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Effect of gamma irradiation on the change of solubility and anti-inflammation activity of chrysin in macrophage cells and LPS-injected endotoxemic mice

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HIGHLIGHTS

- Gamma irradiation leads to the structural modification of chrysin.
- Gamma irradiation improved the solubility of chrysin.
- Gamma-irradiated chrysin significantly inhibited the inflammation mediator.
- Anti-inflammation mechanism involved the modulation of MAPKs and NF-κB.
- Gamma-irradiated chrysin attenuated the endotoxin-induced lethality.

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ABSTRACT

This study evaluated the changes of solubility and anti-inflammatory properties of structurally modified gamma-irradiated chrysin. Chrysin was irradiated at various doses for a physical analysis and determining any structural changes and solubility. As shown through the physical analysis, the main peak of the chrysin was decreased as the irradiation dose increased, and it was concomitant with the appearance of several new peaks, which were highly increased in 50 kGy gamma-irradiated chrysin. The solubility was markedly increased in the gamma-irradiated groups. As shown through a physiological analysis, both gamma-irradiated- (15–50 kGy) and intact-chrysin (0 kGy) did not exert cytotoxicity to bone-marrow derived macrophages. The treatment of LPS-stimulated macrophages with 50 kGy gamma-irradiated chrysin resulted in a dose-dependent decrease in pro-inflammatory mediators, such as iNOS-mediated NO, PGE_2, COX-2, and cell surface marker (CD80 and CD86), as well as pro-inflammatory cytokines (TNF-α and IL-6), when compared to the intact-chrysin treated group. Mechanically, we found that the inhibition of these pro-inflammatory mediators induced by gamma-irradiated chrysin occurred through an inhibition of MAPKs (ERK1/2 and p38) and the NF-κB signaling pathways. Furthermore, the anti-inflammatory activity remained in the LPS-injected animal model. In this model, gamma-irradiated chrysin treatment highly increased the mouse survival, and significantly decreased the serum cytokine (TNF-α, IL-6 and IL-1β) levels. From these findings, the anti-inflammatory action by gamma-irradiated chrysin may be closely mediated with structural modification. It seems likely that gamma irradiation can be an effective tool for improvement of the physical and physiological properties of polyphenols.

1. Introduction

Polyphenols are naturally occurring components of fruits and vegetables and are currently the focus of much nutritional and therapeutic interest against the risk for many complex diseases (Stoclet, 2004; Veeriah et al., 2007). In particular, a large number of researches have supported that dietary polyphenols play a key role in the prevention of inflammation-related diseases

Abbreviations: BMDM, bone-marrow derived macrophage; COX, cyclooxygenase; ERK, extracellular signal-regulated kinase; FITC, fluorescein isothiocyanate; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase; iNOS, inducible nitric oxide synthases; Iκ-B, inhibitor of κB; IL, interleukin; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; mAb, monoclonal Ab; NO, nitric oxide; NF-κB, nuclear factor-κB; PBS, phosphate-buffered saline; PGE_2, prostaglandin E2; TNF-α, tumor necrosis factor-α; pAb, polyclonal antibody

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(Kemperman et al., 2013; Pérez-Jiménez and Saura-Calixto, 2015; Scalbert et al., 2005).

Inflammation is the result of complex and pathologically unbalanced multicellular processes (Gouwy et al., 2005). The inflammatory response is mediated by a range of factors, such as inducible nitric oxide synthases (iNOS)-mediated nitric oxide (NO), prostaglandin E2 (PGE_2), cyclooxygenase (COX), and cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)−1β, and IL-6), in macrophages and some other cells (Korhonen et al., 2005; Takeda and Akira, 2005). Ultimately, the response to these pro-inflammatory stimuli is processed by the activation of mitogen-activated protein kinases (MAPK) and nuclear factor κB (NF-κB) (Comalada et al., 2006; Gonzalez-Gallego et al., 2010). Growing evidence has strongly emerged regarding a promising approach to counteract risk for a number of inflammation-related diseases by higher polyphenol-intake (Byun et al., 2015; Jung et al., 2008; Takeuchi and Akira, 2001).

Among the various kinds of polyphenolic compounds, chrysin (5,7-dihydroxyflavone) is one such flavonoid that forms the main active component of medicinal plants, such as *Oroxylum indicum* and *Passiflora incarnata* (Engelmann, et al., 2005; Harris et al., 2006). Chrysin has exhibited broad-spectrum pharmacological and medicinal properties such as antioxidant, anti-inflammatory, anti-tumor, antihemolytic, and anti-hypertension properties (Lv et al., 2010; Zou et al., 2010). Due to its abundance in plants and their potential beneficial health and nutritional effects along with low systemic toxicity, chrysin is of considerable interest for drug development as well as health food supplements. However, the applicability of chrysin as a drug is often confined by its low solubility, which seriously limits its bioavailability. Therefore, many researchers have made an effort to search for new methods for improvement of the physical- and physiological-activities of chrysin through a structural modification.

Ionizing radiation has been developed for uses in cancer therapy and tool sterilization, because ionizing radiation can penetrate biomaterials (Lee et al., 2012; Sung et al., 2014). Recently, many studies have attempted to apply radiation technology for enhancing biological activities (Byun et al., 2015; Sung et al., 2009). In previous results, gamma irradiation clearly decreased the toxicity of mistletoe lectin (immune cell cytotoxicity, liver toxicity, and mice mortality) without compromising its bioactivity (Sung et al., 2013). Additionally, irradiation can reduce the unfavorable color of aloe extract while increasing the antioxidant activity, and also converts the ginsenoside structure Rb1 into Rg3, which is a more active form for cancer prevention (Kim et al., 2012a). Moreover, irradiated polyphenolic compound (resveratrol) treatment significantly decreases the inflammation reaction in LPS-stimulated macrophage cells by controlling the NF-κB and MAPK expression, and this enhancing activity may be due to the structural modification by ionizing radiation (Byun et al., 2015).

Therefore, in the present study, we focus on the role of gamma irradiation on the physical solubility properties and physiological properties of chrysin as an anti-inflammatory to explore the possibilities of gamma-irradiated chrysin for improvement of its bioavailability in the medical industry.

2. Experimental

2.1. Materials

Chrysin was obtained from Sigma (St Louis, MO, USA). Lipopolysaccharide (LPS) from *Escherichia coli* O111: B4 and anti-β-actin mAb (AC-15) were purchased from Sigma (San Diego, CA, USA). COX-1/2 polyclonal (p) anti-body (Ab), iNOS pAb, anti-phosphorylated extracellular signal-regulated kinase (ERK) 1/2 monoclonal (m) Ab, anti-ERK1/2 pAb, anti-phosphorylated p38 mAb, anti-p38 pAb, anti-phosphorylated inhibitory (I) κB-α pAb, and anti-IκB-α pAb were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Horseradish peroxidase (HRP)-conjugated anti-mouse immunoglobulin G (IgG) Ab and HRP-conjugated anti-rabbit IgG Ab were obtained from Calbiochem (San Diego, CA). Fluorescein isothiocyanate (FITC)-conjugated mAb against CD11b and phycoerythrin (PE)-conjugated mAb against CD80, and CD86 and cytokine for IL-6 and TNF-α and PGE_2 detection kits were all purchased from eBioscience (San Diego, CA).

2.2. Preparation of gamma-irradiated chrysin

Chrysin was dissolved in methanol to reach a concentration of 1 mg/mL for studies on its structural and functional changes. The chrysin solution (1 mg/mL) was then irradiated at 0, 15, 30, and 50 kGy. Gamma irradiation was performed using a cobalt-60 irradiator (point source AECL, IR-221, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) with a 11.1 peta-becquerel (PBq) source strength and operated at a dose rate of 10 kGy/h in the Advanced Radiation Technology Institute, a branch of the Korea Atomic Energy Research Institute (Jeoung-Eup, Korea). Dosimetry was performed using an alanine dosimeter with a 5-mm diameter (Bruker Instruments, Rheinstetten, Germany). The dosimeters were calibrated against an international standard set by the International Atomic Energy Agency (Vienna, Austria). A methanol solution of gamma-irradiated chrysin was evaporated using a rotary evaporator (Tokyo Rikakikai Co. Ltd., Japan) for studies of the physiological activity, and both intact- and gamma-irradiated chrysin powders were then redissolved in dimethyl sulfoxide(DMSO) and stored at −80 °C.

2.3. High-performance liquid chromatography (HPLC) analysis

An agilent HPLC system 1100 with a Diode Array Detector (DAD) was used for the chromatographic separation of gamma-irradiated chrysin. All of the samples were filtered before analysis using HPLC. The injection volume of intact- and gamma-irradiated chrysin solutions (1 mg/mL) were 20 μL. The brief conditions of the HPLC system are as follows: Reverse phase: Agilent Eclipse XDB–C18 column (5 μM pore size and length I.D., 4.6 mm × 150 mm); Mobile phase: A: methanol; B: 0.1% formic acid. Flow rate: 0.8 mL/min, detector: UV – 280 nm. These analyses were conducted using an isocratic elution during 60 min with A–B (75–25).

2.4. Solubility of gamma-irradiated chrysin

To investigate the solubility of gamma-irradiated chrysin, 10 mg of chrysin was suspended into 1 mL of methanol, and simply vortexed for 30 min. This solution was gamma-irradiated at 0, 5, 10, 15, 30, and 50 kGy. Gamma-irradiated solutions containing precipitate of chrysin were simply vortexed for 30 min and centrifuged and then supernatant was removed. The remaining precipitate was collected and the solubility was calculated as percentage when compared to the initial weight of the chrysin.

2.5. Cell culture and generation of bone-marrow derived macrophages

Animal and cell mediated experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Korea Atomic Energy Research Institute (KAERI-IACUC-2013-001). Bone marrow derived macrophages