EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 11
153-155
Year: 2017

SERINE PROTEASE INHIBITORS AS POTENTIAL THERAPEUTIC AGENTS: A PERSPECTIVE

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Article Received on 29/08/2017 **Article Revised on 19/09/2017** **Article Accepted on 09/10/2017**

ABSTRACT
Serine proteases play a major role in various biological processes like cell signalling, digestion, immune responses, blood clotting and extracellular matrix remodelling. The dysregulation of serine proteases during diseased conditions ranging from cancer and inflammatory disorders to degenerative diseases and colitis represents their importance in various physiological conditions. One of the effective ways to regulate their effect is by inhibiting the proteolysis using protease inhibitors. Understanding the role and the underlying mechanism played by serine proteases and their inhibitors in various pathological conditions offer novel strategies for therapeutic intervention. Scientific reports highlight the effectiveness and potency of small cyclic peptides as protease inhibitors. Small cyclic peptides are advantageous over linear or high molecular weight protein inhibitors because of their stability and efficacy. The bioavailability of cyclic peptides is more due to their cell penetrating behaviour.

KEYWORDS: *Serine proteases, protease inhibitor, cyclic peptides, bowman-birk inhibitor.*

INTRODUCTION
Serine proteases play a central role in the body's inflammatory response, as pancreatic enzymes, vasoactive enzymes, mediators of clotting and fibrinolysis and intermediates of the complement system.[1] In instances when inflammatory agents go unchecked or excess anti-inflammatory response elicits immunosuppression, protease inhibitor therapy may serve as a useful medicinal tool to restore balance.[2] In earlier days of research, proteases were viewed as degradation machines, but paradigms have shifted and proteases are now viewed as signaling molecules critical for many biological processes.[3] The major areas of interest for protease-targeted therapies are the cardiovascular, inflammatory, infectious diseases areas, cancer and neurodegenerative disorders. A more detailed look at the proteases currently considered as potential targets shows that endogenous proteases are often linked to chronic diseases and are therefore attractive to pharmaceutical companies.[4]

Proteinase Inhibitors
The protein-proteinase inhibitor is a protein, which combines reversibly with one or more proteinases to form complexes of discrete stoichiometry, in which all the catalytic functions of the proteinase are competitively inhibited. These inhibitors are widely distributed among different botanical families in the plant kingdom.[5] The inhibitors from legume seeds are well studied and documented owing to their importance as a rich source of proteins for both human and animal nutrition. A number of physiological functions have been proposed for these inhibitors, including the control of endogenous proteases, particularly during dormancy of the seed, acting as a defense against the proteolytic enzymes of microbial, insect, avian or mammalian predators and serving as a storage depot particularly of the sulfur containing amino acids. During past few decades, physicochemical properties and the nature of the enzyme interaction with the inhibitor have been investigated. Proteinase inhibitors are essential in nature for the regulation of proteinases in numerous biological processes. Evidence shows that many different protease inhibitors in their pure forms suppress carcinogenesis.[6] Plant-derived protease inhibitors with strong trypsin/chymotrypsin inhibitory activity have been shown to suppress several stages of carcinogenesis.

The inhibitors of the Bowman-Birk family are small serine proteinase inhibitors found in seeds of legumes and in many other plants.[7] All the Bowman-Birk inhibitors (BBIs) exhibit two regions of tandem homology, each with an independent reactive site loop of nine residues formed by two very well conserved cysteine residues. Much evidence shows that many different protease inhibitors in their pure form suppress carcinogenesis.[6] BBI has been extensively studied as an anticarcinogenic protease inhibitor. The soybean derived

BBI is particularly effective in suppressing carcinogenesis in different species (mice, rats and hamsters) when given to animals by several different routes of administration, including diet. BBI suppresses carcinogenesis in animals with genetic susceptibility to cancer.[8] Animal carcinogenesis studies have shown that dietary amounts as low as 0.01% BBI can suppress liver carcinogenesis in mice.[9] The strength of the BBI as a cancer preventive agent lies in its ability to reverse the initiation during tumorigenesis. BBI suppresses carcinogenesis by its ability to inhibit serine proteases or the expression of certain proto-oncogenes.[10]

Soybean BBI quelled the X-ray-induced transformation of mouse embryo fibroblast cells.[11] Potato protease inhibitors (PPI) I and II showed similar *in vivo* effects.[12] The chemoprotective activities of PPI I and II have been attributed to blocking activation of transcription activator protein 1, an inducible eukaryotic transcription factor.[13] Potato carboxypeptidase inhibitor suppressed growth of several human adenocarcinoma cell lines, due to its antagonistic effect on epidermal growth factor expression.[14] Food and Drug Administration (FDA) has granted BBIC from soy with investigational new drug status.[15] Phase I and Phase IIa studies of BBIC in patients with oral leukoplakia have demonstrated clinical activity without detectable side effects after oral administration.[16] BBIC shows *in vitro* inhibition towards tumor cell proliferation, invasion and survival in several models of prostate cancer without adversely affecting normal cells.[17] BBI, a well-characterized soybean peptide, has exhibited broad anticancer activities in clinical trials and cell-based assays. The chemopreventive property of BBI is attributed to proteasome inhibition, presumably *via* an anti-chymotrypsin mechanism.[18,19] The BBI have been extensively studied and their mechanism of inhibition of serine proteases is well established. The BBI has been clearly shown to prevent malignant transformation in many cell lines and in several organ systems. The anticarcinogenic activity has been linked to the chymotrypsin inhibitory potential of BBI. However, the poor bioavailabilty and high susceptibility to proteolysis are significant limitations of BBIs as efficient and potent cancer chemopreventive agents; the limitations are attributed to its large size (8 kDa). The major isoinhibitor from pea, TI1B possesses anti-proliferative effect on HT29 colon cancer cells mediated through protease inhibition.[20] The black eyed pea trypsin/chymotrypsin inhibitor (BTCI) strongly inhibits the proteolytic chymotrypsin, trypsin and caspase-like activities of 20S proteasome. BTCI is associated with cytostatic and cytotoxic effects in MCF-7 breast cancer cells by means of apoptosis.[21] HGI plays a protective role against preneoplastic lesion in colorectal carcinogenic mice model.[22]

Cyclic Peptides

Cyclisation of small peptides is a routine method of improving efficiency by reducing degradation and enhancing binding affinity *via* the minimization of entropic losses. Cyclic proteinase inhibitors have several advantages over their acyclic counterparts. Hence identifying such naturally occurring inhibitors or designing and synthesizing such inhibitors is of importance. Synthetic peptides constructed based on the amino acid sequence of the inhibitory loops were found to have a binding affinity that was three orders of magnitude less than the natural inhibitor.[23] Furthermore, the discovery of 34 amino acid residue cyclic trypsin inhibitors from squash seeds suggest that naturally occurring cyclic proteinase inhibitors may be more prevalent. Matriptase is a type II transmembrane serine protease, found to be upregulated in cancers. SunFlower Trypsin Inhibitor (SFTI-1), a cyclic peptide inhibitor from sunflower seeds exhibits potent inhibitory activity toward matriptase with the K_i of 0.92 nM[24] and has been suggested that SFTI-1 could be used as a molecular scaffold for drug discovery.[25] Cloning and expression of such small cyclic peptides based on the DNA sequences of the BBI is an attractive alternate to isolation and purification of these peptides. The efficacy of these peptides can be tested *in vitro* using various cell lines.

CONCLUSION

Targeting tumor cells aims at more specific agents with less toxicity. Proteases would be ideal therapeutic targets owing to their frequent over expression at different stages of tumor progression. Understanding how inhibition of one protease can affect the overall proteolytic balance of the tumor microenvironment is crucial to design agents that target proteases with maximal impact and minimal toxicity. Diversity and abundance of protease inhibitors in plants make them excellent sources for discovering novel protease inhibitors with specific pharmacological effects. The mechanism of their action and the regulatory network between the proteases and signalling molecules should be appraised. Bioavailability is a challenging factor when promoting a natural compound as therapeutic agent. Cyclization of small peptides would improve the efficacy and stability by escaping degradation and enhancing binding affinity. Cyclic peptides poses to be more potent as the probability of their reaching to target cells are more because of their cell penetrating behaviour.

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