budding targets for developing novel anti-*C. albicans* drugs [26]. Also, studies have proved that pepstatin A and aspartic-type PIs which are used in chemotherapy against HIV, inhibit *C. albicans* with specific effects on SAP activity, fungal proliferation and morphogenesis, binding and interaction with living and nonliving world and modifications of various biochemical factors causing virulence [33]. Therefore, it can be concluded that *C. albicans* can be inhibited by natural or synthetic pharmacological compounds with anti-aspartic protease properties.

PIs as Antiprotozoal Agent

Recent studies have revealed the role of PIs in the inhibition of various pathogenic protozoa including *Leishmania* and *Trypanosoma.* These protozoa exhibit cysteine proteases which are stage regulated and are found to be involved in parasite differentiation and disturbing host's immune response [34]. Therefore, cysteine proteases are the key virulence factor of protozoan parasites [27]. To counter, structure-based drugs have been designed against proteases like cathepsin L-like (CpL) and B-like (CpB) cysteine proteases in *Leishmania* sp [28]. All parasitic protozoa contain numerous proteases including plasmepsins or the aspartic proteases from *Plasmodium* which provide required nutrients for the growth of the parasite. Studies have brought in light the role of two aspartic proteases, plasmepsins I and II found in malaria parasite *P. falciparum*, which have a crucial role in the degradation of hemoglobin within the parasite's lysosomes [35]. There is a rich unexplored area in the form of aspartyl PIs (including HIV PIs) in *T. brucei*. The hydrolysis of a cathepsin D fluorogenic substrate (7-methoxy-coumarin-4-acetyl-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(DNP)-Arg-amide) by *T. cruzi* epimastigote extract and the inhibition of its hydrolysis by pepstatin A suggested the possibility of aspartic peptidase activity as an intracellular target of this inhibitor [35]. With these results, there is a potential of exploiting aspartic peptidases as treatment agents for Chagas' disease.

The recent studies also demonstrate that cysteine protease inhibitors can selectively arrest replication of a microbial pathogen without untoward toxicity to the host. This can be achieved with reasonable dosing schedules and oral administration of the drug and this has been confirmed by the efficacy of cysteine protease inhibitors in the treatment of *T. cruzi, P. falciparum* and *Leishmania major.* Moreover, work on *Trypanosoma brucei*, the agent of African trypanosomiasis, is preliminary but also promising [36]. Henceforth, it can be concluded that the search for novel anti-parasitic agents has turned its way towards PIs for these cysteine and aspartic proteases.

Protease Inhibitors as Antiviral Agent

PI and Human Immunodeficiency Virus

In combination with anti-HIV therapy, inhibitors of HIV proteases have proven to be crucial drugs. These inhibitors were constructed to target mature protease and avoid viral particle evolution by arresting Gag and Gag-Pol processing by mature proteases [37]. An important therapeutic target for the treatment of HIV infection and AIDS is the HIV-1 protease. Therefore, the development of infectious virus can be avoided by the use of PIs. Hence, a number of effective PIs are involved in drugs used to treat HIV/AIDS patients [38].

Nine different PIs have been recognized for clinical use so far. Except tipranavir, all PIs are combative peptido-mimetic inhibitors, mimicking the natural substrate of the viral protease receptors (PR). The peptido-mimetic inhibitors include a hydroxy-ethylene core which blocks cleavage by the viral PR [39].

Darunavir and saquinavir have been shown to be effective at arresting the initial auto processing of full-length Gag-Pol in HIV-1-infected T cells. Thus, two identified HIV-1 PIs that have actions against the primary autocatalytic step of the embedded HIV-1 protease in Gag-Pol at concentrations that may be attained in HIV-1-infected patients [40]. This action at the initial processing step makes it probable to explore advance inhibitors with greater potency leading to imperfect viral maturation at the early stages [41].

PPIs are also potential candidates for anti-HIV treatment following reports that trypsin inhibitors from *Phaseolus lunatus* and *Glycine max* are able to inhibit HIV-1 reverse transcriptase [42].

PI and Herpes Virus

In infected individuals, clinical manifestations are majorly caused by Human Cyto-Megalo Virus (CMV), Herpes

Simplex Virus type 1 and type 2 (HSV-1 and HSV-2). There has been a rise in their number in the last decade due to increase in the number of immuno-compromised patients undergoing chemotherapy, transplantation, or suffering from AIDS. The mode of action of all the currently available antiviral drugs is interference with the synthesis of viral DNA [43]. These include acyclovir, valaciclovir, and famciclovir for treating HSV infection and ganciclovir, foscarnet, and cidofovir for CMV infection. These chemotherapeutic agents except acyclovir have serious side effects. Moreover, there has been an emergence of drug-resistant viral strains against these antiviral agents which has become another problem these days [44]. Thus, the development of new antiviral drugs with different mechanism of action is need of the hour [45].

The CMV protease, assemblin [46], plays a central role in the complex processes of capsid assembly and maturation through its proteolytic processing of the CMV assembly protein precursor [47]. Assemblin-mediated proteolysis is considered to be essential for CMV viability as a mutation in HSV assemblin yields noninfectious virus [48]. Therefore, peptidomimetic inhibitors for assemblin and inhibitors which target virus capsid protease represent a potential therapeutic alternative.

PI and Hepatitis C Virus

There is a high rate of progression to chronicity in cases of Hepatitis C infection [49], which is characterized by liver cirrhosis, progressive liver injury, and, in some cases, hepatocellular carcinoma. Currently, there are no curative antiviral agents available for the treatment of HCV infection but sometimes alpha interferon therapy is used in the treatment of moderate or severe liver disease caused by the HCV, only some patients manifest a sustained response [50]. Additionally, there is no vaccine to prevent HCV infection which might be either due to complex and multiple HCV genomes or due to variation in viral infection within infected person [51, 52]. Therefore, an antiviral therapy that effectively suppresses virus replication is needed. To combat the situation, inhibitors of HCV-encoded enzymes, specifically NS3 protease and NS5B RNA polymerase should be the focus of thorough research for novel drug designing [44].

DISCUSSION AND CONCLUSION

PIs are involved in regulating various normal and abnormal viral activities. This suggests that there is a vast scope of their use in the field of pharmaco-therapeutics especially when they have a strong defensive therapeutic role without side effects. The authors have collectively worked in evaluating the antimicrobial activities of PIs, *Cajanus cajan* Trypsin Inhibitor (CCTI) and Lima Bean Trypsin Inhibitor (LBTI) purified from plant sources. Antimicrobial activity of the isolated PIs was studied against three gram-positive bacteria (*Bacillus subtilis, Aeromonas spp.* and *Staphylococcus aureus*) and five gram-negative bacteria (two of *Escherichia coli* and one each of *Klebsiella pneumoniae, Klebsiella oxytoca* and *Pseudomonas aeruginosa*). The results suggest that CCTI and LBTI exhibit bacteriostatic property (Table **1**). It was found that PIs showed maximum inhibition against *E. coli* (MTCC 443), minimum inhibition against *S. aureus* (MTCC 902) while no effect was found on *B. subtilis* (MTCC 736). Additionally, experiments with animal models for *in vivo* studies for biological characterization to correlate significance in human health will be done in near future. These studies, collectively, form a part of continued efforts in the development of phytodrug for human benefit.

Table 1. Mean Growth inhibition (MGI%) of various bacterial strains in presence of CCTI and LBTI.

S.No.	Bacterial strain	MGI% (CCTI)	MGI%(LBTI)
1.	*E.coli* (ATCC 25922)	31.62	43.06
2.	*K. pneumoniae* (ATCC 700603)	32.2	44.3
3.	*S. aureus* (MTCC 902)	10.07	31.4
4.	*B. subtilis* (MTCC 736)	0	0
5.	*E.coli* (MTCC 443)	69.87	71.72
6.	*P. aeruginosa* (MTCC 2453)	31.56	55.87
7.	*Aeromonas spp.* (A10 MDR)	42.86	62.38
8.	*K. oxytoca* (A13 MDR)	15.91	55.23

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