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Pro-inflammatory Cytokine Production Inhibitory Effects of Broccoli extract

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Abstract[1]

Background/Objectives: This study aims to demonstrate pro-inflammatory cytokine production inhibitory effects using broccoli extract. **Methods/Statistical analysis:** Thus, This paper was carried out to see the production of NO and pro-inflammatory cytokine ,TNF-α and IL-6 using the macrophages of LPS-treated mice. The cytotoxicity test was used 96 AQueous One solution cell proliferation assay. NO was measured using NO Detection Kit, and the production of pro-inflammatory cytokine was measured using ELISA kit. **Findings**: As a result, broccoli extract had no cytotoxicity at 10ug/mL to 1,000 ug/mL and significantly inhibited the production of NO as well as TNF-α and IL-6 which are pro-inflammatory cytokines. In the RAW 264.7 macrpphage cells, Pro-inflammatory cytokine production inhibitory effects are likely to be diversely utilized as basic physiological activity data and functional materials to demonstrate anti-inflammatory properties of broccoli extract.**Improvements/Applications**: In conclusion, this study can be used to a basic data to objectively demonstrate the physiological activity of immunological mechanism associated with the anti-inflammatory action of broccoli extract. However, in-depth research on anti-inflammatory is needed.

Index Terms

Broccoli extract, Cytokine, Anti-inflammatory, NO, TNF- α

I. INTRODUCTION

Inflammatory response is the defense mechanism of tissues for internal homeostasis against internal stimuli such as the production of metabolites inside the body through various pathways including external stimuli or bacterial infections[1]. The primary and secondary mediators of various intracellular inflammatory regulators including pro-inflammatory cytokines such as TNF-α, IL-6, IL-8 and rostagrandin, lysosomal enzymes and free radicals are involved in the inflammatory response[2]. In particular, the transcription factors of inflammatory response are activated by stimuli such as tumor necrosis factor (TNF)-α and lipopolysaccharide (LPS), which are cytokines secreted from macrophages. This induces

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the expression of inducible nitric oxide synthase and cyclooxygenase-2 and the production of nitric oxide and prostaglandin E2 causes inflammation[3,4]. The excessive production of NO, a vasodilator, increases inflammatory response, causes septic shock by excessive vasodilation, inhibits wound healing, and damages nerve tissue, and thus causes different diseases in the body[5,6]. Steroids and nonsteroidal antiinflammatory drugs (NSAIDs) used as therapeutic agents for acute and chronic inflammatory diseases are used in various fields, but they are difficult to use for a long time because of serious concerns about side effects[7-9]. Broccoli (Brassica oleracea var. italica) is an edible green plant in the cabbage family (family Brassicaceae, genus Brassica) whose large flowering head, stalk and small associated leaves are eaten as a vegetable. Broccoli is classified in the Italica cultivar group of the species Brassica oleracea[10]. Among the cabbage family crops Broccoli has antioxidant activity, It contains a large amount of ß-carotene, ascorbic acid, rutin, glutathione, quercetin, and selenium[11]. It is eaten either raw or cooked. Broccoli is a particularly rich source of vitamin C and vitamin K. Contents of its characteristic sulfur-containing glucosinolate compounds, isothiocyanates and sulforaphane, are diminished by boiling, but are better preserved by steaming, microwaving or stir-frying[12,13].

This paper was carried out to see the production of NO of broccoli extract, which is used as anti-inflammatories in various fields, and the production of TNF-α , IL-6, which are pro-inflammatory cytokines, and to examine various applications for basic physiological activity data and functional materials to demonstrate anti-inflammatory properties of broccoli extract.

II. MATERIALS AND METHOD

A. Experimental Material

Broccoli extract used in an experiment was 100% pure natural essential oil, which was certified by an organic certification body (ECOCERT-F-32600). It used products manufactured by NEW DIRECTIONS LABORATORY LTD. Ethanol and broccoli extract were diluted to 4:1 and added to a medium.

B. RAW 264.7 Cell culture

RAW264.7 mouse macrophage cell line was ordered from KCLB (Korea Cell Line Bank, Korea) and used for the experiment. DMEM (Dulbecco's Modified Eagle's Medium) medium was used for cell culture and medium containing 12% FBS and 1.5% penicillin-streptomycin was used. The macrophages were cultured in a CO_2 incubator (37 ° C, 5% CO_2) and subcultured every other day. Mouse macrophage cells were washed twice with fresh medium and stimulated with 10ug/ml LPS.

C. Cytotoxicity

Toxicity of broccoli extract to cells was measured using Desai's method [14]. Cytotoxicity assays are to determine the degree of toxicity by measuring the conversion of MTS into formazan by mitochondrial dehydrogenases using MTS assay method. After RAW264.7 macrophage cells were loading at 1.0×105 cells in 96-well and cultured for 18 hours, broccoli extract was treated at 10 μg/mL, 100 μg/mL and 1,000 μg/mL and cultured in CO2 incubator for 24 hours. After 20 μl of MTS solution was added 24 hours later and reacted in CO2 incubator (37°C, CO_2 5%) for 4 hours, the change in absorbance was measured at 450 nm and then cell viability, which could confirm the cytotoxicity of the control group, was expressed as a percentage.

D. Measurement of NO

The NO concentration was measured in the nitrite concentration using Griess reagent system[15]. RAW 264.7 macrophage cells were seeded in a 96-well at a density of 1.0×105 cells and cultured for 16 hours. The cell were pretreated with broccoli extract 10 μg/mL, 100 μg/mL and 1,000 μg/mL and stimulated with LPS 10ug/mL for 24 hour. The same amount of Griess Reagent as the culture medium was added and incubated at room temperature. Absorbance was measured at 540 nm. The concentration of sodium nitrite was used to determine NO concentration in the culture medium.

E. Effect of TNF-α, IL-6

RAW264.7 mouse macrophages were seeded on a 96-well at a density of 1.0 × 10 5 cells / well and cultured in a CO2 incubator for 18 hours. Then, broccoli extract was treated with 10 μg/mL, 100 μg/mL and 1,000 μg/mL. After incubation for 24 h in a CO2 incubator. TNF-α and IL-6, proinflammatory cytokines contained in the culture medium, were measured using an ELISA

F. Statistical analysis

The experimental were determined using the students' t-test, which were calculated as mean ± standard error (Mean ± SE), were significant when the significance of each group was p <0.05.Study Design

Ⅲ. RESULTS AND DISCUSSION

To confirm the cytotoxicity of broccoli extract, which is known to be nontoxic, RAW 264.7 cells were treated with 10 ug/mL to 1,000 ug/mL of broccoli

extract and then MTS assay was performed. Cell viability was measured at different concentrations. As a result, no toxicity was observed up to a concentration of 1,00 ug/mL. The following experiments were performed at concentrations of 10 μg/mL, 100 μg/mL and 1,000 μg/mL, which did not affect the cell viability of RAW 264.7 cells. As a result, broccoli extract showed more than 98.6±5%, 95.9±3.02% and 94.0±2.97%, respectively, at the concentrations of 10 μg/mL, 100 μg/mL and 1,000 μg/mL, which had no cytotoxicity in figure 1.

The effect of broccoli extract on NO production of RAW 264.7 cells was measured using LPS, which is used as an inflammation inducer in Figure 2. As a result, the concentration of NO was very low in the control group in which only RAW 264.7 cells were cultured, while the concentration of NO in the LPS-treated group was significantly increased. In the experimental group treated with broccoli extract, the production of NO was inhibited in a dose-dependent manner and significant inhibition was observed at 1,000 μg/mL.

To investigate the effects of broccoli extract on the production of proinflammatory cytokine TNF-α and IL-6, RAW 264.7 macrophages were treated with LPS (10 ug / mL) alone, or with LPS and broccoli extract at 10 μg/mL, 100 μg/mL and 1,000 μg/mL. The production of TNF-α and IL-6 after treatment was investigated. According to the investigation results, the production of TNF-α, IL-6 was inhibited in a dose-dependent manner, TNF-α, IL-6 production in the experimental group treated with 1,000 ug/mL was significantly inhibited in Figure 3, 4.

MTS assays were performed to measure the toxicity of broccoli extract through LPS stimulation to RAW 264.7 macrophages. There was no cytotoxicity in the control group and the experimental group treated with broccoli extract for 24 hours.

Different inflammatory regulators in cells, as primary and secondary mediators, are involved in inflammation expressed as a defense mechanism in vivo against external infections through various pathways or internal and external stimuli by metabolites in vivo. They are also responsible for different inflammatory diseases such as allergies, atopy, arthritis, heart disease, brain cardiovascular disease and disorders, and cancer [11]. Inflammation is a body defense mechanism, manifesting symptoms and signs in various ways as the most important mechanism in the body defense mechanisms .

The efficacy was demonstrated through an experiment regarding anti-inflammatory properties using various components and preparations extracted from plants. Inflammation involves a variety of mediators, and in particular, pro-inflammatory cytokines produced from cells such as activated lymphocytes and macrophages include TNF-α, IL-6 and IL-8. TNF-α plays a key role in regulating innate immune responses, as a major mediator of LPS stimulation. TNF-α is produced from Macrophages and Mast Cell and associated with chronic inflammation in vivo, and it shows intracellular toxicity in tumor cells. In the experimental group treated with broccoli extract to investigate the change in the inhibition of IL-6 and TNF-α production, it was observed that the production of NO, TNF-α and IL-6 was inhibited in a dose-dependent manner. According to these study results, broccoli extract has significant anti-inflammatory properties due to pro-inflammatory Cytokine TNF-α production inhibitory effects in LPS-induced inflammatory model[16.17].

To confirm the cytotoxicity of broccoli extract, which is known to be nontoxic, RAW 264.7 cells were treated with 10 ug/mL to 1,000 ug/mL of broccoli extract and then MTS assay was performed. Cell viability was measured at different concentrations[18]. In conclusion, this study can be used as a basic data to objectively demonstrate the physiological activity of immunological mechanism associated with the anti-inflammatory action of broccoli extract, but it is necessary to conduct in-depth studies on anti-inflammation.

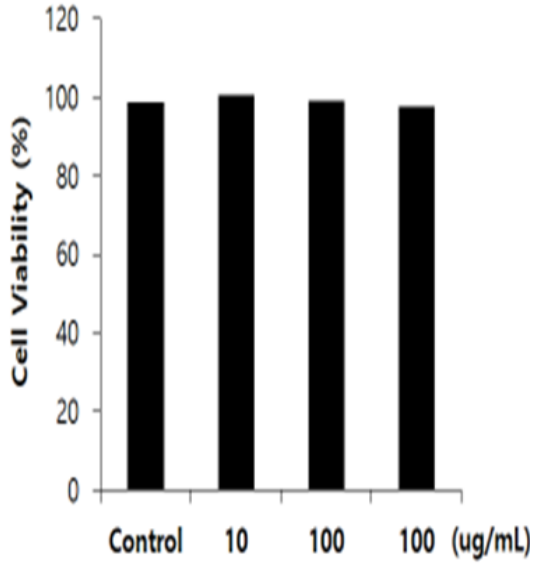

Fig. 1. Effects of broccoli extract on the cell viability of RAW264.7 cells.

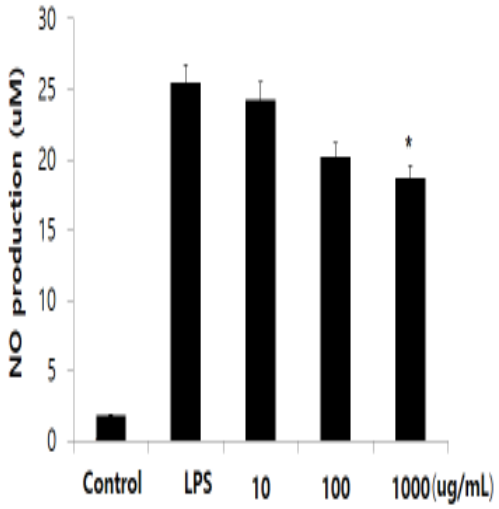

Fig. 2. Effects of broccoli extract on Inhibition of NO production in LPS-stimulated RAW 264.7 cells.

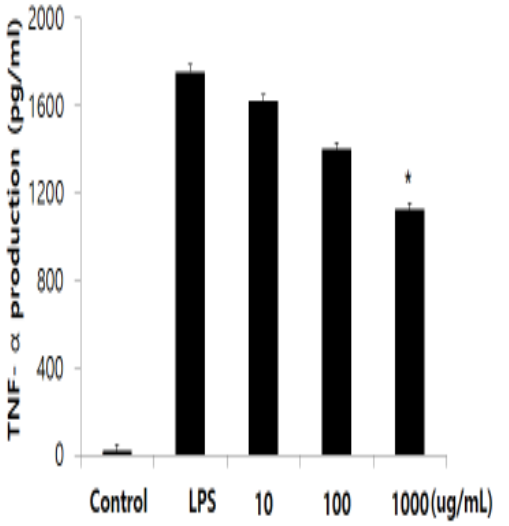

Fig. 3. Effects of broccoli extract on Inhibition of TNF-α production in LPS-stimulated RAW 264.7 cells.

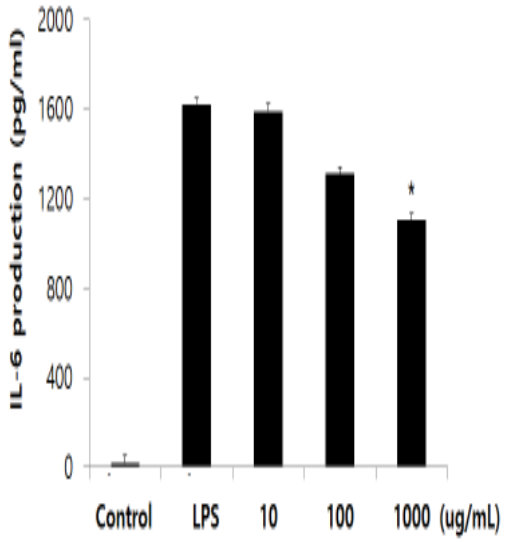

Fig. 4. Effects of broccoli extract on Inhibition of IL-6 production in LPS-stimulated RAW 264.7 cells.

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