Anti-Inflammation of Miso, Japanese Fermented Soybean Paste

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DESCRIPTION

Inflammation of the human intestines results from a lipopolysaccharide (LPS) component in the cell walls of unfriendly gram-negative bacteria. Actually, a small amount of LPS from Escherichia coli inflames the human body. LPS causes septic shock in severe cases. LPS from intestinal bacteria inflamed intestinal mucosa and diarrhea caused by the inflammation. Lactoferrin in milk caused detoxification activity against LPS was known. Whereas, many Japanese or Asian people consume little milk or dairy products because of widespread lactose intolerance. It is, therefore, necessary that they consume non-dairy foods for detoxification of LPS. We were reported about the LPS elimination activity of miso. And the anti-inflammation activity of miso also is expected to prevent diarrhea caused by intestinal inflammation. Nevertheless, few reports have described studies for the inflammation mechanisms of miso. This report describes that fermented foods mioso suggested preventing inflammation activity of miso for alleviation of diarrhea from damaging intestinal mucosa instead of lactoferrin, milk, or dairy products [1-13].

Inflammation of the human intestines results from a lipopolysaccharide (LPS) component in the cell walls of gram-negative bacteria as unfriendly [1]. The LPS comprises three units as follows, O-antigen, core, and lipid A.

Lipid A which composed of phosphorylated liposaccharide, and it calls an endotoxin because it contains intercellular components [2]. Small amounts of LPS in bacteria cause inflammation of mucosa and other tissues in humans. Actually, $2 \times 10^9$ g/kg human of LPS in Escherichia coli 0113 strain causes inflammation in humans [3].

It is known that lactoferrin is one kinds of milk protein and it neutralize/eliminate LPS [4]. LPS maintains residual toxic activity after sterilization at 121°C for 15 hr. It requires detoxification at 250°C over 30 hr [5]. Moreover, LPS causes septic shock [6]. However, Japanese people consume little milk or dairy products because of many Japanese are lactose intolerance [7]. Japanese have taken soymilk instead of milk from the background Japanese culture or physical feature. Thus, milk or dairy products as like soybean product is desired to prevent intestinal inflammation for LPS neutralization/elimination peptide/protein source by many Japanese. Therefore, we focused on miso made from soybean and eaten every day by Japanese.

Miso which is eaten nowadays in Japan is a fermented soybean with koji molded rice (growing Aspergillus oryzae) by salt tolerance lactic acid (Tetragenococcus halophilus) bacteria and salt tolerance yeast (Zygosaccharomyces rouxii). It has taken not only for seasoning but also nutritional source as like protein but also since ancient times. Recently, the health functions have miso such as the estrogen-like activity; isoflavones, anti-oxidation; melanoids, and angiotensin-converting-enzyme inhibition activity, some kinds of polypeptides [8].

Hitherto, Sasaki et al. [9] reported miso aged two years had the highest LPS-neutralizing activity found among some kinds of miso. They also considered that LPS elimination substance is peptide-based because LPS neutralization has a close relation to formol nitrogen in miso. Moreover, the LPS neutralization peptide/protein was purified using Blue Native PAGE method.
and analyzed in soy by nano LC-MS/MS analysis. The homology of the amino acid sequence was analyzed using BLAST. The peptide/protein was identified in soy as 2S albumin subunit which had 18,442 molecular mass, 158 amino acid residue [9].

Thereafter, the anti-inflammation activity of the peptide/protein was assayed by Prostaglandin D2 (PGD2) production from macrophage cells. Prostaglandin (PG) is a hormone-like active substance produced from human cells by the stimulation of LPS. Although it is a homeostatic mechanism, it is pro-inflammatory by its overexpression. PG is bio-converted from arachidonic acid in mammal cells to PG substances of several kinds. Particularly, PGD2 is related to inflammation in humans strongly [10].

Anti-inflammation using a macrophage cell was assayed according to a protocol described earlier [11]. Mouse macrophage cell line RAW264.7 was used for subculture in DME medium containing 10% fetal bovine serum and 1% penicillin. The flask for cell cultivation under 5% CO2 condition at 37°C for 24 hr. After pre-cultivation, PGD2 was analyzed using a LC-MS/MS using reverse phase column and eluting acetonitrile: D.W.: acetic acid (40: 60: 2).

The relative ratio of PGD2 was 100% as the negative control. It shows that LPS caused inflammation of macrophage cells. Data were subjected to statistical analyses by ANOVA testing to assess the equality of all means and Tukey tests for post-hoc analysis. Consequently, macrophages produced PGD2 of the relative ratio of 16.6% in the medium containing 50 mg/mL of LPS neutralization peptide/protein. And also, macrophages produced PGD2 of the relative ratio of 27.8% in the medium containing 0.1% miso extract. All data were inferred as significantly different (P<0.05) from data of the positive control.

Some peptides have inhibitors against cyclooxygenase-2 (COX-2), which is an enzyme for producing PGD2 [12]. It was inferred that the LNP also has inhibition activity against COX-2. Future studies must be conducted to examine the inhibition activity of LNP against COX-2.

As the results, it is obvious that the miso containing the peptide/protein has inflammation activity as same as lactoferrin. There is many proverbs about miso, because of the healthy function of miso has been known from ancient empirically, for example, Eaten miso keeps the doctor away. Moreover, Japanese ancient literature; “Honcho syokkan,” [13], described that miso has effects of “stopping diarrhea.” It is proved clearly of the ancient description, and we suggest that miso peptide/protein is anti-inflammation food instead of lactoferrin in milk.

CONCLUSION
Anti-inflammation of its peptide/protein from miso was isolation using Blue Native PAGE method, and the sequence analysis of it was carried out to analyze by nano LC-MS/MS. Consequently, it was part of peptides on a 2S albumin in soybean. And it is decided that the peptide have anti-inflammation activity, which assayed prostaglandin D2 production from macrophage cell. It is clear that the miso containing the miso peptide/protein has inflammation activity as same as lactoferrin in milk.

REFERENCES