Modulatory effects of a lunasin-enriched soybean extract on immune response and oxidative stress-associated biomarkers

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Bioactive Peptides

Microbial fermentation
Chemical or enzymatic hydrolysis
Digestion or industrial processing

Precursor protein

Animal Origin
Milk
Eggs
Meat

Plant Origin
Cereals
Legumes
(Soybean)

Antimicrobial
Antioxidant
Antidiabetic
Anticancer
Immunomodulatory
Antihypertensive

Multiple Biological Activities
Introduction

Lunasin

Gm2S-1 albumin 2S
43 amino acids

1-22
Unknown function
Possible interaction with deacetylated H4

23-32
Histone H4 binding

33-35
Cell adhesion

36-43
Inhibits H3 acetylation

Sources

Soybean and soy products
Barley
Wheat

Absorption

Biological activities

Pasive paracellular diffusion mechanism

Chemopreventive / Anti-inflammatory / Hypcholesterolemic

Immunomodulatory
Main Objective

Evaluation of the antioxidant and immunomodulatory activity of a lunasin enriched soybean extract in murine RAW 264.7 macrophages

Specific Objective 1
Characterization by electrophoresis and Western-Blot of the protein profile of the lunasin enriched soybean extract

Specific Objective 2
Study of the behavior of the lunasin enriched soybean extract under conditions that simulate the gastrointestinal digestion process

Specific Objective 3
Study of the effect of the lunasin-enriched extract on biomarkers associated with oxidative stress and the immune response
Workplan

Albumin enriched soybean sample (ES)

Lunasin enriched soybean extract (LES)

Characterization
  - SDS-PAGE
  - Western-Blot
  - BCA

Simulated digestion

Antioxidant activity *in vitro*
  - ORAC
  - ABTS

RAW 264.7 Macrophages
  - Cell viability
  - Phagocytosis
  - NO levels
  - ROS production

Cytokines (IL-6, IL-10)
Results: LES characterization

SDS-PAGE electrophoresis

• Similar profiles for ES and LES, although bands in LES showed more intensity

• Identification of 4-138 kDa bands

Presence of the major soybean proteins: β-conglycinin, glycinin and their corresponding subunits

Western-Blot

• Identification of lunasin’s monomeric and dimeric forms

16.42 mg lunasin/g of protein
2.07 mg lunasin/g of extract
**Results: Behaviour under simulated gastrointestinal digestion**

<table>
<thead>
<tr>
<th>Synthetic Lunasin</th>
<th>LES</th>
<th>CD</th>
<th>GD</th>
<th>GID</th>
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60% of the initial lunasin was detected in GD

2.88% of the initial lunasin was detected in GID

**Protease inhibitors** (Bowman-Birk inhibitor or Kunitz inhibitor) potentially present in LES could be responsible for the greater resistance of lunasin to the digestive process
Soluble proteins and small peptides: responsible for the antioxidant activity

The antioxidant activity was higher after the gastrointestinal digestion
Results: Immunomodulatory activity in macrophages RAW264.7

Cell Viability (MTT assay)

Significant and dose-dependent increase in the % of viable cells was observed after first 8 h

Longer treatment times resulted in significant reduction of cell viability
Results: Immunomodulatory activity in macrophages RAW264.7

NO Production (Griess assay)

Induction of the release of NO was provoked by the treatment with both LPS and LES.

For LES, the induction was dose- and time-dependent, reaching values higher than 60 uM at LES doses of 15 ug protein/mL for 24 h.
Results: Immunomodulatory activity in macrophages RAW264.7

Phagocytic Activity

After 8 h of treatment with LES, the phagocytic capacity was significantly increased, with a similar behaviour to that of LPS.

After 16 h, no changes were observed except for 2.5 µg prot/mL of LES.

At 24 h, only the lower concentration caused an increase in phagocytic capacity, while the higher concentrations caused a significant decrease.
Results: Immunomodulatory activity in macrophages RAW264.7

**ROS Production**

LES at concentrations of 0.5, 2.5 and 7.5 µg protein/mL exerted a **protective effect** against oxidative stress, decreasing ROS levels at the **three treatment times**

The **highest concentration** caused an **oxidizing effect** at the three times tested, similarly to LPS.
LES induced the liberation of IL-6 in a dose-dependent manner.

LES induced the liberation of IL-10 in a dose-dependent manner. At LES highest concentration, the levels of IL-10 were even higher than the LPS.
Conclusions

1. An enrichment in soluble proteins and small peptides such as lunasin, whose concentration was 2.07 mg lunasin/g of LES, was achieved. The protease inhibitors could exert a protective effect against digestive enzymes, allowing lunasin to partially resist the digestive process.

2. The small and medium peptides were responsible for the radical neutralizing activity. After the simulated digestive process, the activity increased, indicating that peptides released during digestive process were more potent as antioxidants.

3. LES presented, in a dose- and time-dependent manner, an immunomodulatory activity in RAW 264.7 macrophages as demonstrated by changes in the levels of NO and ROS, and in the phagocytic activity of the cells. LES, also had a dose-dependent effect in the liberation of proinflammatory (IL-6) and antiinflammatory cytokines (IL-10).