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学位論文内容の要旨

Allergy is a disorder of excessive immune response to harmless foreign substances and is commonly classified into four types according to their mechanisms. Type-I allergies are immediate hypersensitivity reactions triggered by antigens such as pollen and foods. Basophilic leukocytes and mast cells play a key role in this response. Antigens bind to IgE antibodies attached to FcεRI receptors on the cell membrane, causing cross-linking of IgEs. This cross-linking initiates intracellular signaling, including protein phosphorylation and calcium influx. Stimulation leads to degranulation, releasing histamine and leukotrienes (LTs) such as LTB₄ into the extracellular space. Histamine and LTs act as chemical mediators, promoting vasodilation, increased vascular permeability, smooth muscle contraction, and leukocyte chemotaxis, resulting in allergic symptoms.

Inflammation is a defense response of the immune system against local tissue damage caused by viral and bacterial infections, allergies, etc. Macrophages play an essential role in the pathogenesis of many inflammatory diseases, where they regulate the inflammatory response through the secretion of pro-inflammatory mediators and pro-inflammatory cytokines. Lipopolysaccharide (LPS), a bacterial cell wall component, acts as a cellular stimulator that binds to Toll-like receptors (TLRs) on the surface of macrophages, leading to the activation of macrophages. Nitric oxide (NO) synthesized by inducible nitric oxide synthase (iNOS), and prostaglandin E₂ (PGE₂), produced by cyclooxygenase (COX), are typical pro-inflammatory mediators involved in a variety of physiological responses that exacerbate inflammatory symptoms in the body. In addition, increased inflammation is accompanied by excessive release of cytokines, including interleukins IL-1β, IL-6, and TNF-α.

Sea buckthorn (*Hippophae rhamnoides* L.) is a deciduous shrub of the Elaeagnaceae family, native to northern Eurasia and cultivated in Hokkaido, Japan. Sea buckthorn berries (SBB) are rich in vitamins, carotenoids, and flavonoids and are used in various food processing applications. SBB has been shown to have antioxidant, antibacterial, and antiviral effects and is expected to have significant therapeutic and medicinal potential. Many compounds such as polyphenols have been isolated from SBB, and the flavonoid profile of SBB consists mainly of various glycosides of isorhamnetin, quercetin, and kaempferol. This study aims to elucidate the effects of anti-allergic and anti-inflammatory effects of sea buckthorn berry extract in mast cells and macrophages.

The SBB extract was prepared with 70% methanol (MeOH), followed by reversed-phase column chromatography with Diaion HP-20 to obtain seven MeOH fractions. The total polyphenol contents of the SBB extract and its fractions were determined by the Folin-Ciocalteu method and their radical scavenging abilities were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Rat basophilic leukemia cell line (RBL-2H3) and mouse bone marrow-derived mast cell line (PB-3c) were used for histamine and LTB₄ release assays, respectively. After the cell stimulation, the chemical mediators released from the cells were determined by HPLC. The effect of SBB on the cell signaling after the stimulation was analyzed by SDS-PAGE and Western blotting using anti-phosphorylated tyrosine

and anti-spleen tyrosine kinase (Syk). Ca^{2+} concentration in the cytoplasm of RBL-2H3 cells was measured using a fluorescent probe. Murine leukemia macrophage cell line (RAW 264.7) were used to investigate the inhibitory effects of SBB extract and its fractions on inflammatory mediators and the underlying mechanisms. NO release from RAW 264.7 after lipopolysaccharide stimulation was measured using 2,3-diaminonaphthalene. The production of inflammatory cytokines and inflammatory mediators such as IL-1 β , IL-6, TNF- α , and PGE₂ in the LPS-stimulated cells was determined by ELISA. The expression level of inducible nitric oxide synthase (iNOS) in the LPS-stimulated cells was analyzed by qPCR and Western blotting using a monoclonal antibody.

The total polyphenol content and DPPH scavenging ability of Fraction (Fr.) 6 was higher than other fractions and extract. Fr.6 showed a significant inhibitory effect on histamine release from the stimulated RBL-2H3 cells, while extract and other fractions had no effect. Fr.6 significantly suppressed LTB₄ production in the stimulated PB-3c cells and had similar activities. Fr.6 decreased total tyrosine phosphorylation and Syk phosphorylation, and Ca^{2+} influx associated with signal transduction in the stimulated cells. Fr.6 significantly suppressed NO release from the LPS-stimulated RAW 264.7 cells. Fr.6 inhibited the production of IL-1 β , IL-6, TNF- α , and PGE₂ and suppressed iNOS expression.

These results suggest that SBB may alleviate symptoms of allergy and inflammation, and the antioxidative polyphenols may contribute to this effect.

審査結果の要旨

花粉症等の I 型アレルギーでは、抗原抗体反応によって肥満細胞等が刺激され、細胞内シグナル伝達を経てケミカルメディエーターが細胞から放出されアレルギー症状を呈する。アレルギー反応や感染によって誘導される炎症では、マクロファージ等から一酸化窒素およびサイトカインが放出されることで炎症症状を呈する。現在、アレルギーおよび炎症症状の抑制は薬剤による対処療法に依存しているが、副作用が少ない食品の摂取による症状緩和が期待されている。シーベリー (*Hippophae rhamnoides* L.) は、ロシアやモンゴルを中心に健康食品として利用されており、様々な生理活性が示唆されている。

本研究は、シーベリー抽出物の抗アレルギーおよび抗炎症効果を培養細胞を用いて調べ、その作用機序の解明を試みたものである。その結果、シーベリー抽出物に抗アレルギー効果（ケミカルメディエーター放出および細胞内 Ca^{2+} 濃度上昇抑制活性）および抗炎症効果（一酸化窒素およびサイトカイン放出抑制活性）が認められた。

これらの結果は、シーベリーの摂取がアレルギーおよび炎症の予防や緩和に寄与することを示唆するものである。本研究の成果は新知見であり、査読付国際英文誌 (*Preventive Nutrition and Food Science*) への掲載が承認されている。

以上より、QIU SHIMAN 氏は北見工業大学 博士（工学）の学位を授与される資格があるものと認められる。