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Original article

New Radiolytic Cyclization Products, Phlorocyclin and Isophlorocyclin Exhibit Anti-inflammatory Effects in LPS-stimulated Macrophages

Tae Hoon Kim[1,*]

[1]Department of Food Science and Biotechnology, Daegu University, Gyeongsan 38453, Republic of Korea

ABSTRACT Phlorocyclin (PC) and isophorocyclin (IPC) are rare benzofuran derivaitves obtained from the representive dihydrochalcone glucoside, phloridzin (PZ) and are a type of neolignan backbone with a potential anti-glycative agents. However, research related to the enhancement of biological functionallites to inflammation of the newly converted products is very limited. This research was directed with the purpose of discovery more effective anti-inflammatory agents in macrophages of newly radiolysis products PC and IPC. The anti-inflammatory capacities of the characterized products in RAW 264.7 and DH82 macrophages treated with lipopolysaccharide (LPS) to stimulate an inflammation response were examined. The pro-inflammatory factors such as prostaglandin E2 (PGE2), tumor necrosis factor-α (TNF-α), nitric oxide (NO), interleukin-6 (IL-6), and IL-10, without cytotoxicity in LPS-stimulated macrophages, were significantly inhibited after treatment with PC and IPC, when compared to PZ. Moreover, PC and IPC decreased the appearance of cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS) proteins in macrophages. The cyclization products modified by radiolysis showed the greatest anti-inflammatory effects in macrophage cells, indicating PC and IPC are a potential candidate for use in anti-inflammatory agents.

Key words: Phlorocyclin, Isophlorocyclin, Anti-inflammatory, Macrophages, Radiolytic cyclization

1. Introduction

Macrophages are cells that participate in inflammatory responses. They are activated by various stimuli or cytokines secreted by immune cells, and induce a variety of inflammatory responses, including pain and swelling, and promote the migration of immune cells to the site of inflammation [1]. The RAW 264.7 and DH82 macrophages promote the expression of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) by lipopolysaccharide (LPS), and the formation of these pro-inflammatory mediators plays a major role in inflammation by participating in the mass production of prostaglandin E_2 (PGE_2) and nitric oxide (NO) [2,3]. Cyclooxygenase-2 (COX-2) is an enzyme that promotes the oxidation of arachidonic acid to PGE_2 from the cell membrane phospholipids [4]. It is highly expressed in macrophages by various stimuli such as LPS and pro-inflammatory cytokines, and PGE_2 generated by this causes tumor cell death to be suppressed and angiogenesis to be induced, which contributes to tumor growth. Additionally, inducible nitric oxide synthase (iNOS) is also stimulated by external stimuli and cytokines, and produces a large amount of NO [5]. As a result, decreasing the expression of COX-2 and iNOS can inhibit pro-inflammatory responses. Inflammation is one of the body's normal defense mechanisms, but excessive inflammation can be the cause of various diseases. Therefore, it is important to regulate inflammation to maintain health [6,7].

Gamma irradiation technology for structural modification of natural products is a new green chemical synthesis method for the development and discovery of new drug candidates [8]. Gamma irradiation produces a variety of free radicals such as hydroxymethyl ($^{\bullet}CH_2OH$), peroxyl ($HOO^{\bullet}$), hydroxyl ($^{\bullet}OH$), and methoxy ($CH_3O^{\bullet}$) radicals, which can change the molecular modification of the compound and improve its functionality [9]. Recently, the structure of a compound that has been structurally transformed by gamma irradiation of flavonoids, including lute-

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***Corresponding author:** Tae Hoon Kim
Tel. +82-53-850-6533, Fax: +82-53-850-6539, E-mail: skyey7@daegu.ac.kr

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olin and chrysin, has been identified, and it has shown excellent anti-inflammatory activity *in vivo* and *in vitro* test [10,11].

Phloridzin is representative dihydrochalcone glucoside and is usually found in unripe fruits and root bark of apple (*Malus pumila*) and has been shown to possess a wide range of powerful biological capacities including antioxidant, anti-diabetic, and anti-tumor effects [12,13]. Recently, we have conducted research that radiolytic transformation technology (RTT) of phloridzin in methnolic conditions led to the formation of the new neolignans, named phlorocyclin and isophlorocyclin, which was purified by chromatographic isolation [14]. The phlorocyclin and isophlorogcyclin contain rare dihydrobenzofuran skeleton by cyclization between ketone with hydroxyl moieties of phloridzin. These unique neoligan derivatives exhibited potent anti-glycation and radical scavenging activities. In the present study, we investigated the anti-inflammatory activity of phlorocyclin and isophlorogcyclin related its potential inhibitors by focusing on the pro-inflammatory mediators in murine and canine macrophages.

2. Materials and Methods

2.1. Chemicals and instruments

Phloridzin (PZ), methanol (MeOH), ethanol (EtOH), acetonitrile (MeCN), and formic acid (HCOOH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). NMR spectroscopic data were recorded at room temperature on an Varian VNS600 instrument (Varian, Palo Alto, CA, USA) using acetone-d_6 (δ_H 2.05, δ_C 29.8) as solvent and tetramethysilane (TMS) as internal standard, and the coupling constant (J) are given in Hz. Fast atom bombardment mass (FABMS) spectrometer was performed on a JMS-700 spectrometer (JEOL, Tokyo, Japan) operated in the postive-ion mode. Optical rotation and CD spectral data were conducted on JASCO P-2000 polarimeter and JASCO J-715 spectrometer (JASCO, Tokyo, Japan), respectively. Column chromatography was performed using Toyoperal HW-40 (coarse grade; Tosoh Co., Tokyo, Japan) and Sephadex LH-20 (particle size 25-100 μm; GE Healthcare Biosciences AB, Uppsala, Sweden) gel columns.

2.2. Preparation of irradiated sample

Gamma radiation was carried out at room temperature, using a cobalt-60 irradiator (Point source AELC, IR-79, MDS Nordion International Co. Ltd.) at the Advanced Radiation Technology Institute (Jeongup, South Korea). The source strength was about 320 kCi with dose rate of 10 kGy/h. Pure phloridzin (100 mg) dissolved methanol (100 mL) in chapped glass bottle was directly exposed with ranging 10 to 70 kGy dose. The methanolic solution of irradiated samples was immediately evaporated to remove the solvent and dried. HPLC (Shimadzu LC-20AD, Shimadzu, Tokyo, Japan) anlaysis was accompanied on a YMC-Pack ODS A-302 column with 4.6 mm i.d. × 150 mm, particle size 5 μm (YMC Co., Kyoto, Japan) using an gradient mode of 10% to 100% CH_3CN in 0.1% formic acid (flow rate 1.0 mL/min) (Fig 1A and B).

2.3. Isolation of purification of cyclization products

The dried phloridzin with irradiated at 50 kGy dose showed the most enhanced NO production inhibitory effects in LPS-induced RAW264.7 cells, compared to the of original phloridzin (Fig. 1C and D). The drided reactants (95 mg) was subjected to colunm chromatography over Sephadex LH-20 gel column (1.0 cm i.d. × 38 cm) with aqueous EtOH to yield pure phlorocyclin (PC) (25 mg, t_R 10.8 min) and isophlorocyclin (IPC) (37 mg, t_R 11.1 min). Chemical structures of these gernerated products PC and IPC were elucidated by NMR and FABMS spectroscopic analyses with the literature values (Fig. 2).

2.4. Cell culture

The RAW 264.7 cell line (mouse) was obtained from the Korean Cell Line Back (Seoul National University, Seoul, Korea) and DH82 cell line (canine) was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The macrophage cells were cultured under sterile conditions at 37°C in a humidified atmosphere containing 5% CO_2. The macrophage cells culture medium consisted of Dulbecco's modified eagle medium (DMEM, GIBCO Invitrogen Corp., Carlsbad, CA, USA) of added using 10% fetal bovine serum (FBS, GIBCO Invitrogen Corp.), and 1%/streptomycin/penicillin (GIBCO Invitrogen Corp.).

2.5. Cell viability assay

The effects of the newly cyclized products from IR-PZ on cell viability in RAW 264.7 and DH82 cells was estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) protocol [15]. Cells were showed at a density of 5×10^4 cells/well into 96-well plates and incubated for 24 h. The cells were treated with PC and IPC (50, 100, and 200 μM) in dissolved free medium, and cultured at 37°C for 24 h. A MTT (0.5 mg/mL) solution was provided to each well plates and the

plates were maintained at 37°C for 3 h to allow the response to take place before elimination of the culture medium, then the produced formazan blue was dissolved in dimethyl sulfoxide (DMSO). Cytotoxicity was verified with a spectrophotometer and the absorbance was detected at 570 nm and the control was considered 100%.

2.6. Determination of pro-inflammatory mediators and cytokine productions

The RAW 264.7 and DH82 cells were seeded in a 96-well plate at a density of 5×10^4 cells/well and cultured at 37°C for 24 h. Incubated cells were preliminary treated with different concentrations of phlorocyclin and isophlorocyclin for 2 h before incubating with LPS (0.1 or 1.0 μg/mL) for 24 h at 37°C. Nitric oxide yield was estimated by the change of a macrophages culture supernatant by a Griess reagent [16]. The culture supernatant (100 μL) was mixed with the Griess reagent (100 μL) at room temperature and shaken gently for 20 min. Finally, a microplate reader was employed to detect the absorbance of the reactants at 548 nm. The level of PGE_2 and cytokines was determined by ELISA using commercial reagent kits (BD Biosciences) according to the manufacturer's instructions.

2.7. Western blot analysis

Sample treated RAW 264.7 and DH82 macrophage cells were collected, lysed using radioimmunoprecipitation buffer (RIPA; Rockland Immunochemicals, Inc., Limerick, PA, USA). Cell lysates containing an equal amount of protein (30 μg) were prepared and separated by 10% SDS-PAGE and 10 μg of cytosolic fractions and 15 μg of both mitochondria and debris fractions were loaded to 12% SDS-PAGE and shifted to polyvinylidene difluoride membranes (PVDF; Merck Millipore). Then, the membranes were blocked with 5% non-fat milk in Tris-buffered saline containing Tween-20 (TBST) at RT for 1 h. The membranes were then searched overnight at 4°C with primary antibodies for dilution of 1:1,000 for anti-COX-2 (cat. #4842), anti-iNOS (cat. #2977), and anti-GAPDH (cat. no. 2118) (all primary antibodies; Cell Signaling Technology, Danvers, MA, USA).

2.8. Statistical analysis

All data were evaluated by the Student's two-tailed *t*-test and studied results were measured statistically significant when the $*p<0.05$ and $**p<0.01$. Experiment data were conducted at least of three times repetition independently.

3. Results

3.1. Characterization of cyclization products

The phloridzin dissovled in methanolic solution was directly exposed to gamma irradiation, and the radolytic modification pattern was checked using a revers-phase HPLC instruments [14]. After irradiation with gamma irradiation at 50 kGy dose, phloridzin was nearly decreased and the newly generated peaks at a t_R 10.8 and 11.1 min detected two major products (Fig. 1A and B). The IR-phloridzin samples containing newly cyclized products exhibited improved inhibitory activities on nitric oxide (NO) yield in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells compared with original phloridzin (Fig. 1C and 1D). In the present study, column chromatography isolation of the IR-phloridzin reactant led to the isolation of two cyclized products and identified as a phlorocyclin (PC) and isophlorocyclin (IPC) by a careful interpretation of their spectroscopic data (NMR, MS) using the literature values. The structural characteristics are detailed show below (Fig. 2).

Phlorocyclin (PC): Yellowish gum, $[\alpha]^{20}_D$ −45.3° (*c* 0.1, MeOH); CD (MeOH) Δε (nm): 233 (−35.1), 275 (−14.2) nm; ^{1}H-NMR (acetone-d_6, 600 MHz): δ 1.70 (1H, m, H-8), 2.18 (1H, m, H-8), 2.53 (2H, t, *J* = 7.8 Hz, H-7), 3.18 (1H, ddd, *J* = 8.4, 4.2, 1.2 Hz, H-9), 3.40 (1H, m, H-4"), 3.44 (1H, m, H-5"), 3.48 (1H, m, H-2"), 3.52 (1H, t, *J* = 8.4 Hz, H-3"), 3.67 (1H, dd, *J* = 12.0, 6.0 Hz, H-6"), 3.89 (1H, dd, *J* = 12.0, 2.4 Hz, H-6"), 4.88 (1H, d, *J* = 7.8 Hz, H-1"), 5.72 (1H, d, *J* = 1.2 Hz, H-10), 5.95 (1H, d, *J* = 1.8 Hz, H-6'), 6.25 (1H, d, *J* = 1.8 Hz, H-4'), 6.70 (2H, d, *J* = 8.4 Hz, H-3, 5), 7.06 (2H, d, *J* = 8.4 Hz, H-2, 6), ^{13}C-NMR (acetone-d_6, 150 MHz): δ 32.5 (C-7), 34.1 (C-8), 48.3 (C-9), 62.4 (C-6"), 71.1 (C-4"), 74.3 (C-2"), 77.5 (C-5"), 78.0 (C-3"), 92.6 (C-6'), 96.2 (C-4'), 101.7 (C-1"), 107.2 (C-10), 109.6 (C-2'), 115.7 (C-3, 5), 130.1 (C-2, 6), 133.5 (C-1), 156.0 (C-4), 155.7 (C-1'), 159.7 (C-5'), 160.8 (C-3'); FABMS *m/z* 473 $[M + Na]^+$ [14].

Isophlorocyclin (IPC): Yellowish gum, $[\alpha]^{20}_D$ −152.9° (*c* 0.1, MeOH); CD (MeOH) Δε (nm): 236 (+28.5), 275 (+13.9) nm; ^{1}H-NMR (acetone-d_6, 600 MHz): *δ* 1.70 (1H, m, H-8), 2.06 (1H, m, H-8), 2.57 (2H, t, *J* = 7.8 Hz, H-7), 3.24 (1H, ddd, *J* = 8.4, 4.2, 1.2 Hz, H-9), 3.38 (1H, t, *J* = 8.4 Hz, H-4"), 3.46 (1H, m, H-5"), 3.48 (1H, m, H-2"), 3.52 (1H, t, *J* = 8.4 Hz, H-3"), 3.67 (1H, dd, *J* = 12.0, 6.0 Hz, H-6"), 3.89 (1H, dd, *J* = 12.0, 2.4 Hz, H-6"), 4.88 (1H, d, *J* = 7.8 Hz, H-1"), 5.71 (1H, d, *J* = 1.2 Hz, H-10), 5.95 (1H, d, *J* = 1.8 Hz, H-6'), 6.23 (1H, d, *J* = 1.8 Hz, H-4'), 6.72 (2H, d, *J* = 8.4 Hz, H-3, 5), 7.04 (2H, d, *J* =

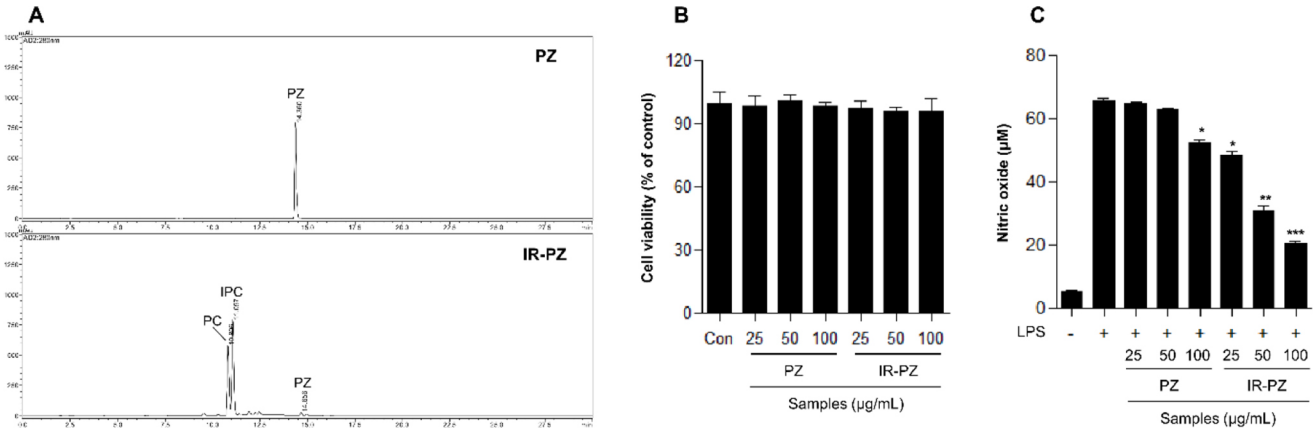

Fig. 1. Effects of gamma-irradiation on cell viability and nitric oxide production of phloridzin in LPS-induced RAW 264.7 cells. (A) Radiolytic transformation was determined by HPLC analysis. (B) Cell viability was measured by MTT assay. (C) Production of nitric oxide in culture medium measured by Griess assay. The data represent the mean ± SEM (n = 3). *p<0.05, **p<0.01, and ***p<0.001 represent significant differences compared to only LPS-treated group. PZ: phloridzin, IR-PZ: Irradiated phloridzin.

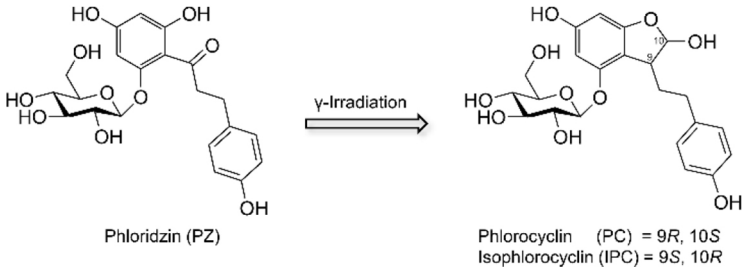

Fig. 2. Structures of phlorocyclin and isophlorocyclin.

8.4 Hz, H-2, 6), ^{13}C-NMR (acetone-d_6, 150 MHz): δ 32.6 (C-7), 34.9 (C-8), 48.2 (C-9), 62.5 (C-6''), 71.1 (C-4''), 74.4 (C-2''), 77.5 (C-5''), 77.6 (C-3''), 92.6 (C-6'), 96.0 (C-4'), 101.5 (C-1''), 107.4 (C-10), 109.3 (C-2'), 115.8 (C-3, 5), 130.0 (C-2, 6), 133.7 (C-1), 156.1 (C-4), 155.7 (C-1'), 159.7 (C-5'), 160.8 (C-3'); FABMS m/z 473 $[M + Na]^+$ [14].

3.2. Quantification of cyclization products

Structural cyclization of phloridzin induced by gamma irradiation at 50 kGy dose resulted in agents with potent anti-inflammatory abilities. The cyclized compounds produced according to the different dose of gamma irradiation were detected by HPLC (Fig. 3A-D). In the irradiated reactant at 10 kGy dose (Fig. 3A), only phloridzin was detected, but very small amount of the newly transformed peaks (t_R 10.8 and 11.1 min) was detected in the irradiated samples with 30 kGy dose (Fig. 3B). Furthermore, in the 50 kGy dose samples, phloridzin was reduced, and two major compounds were detected (Fig. 3C). Quantitative analysis displayed that the contents of the most potent phlorocyclin and isophlorocyclin in irradiated samples at 50 kGy dose were 385.7±1.2 and 415.6±1.8 mg/g, respectively (Fig. 3E).

3.3. Effects of cyclization products on cell viability

The potential anti-inflammatory effects of cyclization products from phloridzin modified by gamma irradiation were examined to assess prostaglandin E_2 (PGE_2) and nitric oxide (NO) productions in macrophage cells (Fig. 5). We first assessed cell viability by MTT assay, as previously described, following exposure to three different concentrations of phloridzin, phlorocyclin, and isophlorocyclin for 24 h. Phloridzin and cyclized products (phlorocyclin and isophlorocyclin) up to 200 μM had no effect on viability of RAW 264.7 (Fig. 5A) and DH82 (Fig. 5D) macrophage cells.

3.4. Effects of cyclization products on NO and PGE_2 production

To determine the inhibitory effects of cyclized products on

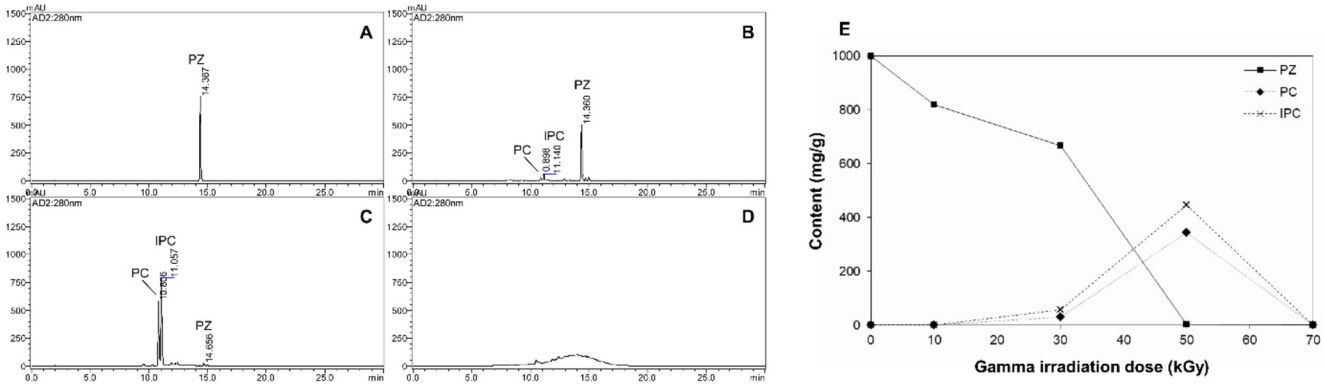

Fig. 3. HPLC chromatograms and content of major products of IR-PZ at different dose. (A) 10, (B) 30, (C) 50, and (D) 70 kGy. For analytical conditions, see the Materials and methods section. (E) Absolute content (mg/g) of individual components in the irradiated solutions. PZ: phloridzin, PC: phlorocyclin, IPC: isophlorocyclin.

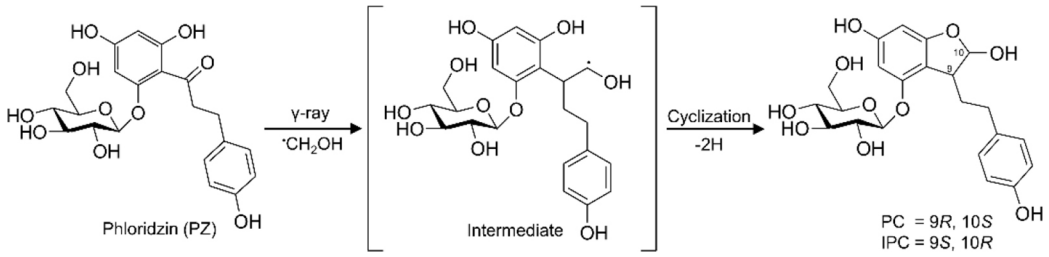

Fig. 4. A plausible mechanism of the cyclization of phloridzin modified by γ-ray

LPS-stimulated NO production in RAW 264.7 cell, NO levels in the cell culture medium were measured by the Griess reagent assay. As shown in Fig. 5B, treatment of LPS with phloridzin resulted in a significantly higher induction of NO production compared with the control group. However, treatment with the novel neolignans, phlorocyclin and isophorocyclin significantly inhibited NO production in a concentration dose-dependent manner. PGE_2, another important inflammatory mediator, was also measured by ELISA. The results indicated that phlorocyclin induced potent inhibition of PGE_2 production at concentrations of 100 and 200 μM, by approximately 585 and 659 pg/mL, respectively. Interestingly, another structurally similar isophlorocyclin, with 9*S*, 10*R*-stereostructure, was found to show relatively weaker inhibitory effects in PGE_2 production assay than phlorocyclin (Fig. 5C). Phlorocyclin and isophlorocyclin also showed improved inhibition of NO and PGE_2 production compared to PZ in DH82 macrophage cells (Fig. 5E and F).

3.5. Effects of cyclization products on COX-2 and iNOS protein expression

The new cyclization products, phlorocyclin and isophlorocyclin showed potent inhibitory effects of inflammatory factors against LPS-stimulated RAW 264.7 and DH82 macrophage compared to the original PZ (Fig. 5). Western bolt studies were conducted to evaluated whether the inhibition capacities of cyclized products on the pro-inflammatory factors PGE_2 and NO were associated with the modulation of the expressions of COX-2 and iNOS (Fig. 6). In LPS-stimulated RAW 264.7 and DH82 macrophage cells, COX-2 and iNOS protein was remarkably upregulated, but LPS-treatment with phlorocyclin and isophlorocyclin showed these downregulations of protein levels, compared to the PZ (Fig. 6). These results indicate that the inhibitory effects of phlorocyclin and isophlorocyclin against LPS-mediated PGE_2 and NO productions are triggered by COX-2 and iNOS suppression.

3.6. Effects of cyclization products on cytokine levels

Macrophages upon stimulation by LPS produce several pro-inflammatory cytokines such as IL-6, IL-10, and TNF-α [16]. Accordingly, we examined the effects of new cyclization products from phloridzin modified by gamma irradiation on LPS-stimulated production of TNF-α, IL-6 and IL-10 (Fig. 7). Isophlorocyclin, having 9*R*, 10*S*-configuration at the chiral centers, was exhibited potent inhibitory effects of TNF-α pro-

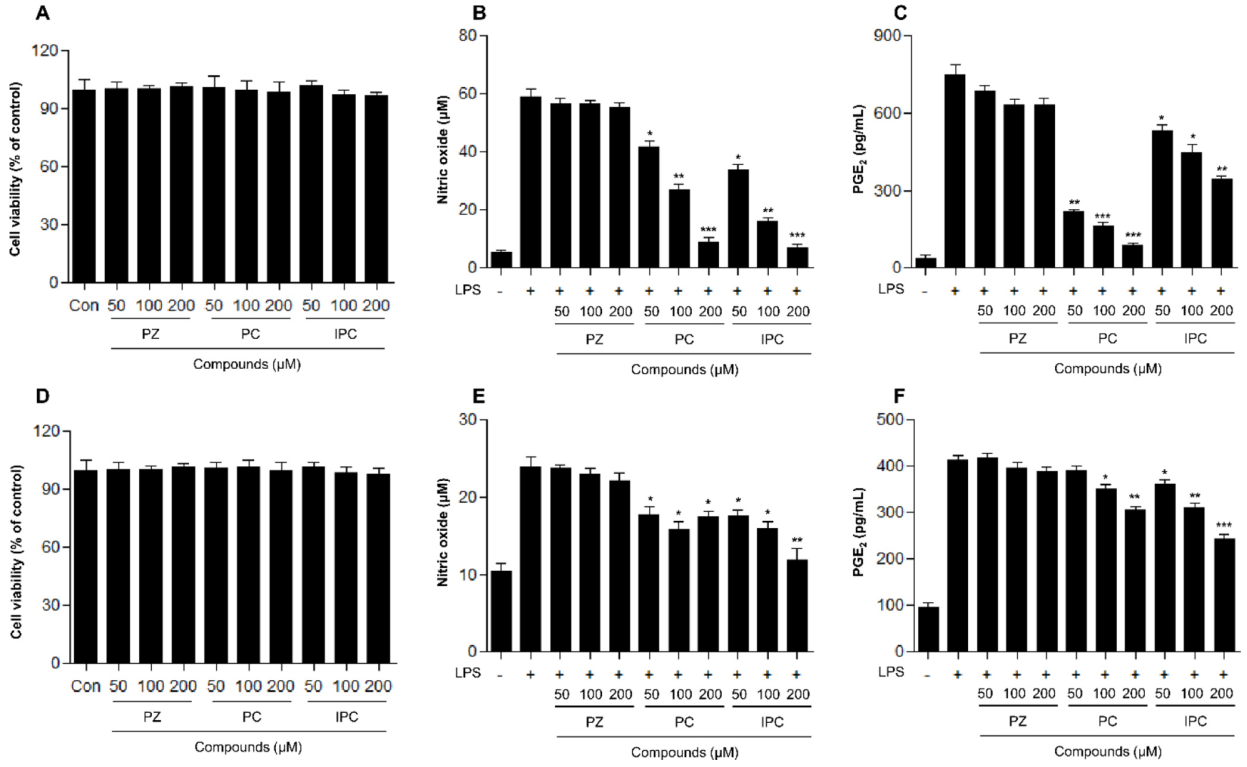

Fig. 5. Effects of cell viability, NO and PGE_2 production of phlorocyclin and isophlorocyclin in LPS-induced RAW 264.7 (A, B, C) and DH82 (D, E, F) macrophage cells. (A, D) Cell viability was determined using MTT assay. (B, E) Production of nitric oxide in culture medium measured by Griess assay. (C, F) Production of PGE2 in culture medium measured by ELISA. The data represent the mean ± SEM (n = 3). *p<0.05, **p<0.01, and ***p<0.001 represent significant differences compared to only LPS-treated group. PZ: phloridzin, PC: phlorocyclin, IPC: isophlorocyclin.

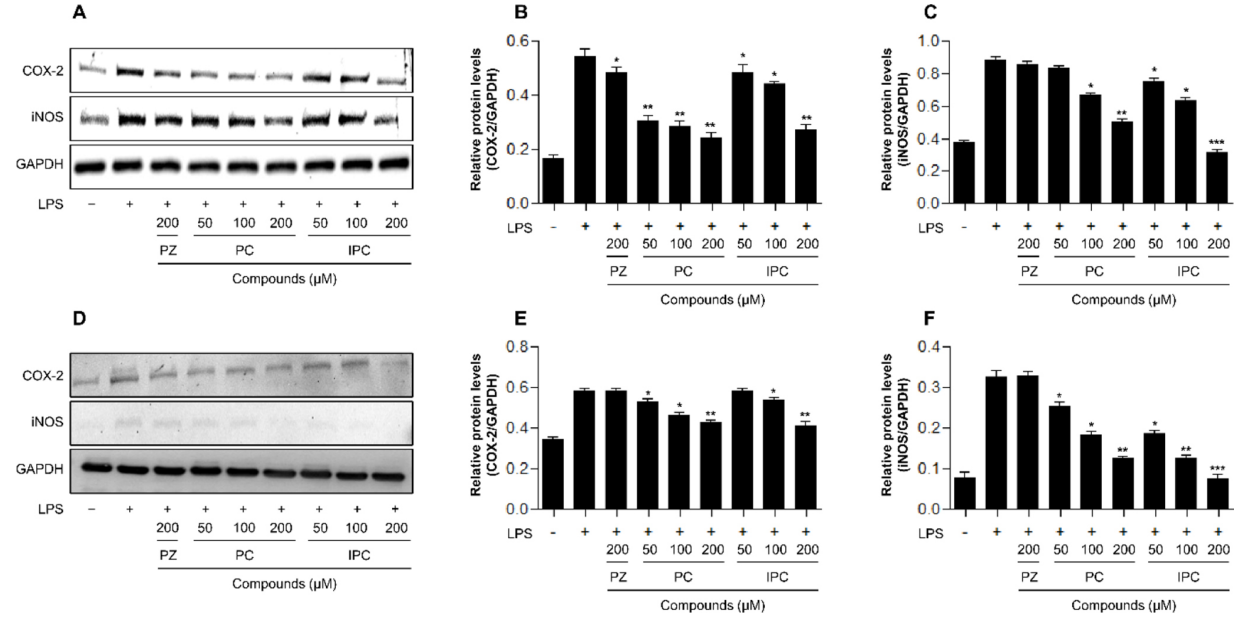

Fig. 6. Effects of expression of COX-2 and iNOS protein of of phlorocyclin and isophlorocyclin in LPS-induced RAW 264.7 (A, B, C) and DH82 (D, E, F) macrophage cells. (A, D) Protein expression of COX-2 (B, E) and iNOS (C, F) induced by samples treatment of macrophage cells for 24 h. The data represent the mean ± SEM (n = 3). *p<0.05, **p<0.01, and ***p<0.001 represent significant differences compared to only LPS-treated group. PZ: phloridzin, PC: phlorocyclin, IPC: isophlorocyclin.

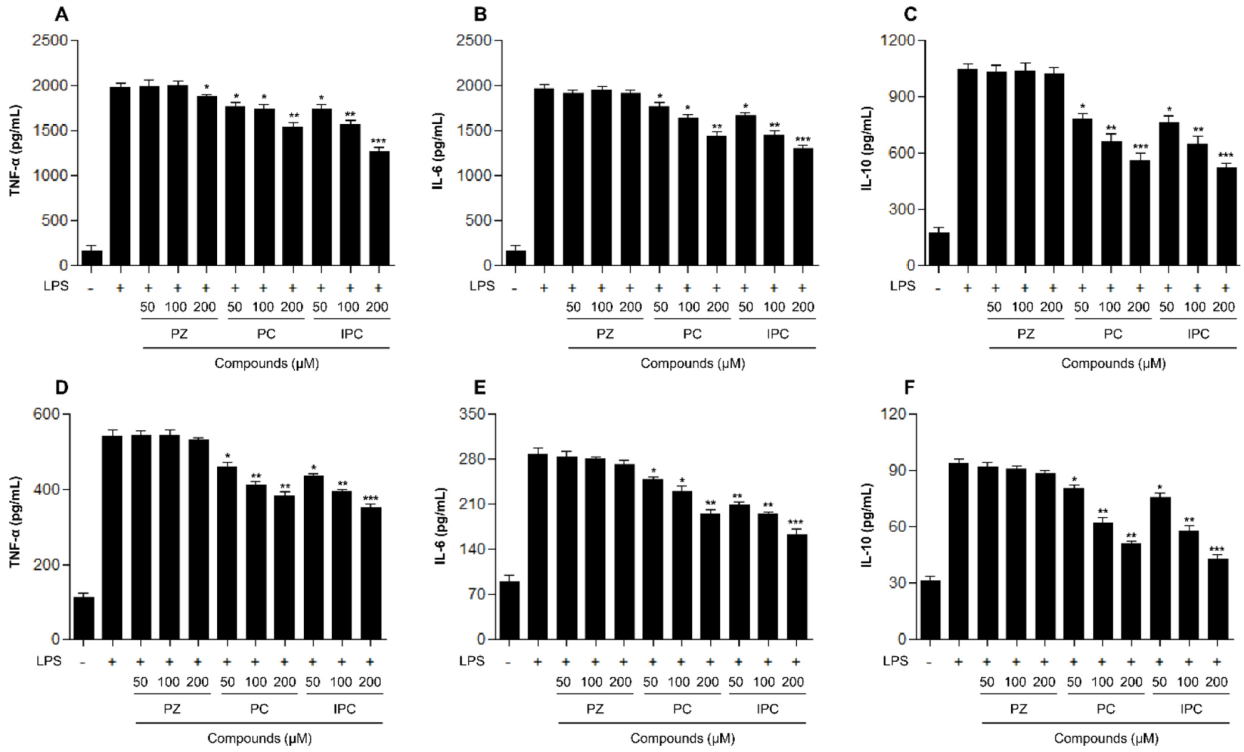

Fig. 7. Effects of cytokine production of phlorocyclin and isophlorocyclin in LPS-induced RAW 264.7 (A, B, C) and DH82 (D, E, F) macrophage cells. Cytokine productions of culture media was measured by ELISA. (A, D) TNF-α, (B, E) IL-6, (C, F) IL-10. The data represent the mean ± SEM (n = 3). *p<0.05, **p<0.01, and ***p<0.001 represent significant differences compared to only LPS-treated group. PZ: phloridzin, PC: phlorocyclin, IPC: isophlorocyclin

duction in LPS-stimulated macrophage cells. On the other hand, diastereoisomer phlorocyclin, with 9*S*, 10*R*-configuration, was found to exhibit relatively weaker inhibitory effect in TNF-α production assay than isophlorocyclin (Fig. 7A and D). In the LPS-induced RAW 264.7 and DH82 macrophage cells, the novel cyclized products, phlorocyclin and isophlorocyclin also exhibited inhibitory activities of the production of inflammatory mediators IL-6 and IL-10 in a concentration-dependent manner than compared to the original phloridzin (Fig. 7B, C, E, and F).

4. Discussion

Radiolytic transformation technology is very simple and economical method for developing functional materials. Previously, many researchers have reported that reactive oxygen species (ROS) and free radicals such as, hydroxymethyl ($^{\bullet}CH_2OH$), methoxy ($CH_3O^{\bullet}$), hydroxyl ($^{\bullet}OH$), superoxide anion ($O_2^{\bullet-}$), and peroxyl ($HOO^{\bullet}$) produced during gamma-ray in methanolic solution have a closely correlation with inducing chemical change and enhancement of biological effects in the natural products [17]. In this study, gamma irradiation of phloridzin in methanolic condition characterized the chemical structure of novel neolignans induced by hydroxymethylation with $^{\bullet}CH_2OH$ radical and cyclization through spectroscopic methods, including NMR and mass spectrometry [14]. The new cyclization products, phlorocyclin and isophlorocyclin exhibited maximum conversion approximately 80% when gamma irradiation with phloridzin at 50 kGy dose. However, high level gamma-ray dose induced degradation of the newly generated phlorocyclin and isophlorocyclin, so the optimal irradiation condition was confirmed to 50 kGy dose.

Cytotoxicity of the phlorocyclin and isophlorocyclin were measured based on RAW 264.7 and DH82 macrophage cell viability at concentrations up to 200 μM. They effectively inhibited the production of pro-inflammatory mediators NO and PGE_2 in LPS-stimulated macrophage cells than compared to the mother phloridzin. The novel neolignans, phlorocyclin and isophlorocylin significantly decreased iNOS and COX-2 protein expression in macrophages through western blot analysis, supporting our findings. Besides, in the evaluation of inhibitory capacities

of the production of inflammatory cytokines including TNF-α, IL-6 and IL-10, phlorocyclin and isophlorocyclin showed significantly improved anti-inflammatory activity compared to phloridzin.

5. Conclusions

The results suggest that PC and IPC exhibit higher anti-inflammatory capabilities toward the production of PGE_2 and NO LPS-induced macrophages than PZ. In addition, newly cyclized PC and IPC decreased the expression of COX-2 and iNOS proteins and suppressed cytokine production. This investigation revealed the modification of dihydrochalcone glucoside using ionizing radiation and provided a unique approach to the semi-synthesis of neolignane glucosides with highly enriched potency for anti-inflammatory capacity in humans and companion animals. The application of radiation technology to natural products is essential for the advent of the medicine discovery and development industry to improve bioactivity and safety.

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