A Mini Review on New Pharmacological and Toxicological Considerations of Protease Inhibitors' Application in Cancer Prevention and Biological Research

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ABSTRACT

Proteases are enzyme complexes that play a crucial role in the degradation of many proteins involved in regulation of cell cycle including G1, S and G2/M phases, apoptosis, cells growth and activation, adhesion, invasion, cell migration and metastasis, protein secretion, cellular interactions and signal transduction, phagocytosis and angiogenesis as well as tissue formation and stabilization. Thus, they show complete anticancer mechanisms. Proteases may be classified by their catalytic mechanisms into aspartic, serine, threonine, metallo- and cysteine proteases and are localized at the cellular surface or within specific sub cellular structures, in particular the lysosomes. In vitro and in vivo studies have reported the anticancer properties of protease inhibitors (PIs) but little is known about the clinical use of natural or chemical PIs, alone or in combination with other chemotherapies in humans. Thus, understanding the mechanisms of these drugs in prevention of cancer could result in them being used for treating the diseases. Furthermore, PIs can attenuate the drug toxicity in studies. They can also promote the defense mechanism, including induction of superoxide dismutase (SOD) and catalase (CAT) activity. These properties, on the one hand, suggest a wide spectrum of clinical applications for treatment and prevention of various cancers and on the other hand, further clinical and biological studies need to know the accordance mechanisms and discover new natural PIs. Thus, studying PIs will open a new anticancer strategy in treatment of various cancers in future.

Key words: Cell cycle regulation, apoptosis, natural and chemical protease inhibitors, cancers, toxicity

INTRODUCTION

PIs are based on special protease types in which inhibitors are classified into aspartic protease, cysteine protease, metalloprotease, serine protease (serpins), threonine protease, trypsin and Kunitz STI protease inhibitors (Rawlings et al., 2004). PIs are localized at the cellular surface or sub
cellular structures, in particular the lysosomes. PIs act as a crucial element in various physiological reactions from simple digestion of food proteins to highly-regulated event including immune system, angiogenesis, apoptosis pathways and cell growth, differentiation, vasculogenesis, as well as effecting cell migration and metastasis which are important in developmental and repair processes (Buhling et al., 2006).

PIs were commonly used to treat viral infection, including HIV (Chow et al., 2009; Danwe et al., 2005; Judith et al., 2007; Van Heeswijk et al., 2001) and hepatitis C for a long time (Nelson, 2011; Ghosal et al., 2011). PIs can inhibit the viral development by preventing the production of proteins which are important to assemble new virus particles (virions) (Chow et al., 2009; Van Heeswijk et al., 2001).

PIs have been recognized as a special approach in anticancer therapy and mostly can be isolated from plants (Rahimi et al., 2011; Khan et al., 2008; Tochi et al., 2008) or bacteria (Yadav et al., 2010; Hossain et al., 2007) as their natural sources. Studies in vitro and in vivo have reported that PI compounds have anticancer properties on their own or synergistically with other compounds. In one study a combination of natural compounds such as gambogic acid with PIs, including MG132 or MG262 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal), have shown inhibitory effects on growth of malignant cells and tumors in allograft animal models with no observed systemic toxicity in the animals (Huang et al., 2011). PIs have been shown to have a potent anticancer effect against various cancers like leukemia, lymphomas, melanoma, hepatocellular carcinoma, prostate, lung, breast, ovarian, cervical, colorectal and gastric cancer (Table 1, 2). Different cancer cell lines have been used for evaluating the anticancer effect of PIs (Table 1, 2). A PC3 prostate cancer cell line is one of the examples in which multiple death signaling pathways can be regulated by PIs (Yang et al., 2006). Thus, PIs represent a complete anticancer effect. Furthermore, except potential attenuation of chemotherapeutic drug toxicities properties, they have proved to be beneficial anticancer drugs, alone or in combination with other anticancer drugs, in new treatment strategy.

Anticancer properties of chemical protease inhibitors: Cancer drugs development is slow and costly. Therefore, an approach to accelerate the availability of new drugs is to reposition drugs approved for other indications as anticancer agents. Studies are investigating whether PIs could possibly be used to treat cancers. They are, also, evaluating its position in future. PI drugs such as nelfinavir and atazanavir are able to kill tumor cells in vitro (Gills et al., 2007; Pyrko et al., 2007) and in vivo (Pyrko et al., 2007), however, this effect has not been completely tested in humans yet. Therefore, wide spectrum research is necessary to be designed according to PIs in human to evaluate its beneficial role. PIs may be targeted for p-glycoprotein 1 (P-gp) also known as multidrug resistance protein 1 (MDR1) or cluster of differentiation 243 (CD243) or ATP-binding cassette sub-family B member 1 (ABCB1), but they interestingly have interaction with P-gp and can inhibit its effect and remain in cells for a long time or they may inhibit the effluxes of other anticancer drugs for more effect and can potentiate its anticancer properties (Washington et al., 1998; Olson et al., 2002). Ritonavir has been reported as the modulation effect on pharmacokinetic (PI5) properties of mirtazapine and citalopram and can increase the peak of these two antidepressants in mice, then Therapeutic Drug Monitoring (TDM) have been necessary when these combinations are needed (Thakar et al., 2012). Inhibitors of protease, can now front-line drugs for the treatment of various cancers in human. Table 1 shows the special considerations for chemical PIs in cancer treatment. Anti metastasis and invasion, cell cycle regulation, differentiation
Table 1: Chemical protease inhibitors and their probable anticancer mechanisms in cancer cell lines

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<td>Donia et al. (2011)</td>
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<td>Gupta et al. (2005)</td>
<td>SQ20B, T24, MIAFACA2 and A549</td>
<td>Lung cancer</td>
<td>Viability decreasing in tumor cells via inhibiting phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway</td>
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<td>Gills et al. (2008)</td>
<td>A549, H460 and MCF7</td>
<td>Lung and breast cancer</td>
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<td>Srirangam et al. (2011)</td>
<td>A549, H522, H23 and K-ras wild-type line H838</td>
<td>Lung and breast cancer</td>
<td>G0/G1 cell cycle arrest and induction of apoptosis, down-regulation of cyclin-dependent kinases (cyclin D1) and retinoblastoma protein phosphorylation and inhibition of survivin messenger RNA and protein levels</td>
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<td>Kumar et al. (2009)</td>
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<td>Srirangam et al. (2006)</td>
<td>MCF7, T47D, MDA-MB-436 and MDA-MB-231</td>
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<td>G1 cell cycle arrest, depletes cyclin-dependent kinases 2, 4 and 6 and cyclin D1 but not cyclin E and depletes phosphorylated Rb and Ser 473 Akt</td>
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<td>Ikezoe et al. (2006)</td>
<td>HL-60 cells</td>
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<td>Bruning et al. (2011)</td>
<td>SiHa</td>
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<td>Libertini et al. (2007)</td>
<td>LNCaP</td>
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<td>Studies</td>
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<td>Li <em>et al.</em> (2011)</td>
<td>HCT116 and SW480</td>
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<td>Cell death caused by mediating caspase dependent apoptosis and cytoplasmic vacuolization. Activation of p53, Bax and NF-κB pathway occurred in accordance to increase in (reactive oxygen species) ROS levels and BCL2 reduction</td>
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<td>Dai <em>et al.</em> (2010)</td>
<td>PC-3, DU145 and CL1</td>
<td>Prostate cancer</td>
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<td>Diterpenoid epoxide triptolide and the quinone triterpene cadastrolare</td>
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<td>Liu <em>et al.</em> (2011)</td>
<td>MCF7</td>
<td>Breast cancer</td>
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<td>Curcumin, Quercetin and Enterolactone</td>
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<td>Shankar and Srivastava (2007)</td>
<td>HCC-J5 and THP-1</td>
<td>Hepatocellular carcinoma and leukemia</td>
<td>Increasing in p53, p21, Bax, calreticulin, caspase 12, caspase 3 and GADD153 expression were occurred, but Bcl-2, Cdc2 and Cdc25c were decreased</td>
<td>Tanshinone IIA (Tan-IIA)</td>
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<td>Bachmeier <em>et al.</em> (2010)</td>
<td>Le174-T</td>
<td>Colon carcinoma</td>
<td>Anti cancer metastasis activity through extracellular signal-regulated kinase pathway, including inhibition the expression of metalloproteinase-2 and 9</td>
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<td>Xu <em>et al.</em> (2010)</td>
<td>DU145</td>
<td>Prostate cancer</td>
<td>Inhibition of signal transducer and activator of transcription 3 (STAT3) and its downstream protein expression cyclin D1, survivin and Bcl-2L</td>
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Anti-cancer mechanisms emphasize the complete anti-cancer properties of PIs which represent a potential strategy for the treatment of human cancers in the future. Other PI drugs and accordance anti-cancer mechanisms in vitro are presented in Table 1.

**Anticancer properties of natural protease inhibitors**: Different cellular events, including DNA repair in cell cycle and survival and apoptosis including p27, a key regulator for G1 to S transition in the cell cycle, can be regulated by natural drugs or their consistent (Yang et al., 2009). In one study, pro-apoptotic regulator agent BAX has been increased by Shiokinin, a natural naphthoquinone of Chinese medicine Zi Cao (gromwell) (Yang et al., 2009) and decreased in the levels of anti-apoptosis regulator agent Bcl-2 in another study (Sohrabi et al., 2009; Dai et al., 2010). Changes in the activity of nuclear factor kappaB (NF-κB), an important inflammatory agent (Hemalatha et al., 2012; Mrholiæ et al., 2011; Arathy and Sreekumar, 2009) and pro-survival factor that is pivotaly regulated by the proteasome, has been reported by natural drugs (Rahimi et al., 2010).

5,6,3′,4′-tetrahydroxy-7-methoxyflavone, a novel natural PI which is known for its anti-cancer effect, is extracted from Anisomeles ovata (Chang et al., 2010). Celastrol is another natural PI that exhibits promising anti-cancer property by inhibiting the constitutive NF-κB activity and modulating the Bcl-2 family proteins, suppressed cell migration and invasion, increasing caspase dependent apoptosis and decreasing angiogenesis in androgen-independent prostate cancer cell lines PC-3, DU145 and CL1 (Dai et al., 2010). Bowman-Birk protease inhibitor which is obtained from Vigna unguiculata seeds has shown an inhibitory effect on trypsin and chymotrypsin, two enzymes belonging to the serine family of proteases in which regulated the immune function, blood clotting and inflammation (Joanitti et al., 2010; Srinivasan and Durairaj, 2007). It has been suggested that this preparation reduces cancer cells’ ability to duplicate and spread within 72 h, leading to apoptosis or cell death anti-cancer effect against MCF-7 breast cancer cells by reducing this cell viability and proliferation through S and G2/M phase arrest (Joanitti et al., 2010). Furthermore, DNA fragmentation, mitochondrial protection and cytoplasm acidification were also reported (Joanitti et al., 2010). Study has indicated that a type of blue-green algae known as Microcystis aeruginosa may be a potential new source of natural protease inhibitors containing two previously unknown inhibitors of the trypsin protease, including micropeptins EI992, EI964 and a modified linear peptide aeruginosin EI461 which is isolated from its hydrophilic extract (Ploutno et al., 2002). Green tea which grows in the north of Iran and has proved to be hepatoprotective and antioxidant, contains a type of polyphenol called epigallocatechin gallate (EGCG) which has shown PI and anticancer activities against human breast cancer cells (Balouchzadeh et al., 2011; Dou et al., 2008). Ellagic acid and punicalagin, from Punica granatum (pomegranate) have been also reported an inhibitory effect on serine PI, including alpha-secretase (TACE), chymotrypsin, trypsin and elastase (Kwak et al., 2005). Catechin, queretin, kaempferol, equol and epicatechin (epigallocatechin-3-gallate) are the other constituents of pomegranate which may also have protective effect (Park et al., 2010). Different anatomical compartments of pomegranate, including seed, juice, peel, leaf, flower, bark and roots may have anti-cancer activity through counteraction with tumor cell proliferation, invasion, cell cycle and angiogenesis (Lansky and Newman, 2007; Rahimi et al., 2011). Pineapple (Ananas comosus) is another plant which contains PIs and can be used for its therapeutic properties including inhibition of malignant cell growth, inflammation, thrombus formation, control of diarrhea, dermatological and skin
debridement (Tochi et al., 2008). Collectively, further studies are necessary to be arranged for detection of new natural Pls in plants and recognizing the accordance anticancer mechanisms. Thus, working on natural Pls will be dispread in the world, because of their low toxicity and cost, good response and anticancer properties. Other reported anticancer mechanisms of natural Pl in vitro are shown in Table 2.

**Toxicological overview of protease inhibitors:** Further, beneficial effects of Pls are in accordance with reduction in toxicity of anticancer or chemotherapeutic drugs in combination. Indinavir, nelfinavir, saquinavir and ritonavir are currently Pls which are administrated to ameliorate doxorubicin induced cardiomyopathy which are mediated by stimulation of Toll-Like Receptors (TLR) 2 and 4 expression on cardiomyocytes (Kast et al., 2007). Combination of PI bortezomib/PS-341 in multiple myeloma cells refractory with multiple prior therapies, including melphalan, dexamethasone and thalidomide can reduce drug resistance and attendant toxicity and improve patient outcome in multiple myeloma (Chauhan et al., 2005). Pls may be considered much because of a better bio-distribution, lower toxicity and inhibitory quality on matrix metalloproteinases (MMP), indicator factors of metastases and the proteasome for a more effective anticancer therapy (Toschi et al., 2011). Furthermore, reduced glutathione, a major defense mechanism against Reactive Oxygen Species (ROS) (Nili-Ahmadabadi et al., 2011) has been restored by rosmarinic acid, a main phenylpropanoid constituent of *Prunella vulgaris* L. Pls' other protective roles are served as the reducer of Malondialdehyde (MDA) level and liver enzyme activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), as well as a lactate dehydrogenase (LDH) which can be induce by alcohol or other poison. Increase in the level of anti oxidative mechanisms, including superoxide dismutase (SOD) and catalase (CAT) activity (Polat et al., 2008). Furthermore, inflammatory cytokines which are important in induction of various diseases (Kalani et al., 2011; Rahimi et al., 2010), have been decreased with aprotinin (Homi et al., 2010). Pls may also be considered much more from the perspective of toxicology and have beneficial properties in comparison to other anticancer drugs.

**DISCUSSION**

Pls represent an important role in regulation of various cellular physiological and biological processes, including cell cycle, cell death, differentiation and the immune response (Fan et al., 2001; Buhling et al., 2006). Previous studies have shown the anticancer effect and probable mechanisms of the natural and chemical Pls in vitro (Table 1, 2).

Pls, like MG132, have shown apoptosis effects against gastric cancer cell lines AGS (p53 wild-type) and MKN-28 (p53 mutant) through a time and dose-dependent stimulation of caspase-3 which results in the release of cytochrome C from mitochondria into the cytosol, as a consequence of up-regulation of BAX. Furthermore, over-expression of all protease-associated proteins, including p53, p21 (wafl) and p27 (kip1) were demonstrated 4 h after protease inhibition. These results indicate a potential effect of Pls as anticancer drugs in gastric cancer (Fan et al., 2001). Two novel analogs, CH05-0 and CH05-10 of Indinavir, a Human Immunodeficiency Virus (HIV) protease inhibitor, inhibits the growth of cancer cells in vivo by induction of G1 cell cycle arrest, caspase-dependent apoptosis, stimulation of endoplasmic reticulum stress and unfolded protein response. In addition, no cytotoxic effect was observed against cancer cells in vitro (You et al., 2010). Also, acetyl salicylic acid (aspirin) induces synthesis of maspin, a member of the serine protease inhibitor which has been shown to inhibit the incidence of breast cancer.
metastasis and invasion in patients who have previously received anticancer therapies (Bhattacharyya et al., 2010).

A 17.5-kDa PI isolated from Chinese mini-black soybeans can inhibit HIV-1 reverse transcriptase (IC (50) = 3.2 and 5.5 μM), proliferation of breast cancer cells (IC (50) = 9.7 and 3.5 μM) and hepatoma cells (IC (50) = 35 and 6.2 μM), with relatively high potencies (Ye and Ng, 2011). Furthermore, PIs show trypsin inhibitory activity which is involved in carcinogenesis and promotes proliferation, invasion and metastasis of tumors and are found in various cancers (Ye and Ng, 2011; Soreide et al., 2006). Co-expressed MMP-2, MMP-7 and MMP-9 with trypsin seem to be of particular importance in proliferation, progression and invasion. In addition, MMPs are zinc-dependant endopeptidase which may play a role in both conversion from adenoma to carcinoma and in the initiation of invasion and metastasis (Wongsawatkul et al., 2011; Soreide et al., 2006; Xu et al., 2007). MMP and proteinase-activated receptor-2 (PAR-2) which are stimulated by trypsin, may activate the mitogenic MAPK-ERK pathway through activation of the epidermal growth factor receptor (Soreide et al., 2006). Such results have shown the molecular mechanisms of proliferation, invasion and metastases that are concerned with the role of trypsin in cancers consisting of colorectal cancer. Thus, PIs which have trypsin inhibitory activity may reduce cancer progression and may be regarded as a potential target of therapy. PIs can be also targeted for treatment of other diseases such as diabetes (Naderi et al., 2006).

PIs, as modulators in chemotherapy, may have adverse effects, on the one hand, through inhibition of worse events, including inflammatory cytokine production and liver enzyme activity and on the other hand, through promotion of protective mechanisms against oxidative stress, including superoxide dismutase (SOD) and Catalase (CAT) induction has been shown in accordance to PIs occupation. Furthermore, change in a pharmacokinetic event to the beneficial way like down regulation of P450 to induce active toxic metabolite is the other protective mechanism in which mediated by PIs (Ikezoe et al., 2006).

CONCLUSION

In numerous studies, the role of natural and chemical PIs in cancers have been reported in vitro and in vivo, but there is little information about the use of these compounds in inhibiting cancer in humans and the mechanisms of their actions. Thus, it will be a wide open spectrum of biological and clinical studies. Clinical trials, involving the use of these compounds, will be necessary in the future. Therefore, we propose that by making sure these compounds are devoid of toxicity, they can be used as potent anticancer drugs in the future.

REFERENCES


