LUNASIN AND ITS VERSATILE HEALTH-PROMOTING ACTIONS

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INTRODUCTION

Due to high prevalence of carcinogenic, cardiovascular and metabolic syndrome-associated disorders all over the world, growing scientific studies have been aimed to investigate various natural plant peptides in respect to prevention and treatment of the chronic diseases (Hernández-Ledesma et al., 2013; Liu et al., 2014; Alaswad and Krishnan, 2016; Fernández-Tomé and Hernández-Ledesma, 2016; Shidal et al., 2016; Janardhanan and Varier, 2018). The bioactive peptides are naturally present in foods or released from the source protein by processing conditions or during gastrointestinal digestion (Fernández-Tomé and Hernández-Ledesma, 2016). These specific protein fragments possess high tissue affinity, specificity and efficiency in promoting health (Daliri et al., 2017) related to their antihypertensive, antioxidative, antiobesity, immunomodulatory, antidiabetic, hypocholesterolemic and anticancer properties (Singh et al., 2014). One of the most promising and potential anticancer candidate, which was first isolated in 1987 in Japan during the screening of protease inhibitors from soybean is peptide lunasin (Liu et al., 2014). Besides anticancer activity, it has inherent antioxidative and anti-inflammatory effects that could contribute to its chemopreventive action, and it could also play a vital role in regulation of cholesterol biosynthesis in the body (Lule et al., 2015). These various biological functions of the peptide have been clearly demonstrated both in vitro and in vivo assays (Fernández-Tomé and Hernández-Ledesma, 2016).

LUNASIN STRUCTURE

Lunasin (from the Tagalog word “lunas” for cure; Hernández-Ledesma and de Lumen, 2008) has been described as 43 amino acid peptide with a molecular weight of 5.5 kDa (Liu et al., 2014; Wynn et al., 2017). It corresponds to a small sub-unit peptide encoded within the soybean 2S albumin GM2S-1 gene (Seher et al., 2012; Lule et al., 2015) coding also methione-rich protein, a signal peptide, and a linker peptide (Fernández-Tomé and Hernández-Ledesma, 2016). The unique amino acids sequence of lunasin molecule: SKWQHQDSCRRKQLQGVNLTPC-EKHIMEKIQG-RGD-DDDDDDDD (Galvez and de Lumen, 1999) with demonstrated activities of all fragments is documented in Figure 1. In summary, the peptide is composed of four regions: (I) fragment with unknown functions, (II) a predicted helix with structural homology to a conserved region of chromatin binding protein for binding to histones H3/H4, (III) a cell adhesion motif containing arginine (Arg), glycine (Gly) and aspartic acid (Asp) residues called RGD module, and (IV) a critical fragment with antimotic functions consisting of eight aspartic acids at the C-terminus (Galvez and de Lumen, 1999; Lam et al., 2003; Hernández-Ledesma and de Lumen, 2008; Hernández-Ledesma et al., 2009a). There is accumulating evidence that the last three functional fragments of the peptide are highly responsible for lunasin specific properties. The RGD tripeptide functions as an extracellular matrix to promote the intracellular accessibility of lunasin (Wan et al., 2017). The important component of lunasin uptake appears to be mediated by internalization via the integrin recycling pathway, with the integrin αVβ3 being a key factor (Cam and de Mejia, 2012; Inaba et al., 2014; McConnell et al., 2015). However, it was shown that the role of RGD motif for cellular internalization is cell-line specific, required in C3H 10T1/2 mouse embryo fibroblasts cells (Galvez et al., 2001) but unnecessary in mouse fibroblast NIH3T3 cells (Lam et al., 2003). Beyond this, the RGD motif potentiates the ability of lunasin to compete with integrins to bind with the extracellular matrix consequently suppressing the integrin-mediated signalling pathway, e.g., those that modulates proliferation, migration, invasion (Jiang et al., 2016) and apoptosis of the cells (Lu et al., 2006). Antimotic effect of lunasin has been attributed to the binding of its poly-D carboxyl end with high negatively charged 8 D-residues to regions of positively charged deacetylated histones and cellular regions, such as hypoacetylated chromatin found in telomeres and kinetochores in centromeres (Fernández-Tomé and Hernández-Ledesma, 2016). As a result, the kinetochrome complex does not form properly, and the microtubules fail to

ABSTRACT

The present review focuses on the potential health benefits of lunasin reported to date. Epidemiological studies on human and animals have demonstrated lower incidence of various types of cancer and other chronic diseases related to consumption of diets containing high amounts of soybean products. Naturally present in soybean, lunasin is the most isolated bioactive peptide with 43 amino acid residues, some of them creating an RGD motif, chromatic-binding helical region and an aspartic acid tail. Due to the three multiple functional domains, the intriguing peptide serves as promising candidate in exerting many-sided health-beneficial actions, such as antioxidant capacity, anti-inflammatory, hypocholesterolemic, and anticancerous activities. Depending on the soybean genotype, the lunasin content in the crop varies from 0.5 - 8.1 mg/g of seed. Besides soybean, it has also been identified in wheat Solanum nigrum, rye, barley, amaranth seeds, triticate and quinoa. In the current review, the structure, bioavailability, and molecular mechanisms involved in health-promoting effects of lunasin have also been summarized.

Keywords: lunasin; soybean peptide; antioxidant capacity; anti-inflammation; hypocholesterolemic activity; anticancer activity
LUNASIN’S BIOAVAILABILITY

Biological activity of lunasin depends on its concentration in plant sources, which in turn is affected by cultivar, environmental factors, and processing conditions (Wang et al., 2008). Indeed, the genotype of soybean, as a principal source of the peptide, has been demonstrated to be the main factor of lunasin quantity in soybean seeds (Hernández-Ledesma et al., 2013) ranging from 0.5 – 8.1 mg lunasin/g seed or 4.4 – 70.5 mg lunasin/g protein (Liu et al., 2014). Also, the stages of seed development and sprouting influence the lunasin production in this crop. Increased lunasin levels are notable during seed maturation and decreased ones during sprouting period (Park et al., 2005). Besides soybean, lunasin has also been identified in wheat (0.21-0.29 mg/g seed; Jeong et al., 2007a), Solanum nigrum L. (1.81 mg/g seed; Jeong et al., 2007b), rye (0.05-0.15 mg/g seed; Jeong et al., 2009), barley (0.013-0.059 mg/g seed; Jeong et al., 2010a), amaranth seeds (0.0095-0.0121 mg/g protein; Silva-Sánchez et al., 2008), triticale (0.43-6.46 mg/g seed; Nakurte et al., 2012) and recently from quinoa (0.001-0.005 mg/g seed; Ren et al., 2017). According to de Mejia et al. (2003), commercial soy products contain reasonable amounts of lunasin ranging from 5.48 mg lunasin/g protein (defatted soy flour) to 16.52 mg lunasin/g protein (soy concentrate). In addition to this, the daily consumption of 25 g soy protein, recommended by Food and Drug Administration (FDA) to reduce coronary heart disease risk (FDA, 1999) supplies approximately 250 mg of lunasin (de Mejia et al., 2003).

Bioavailability of the lunasin is one of the most important assumptions for its therapeutic purposes. Lunasin is very heat-stable peptide, retaining its bioavailability even after 5 minutes of boiling (de Lumen, 2005). Despite its peptide origin it was found that after oral ingestion the bioactive peptide is not completely degraded in the gastrointestinal tract but in intact and bioactive form it reaches various tissues (Hernández-Ledesma et al., 2009a; Muceniece et al., 2016), especially the targets for the most common malignant tumors (i.e., lungs, mammary glands, prostate; Hsieh et al., 2010a). The results of the study by Hsieh et al. (2010a) performed on CD-1 mice receiving 3H-labelled synthetic lunasin mixed with lunasin-enriched soy by gavage have revealed that 30 % of ingested lunasin was distributed in active form into lungs, liver, kidney, mammary glands, prostate and other investigated tissues and organs. Intact state of the intriguine peptide was also extracted from blood and liver of rats fed with lunasin-enriched soy (Jeong et al., 2007c) and lunasin-enriched wheat (Jeong et al., 2007a). Lunasin bioavailability was evaluated in humans, as well. In healthy men who consumed 50 g of soy protein, an average 4.5 % of biologically active lunasin from the total ingestion was absorbed into the plasma (Díaz et al., 2009a). Based on in vitro bioavailability studies simulating gastrointestinal digestion it has been found that lunasin is capable of resistance to function of gastrointestinal enzymes (pepsin, pancreatin) due to naturally present protease inhibitors in food, such as Bowman-Birk protease inhibitor (BBI) and Kunitz trypsin inhibitor (KTI; Jeong et al., 2007b; Park et al., 2007). Hence, both synthetic as well as from food purified lunasin are markedly hydrolysed by pepsin or pancreatin (Fernández-Tomé and Hernández-Ledesma, 2016). On the cellular level it was reported that internalization of lunasin into the mammalian cells started very early within 5 minutes after in vitro treatment. The cellular internalization of the peptide is primarily mediated by endocytic mechanisms involving integrin signalling, clathrin-coated structures and macroinosomes (Caswell et al., 2009; Cam et al., 2013). After the cell entering, lunasin is initially found in the cytoplasm and in approximately of 18 hours of administration it is localized in the nucleus (Lam et al., 2003).

HEALTH BENEFICIAL EFFECTS OF LUNASIN AND THEIR MOLECULAR MECHANISMS

Antioxidant activity

The antioxidant capacity of lunasin mediated through different mechanisms of action has been demonstrated on various in vitro cell models. In lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, lunasin treatment led to a significant decreased production of reactive oxygen species (ROS) by the cells due to lunasin-induced inhibition of linoleic acid oxidation and 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity of the peptide (Hernández-Ledesma et al., 2009b). The authors hypothesize that the role of lunasin as linoleic acid oxidation inhibitor might be linked to its ability to react with peroxyl radicals resulting in suppression of the propagation cycle of lipid peroxidation. In the study by García-Nebot et al. (2014), the ability of lunasin to neutralize peroxyl and superoxide radicals in intestinal Caco-2 cells challenged by hydrogen peroxide and tert-buthylhydroxide has been proven. Additionally, protective effect of lunasin peptide purified from Solanum nigrum L. against DNA oxidative damage have been reported to be associated with suppressing the generation of hydroxyl radical followed by blocking fenton reaction between Fe2+ and H2O2 by chelating iron ferrous ions (Jeong et al., 2010b). According to Zou et al. (2016), the strongest impact on antioxidant activity of peptides (including lunasin) has the composition and the sequence of amino acids in their molecules. Potent action of lunasin is assigned to the presence of Gly, leucine (Leu), phenylalanine (Phe) and proline (Pro) with potent activity against lipid peroxidation (Saiga et al., 2003; Hernández-Ledesma et al., 2009b), and tryptophan (Trp) and cysteine (Cys) residues which were suggested to be responsible for ABTS scavenging activity of the peptide (Hernández-Ledesma et al., 2005; Hernández-Ledesma et al., 2009b).

Anti-inflammatory activity

The anti-inflammatory properties of lunasin have been evidenced through in vitro studies with LPS-stimulated RAW 264.7 murine macrophages (de Mejia and Día, 2009; Hernández-Ledesma et al., 2009b) or LPS-induced THP-1 human macrophages (Cann and de Mejia, 2012) suggesting its actions on key chemical mediators involved in the inflammatory response. In these studies, the capability of lunasin or lunasin-like peptides to inhibit the production of pro-inflammatory cytokines and markers, such as tumor necrosis factor-α (TNF-α), interleukine-6 (IL-6), interleukine 1-β, cyclooxygenase-2, nuclear factor-kappa B (NF-kB), prostat glandin L, nitric oxide (NO) and others have been observed. The down-regulation of expression of the genes associated with uncontrolled and aberrant inflammation in the macrophages is mediated by lunasin-induced...
suppression of NF-κB pathways (de Mejia and Dia, 2009). The inhibition of Akt-mediated NF-κB activation was shown to be through potential interactions of lunasin with αVβ3 (Cam and de Mejia, 2012) and α5β1 integrins (Dia and de Mejia, 2011a). In addition to this, the mechanism of action by which lunasin or lunasin-like peptides exert their anti-inflammatory activities is based on the cell adhesion motif composed of RGD residues in their molecule structure that binds integrins with high specificity resulting in anti-inflammatory effects (Kuphal et al., 2005). Moreover, the alleviation of allergic airway inflammation symptoms manifested by markedly reduced Th2 cytokine expression, reduced inflammatory infiltration and cell metaplasia were found in 2 murine models with induced asthma after lunasin inhalation (Yang et al., 2015). The observation suggests lunasin as an anti-inflammatory agent which can be potentially used in asthma therapy or as an adjuvant to enhance the efficacy of allergy immunotherapy.

Anticancer activity

Lunasin is primarily known as a bioactive peptide due to its ability to exhibit potential anticancer effects (Wan et al., 2017). Chemoprotective properties of lunasin have been confirmed on various cell models in vitro, such as murine NIH/3T3 fibroblast cells (Lam et al., 2003), L1210 leukemia cells (de Mejia et al., 2010), HT-29 colon cancer cells (Dia and de Mejia, 2010), KM12L4 human colon cancer cells (Dia and de Mejia, 2011a), prostate epithelial RWPE-2 cells (Galvez et al., 2011), human liver HepG2 cells (Fernández-Tomé et al., 2014), and MCF-7 and MDA-MB-231 breast cancer cells (Jiang et al., 2016). Lunasin peptide has been shown in in vitro experiments with mammalian cells to suppress transformation of normal cells to cancer ones by oncogenes, such as E1A (Galvez et al., 2001) and Ha-ras (Jeong et al., 2002), and chemical carcinogens 7,12-dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene (Galvez et al., 2001; Hsieh et al., 2010b). This lunasin’s ability was also confirmed on DMBA-induced skin tumorigenesis in a mouse model (Galvez et al., 2001). Moreover, the study by McConnell et al. (2015) revealed that highly purified soybean-derived lunasin has inhibitive effect on anchorage-dependent growth of non-small lung cancer cells (NSCLC), whereas it does not influence two normal bronchial epithelial cell lines. This fact indicates cell-specific aspect of lunasin. The potential underlying mechanisms of lunasin in the prevention and treatment of cancer have been systematically investigated. It was found that all of three unique domains present in sequence of the peptide, i.e., RGD motif, helical domain and poly-aspartic acid tail are implicated in its anticancer activity (Shidal et al., 2017). The tripeptide RGD motif competes with extracellular matrix to interact with integrins α5β1, αvβ3 and αvβ5 (Jiang et al., 2016), widely expressed in different cancer types (Russo et al., 2012), which is crucial in angiogenesis, tumor progression and metastasis. Other mechanisms by which lunasin have shown its anti-neoplastic effects are mediated via its activity inhibiting histone acetylation and its affinity for hypoacetylated chromatin (Fernández-Tomé and Hernández-Ledesma, 2016). Suppressed histone acetylation is a result of lunasin competition with different histone acetyltransferase enzymes (HATs), such as Gcn5 and Pcaf, consequently repressing the cell cycle progression (Jeong et al., 2007a; Jeong et al., 2007b). Since deacetylated histones are absent from wild-type cells, lunasin is not able to disrupt their proliferation or cause their apoptosis, explaining its cell-specific chemopreventive properties (Wan et al., 2017). Antiproliferative and apoptosis-inducing effects of lunasin have been demonstrated in several established cancer cell lines, including leukemia (de Mejia et al., 2010), breast (Hsieh et al., 2010c) and colon (Dia and de Mejia, 2011a). The mechanisms of these actions were attributed to lunasin-induced activation of caspase-3 in leukemia L1210 cells (de Mejia et al., 2010), expression of the pro-apoptotic nuclear clusterin in colon cancer HT-29 cells (Dia and de Mejia, 2010), and reduced expression of cyclin D1/D3, and down-regulation of protein kinase B (PKBα) and activator protein 1 (AP-1) in human breast cancer MDA-MB-231 cells (Hsieh et al., 2010c). The study by Jiang et al. (2016) have shown that the metastasis of breast cancer cells was suppressed by lunasin through integrin-mediated FAK/Akt/ERK and NF-κB signaling pathways followed by down-regulation of the activity and expression of matrix metalloprotease-2/9, possibly via competing with αvβ3 or αvβ5/α5β1 integrin. Human breast cancer MCF-7 cells treated with lunasin have been found to increase their expression and protein levels of the tumor suppressor phosphatase and tensin homolog PTEN, and to enhance PTEN nuclear localization leading to the induction of PTEN-mediated cellular apoptosis (Pubona et al., 2013). Mechanistic studies using lunasin-sensitive NSCLC cell lines revealed the lunasin blocks cell cycle progression at the G1/S phase interface without inducing apoptosis (McConnell et al., 2015). The authors found that lunasin altered the expression of the G1 specific cyclin-dependent kinase complex components, increased levels of p27Kip1, reduced levels of phosphorylated Akt, and ultimately inhibited the sequential phosphorylation of the retinoblastoma protein (RB). These effects are linked to the inhibition of integrin signalling through αv-containing integrins, strongly supporting the hypothesis that direct effects on integrin signalling represent a major mode of action responsible for lunasin’s anticancer activity (Davies and Iannaba, 2016). In human metastatic colon KM12L4 cells, lunasin-arrested cell cycle in G2/M phase with concomitant increase in the expression of cyclin-dependent kinase inhibitors p21 and p27, and lunasin-induced mitochondrial pathway of apoptosis were reported (Dia and de Mejia, 2011b).

Hypocholesterolemic activity

Lunasin has also been previously proven to possess cholesterol lowering activity that is positively associated with cardiovascular system. The peptide exerts its action for lowering LDL cholesterol levels by directly inhibiting gene expression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase to lower cholesterol biosynthesis and by increasing LDL receptor (LDLR) levels to enhance clearance of plasma LDL cholesterol (Galvez, 2012). The underlying mechanism is related to lunasin’s ability to bind specifically to histone H3 and to inhibit H3-Lys14 acetylation by the PCAF (p300/CBP associated factor) histone acetylase enzyme. The acetylation of H3 by PCAF is required for transcriptional activation of HMG-Co-A reductase, the rate-limiting enzyme for cholesterol biosynthesis in the liver. Therefore, lunasin significantly reduced HMG expression in HepG2 liver cells grown in cholesterol-free media by inhibiting PCAF acetylation of H3-Lys 14. Gu et al. (2017) have revealed two mechanisms by which lunasin increases cell-surface LDLR level and enhanced LDL uptake in HepG2 cells and ApoE− mice suggesting its potential role in cholesterol homeostasis maintenance. One of them is mediated via lunasin-induced inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) expression by down-regulating hepatocyte nuclear factor-1α (HNF-1α). Second one is attributed to PI3K-Akt-mediated activation of SREBP-2 pathway.

CONCLUSION

Epidemiologic studies indicate that bioactive compounds from plant resources have various biological functions with beneficial effect on health of living organisms. Many phytochemicals act in the prevention and/or treatment of numerous diseases such as cancer. Nowadays, peptides isolated from soya present the study subject of numerous research teams. Lunasin as one of these polypeptides has a specific amino acid sequence which ensures its unique properties. The substance is becoming an increasingly attractive molecule for research purposes mainly due to its biological availability in organism, its antioxidant, anti-inflammatory, anti-cancer and hypocholesterolemic effects potentially usable for therapeutic purposes. For that reason, there is a growing need for application of advanced molecular and proteomic tools to decipher molecular mechanisms of lunasin action in modulation of various diseases and to determine its safety and effectiveness.

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