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Taxifolin glycoside inhibits dendritic cell responses stimulated by lipopolysaccharide and lipoteichoic acid

Yun Jeong Kim, Sun Eun Choi, Min Won Lee and Chung Soo Lee

Abstract

Antigen-presenting dendritic cells may play an important role in the pathogenesis of atopic dermatitis. Taxifolin is demonstrated to have anti-inflammatory effects. The present study was designed to assess the effect of taxifolin glycoside against stimulated responses of dendritic cells isolated from mouse bone marrow and spleen. Dendritic cells exposed to lipopolysaccharide, lipoteichoic acid or interleukin (IL)-1β exhibited increased production of IL-12 p70 and tumour necrosis factor alpha, increased formation of reactive oxygen species (ROS) and nitric oxide (NO), and elevation of intracellular Ca^{2+} levels. Treatment with taxifolin glycoside inhibited responses stimulated by the microbial products or IL-1β in dendritic cells in a dose-dependent manner. Taxifolin glycoside had a significant inhibitory effect on the production of cytokines, formation of ROS and NO, and change in intracellular Ca^{2+} levels in dendritic cells of bone marrow and spleen. The results show that taxifolin glycoside seems to inhibit the dendritic cell responses stimulated by microbial products and IL-1β, suggesting that taxifolin glycoside may exert an inhibitory effect against dendritic-cell-mediated immune responses.

Introduction

Atopic dermatitis is a chronically relapsing pruritic inflammatory skin disease. Genetic background and environmental factors such as food allergens and microbial antigens, which may interact with susceptible genes in atopic dermatitis, are suggested to be implicated in the development of recurrent itchy eczematous skin disease in patients with atopic dermatitis (Wollenberg et al 2000; Novak & Bieber 2005; Boguniewicz & Leung 2006). These factors induce defects in the immune response, including an imbalance in the spectrum of Th1/Th2 responses, disturbed prostaglandin metabolism, and immunoglobulin-E-mediated facilitated antigen presentation by epidermal dendritic cells.

It has been suggested that dendritic cells play an important role in the pathogenesis of atopic dermatitis (Wollenberg et al 2000; Wuthrich & Schmid-Grendelmeier 2003; Granucci et al 2005). Dendritic cells are highly active antigen-presenting cells, which induce and regulate the immune response against antigens, and function as initiators of protective immunity (Banchereau et al 2000). The innate immune system recognizes the pathogen-associated molecular patterns through Toll-like receptors expressed on the surface of immune cells (Takeda et al 2003). The activation of Toll-like receptors results in the maturation of dendritic cells, leading to the production of pro-inflammatory cytokines. Dendritic cells respond to microbial products such as lipopolysaccharide through activation of Toll-like receptors and thus produce various cytokines that may evoke the T-cell-mediated immune response in atopic dermatitis (Takeda et al 2003; Baker 2006; Miraglia del Giudice et al 2006). Lipopolysaccharide has been shown to react with Toll-like receptor 4, and lipoteichoic acid with Toll-like receptor 2.

The flavonoid taxifolin is isolated from various plants such as *Rhododendron mucronulatum, Rhizoma smilacis glabrae* and *Silybum marianum* (Takahashi et al 2001; Kim et al 2003; Chen et al 2007). At present this compound is commercially available. Taxifolin has anti-inflammatory and anti-cancer effects. Taxifolin prevents hydrogen-peroxide-induced cell death in human keratinocytes and mouse fibroblasts (Svobodová et al 2006). It also inhibits cerebral ischaemia–reperfusion injury in rats through suppression of leucocyte infiltration and by inhibition of cyclooxygensase-2 and the expression of inducible

Department of Pharmacology, College of Medicine, Chung-Ang University, Seoul 156-756, South Korea

Yun Jeong Kim, Chung Soo Lee

Pharmacognosy Laboratory, College of Pharmacy, Chung-Ang University, Seoul 156-756, South Korea

Sun Eun Choi, Min Won Lee

Correspondence: Chung Soo Lee, Department of Pharmacology, College of Medicine, Chung-Ang University, Seoul 156-756, South Korea. E-mail: leecs@cau.ac.kr

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nitric oxide (NO) synthase (Wang et al 2006). Taxifolin prevents the lipopolysaccharide-induced production of tumour necrosis factor (TNF-α) by murine macrophages (Ueda et al 2004). It causes inhibition of cell growth and cell death in prostate, breast and colorectal cancer cell lines (Shen et al 2004; Brusselmans et al 2005).

Taxifolin has been demonstrated to have anti-inflammatory effects, although effects against stimulated responses in dendritic cells remain uncertain. The aim of the current study was therefore to assess the effect of taxifolin glycoside against activated responses in dendritic cells and thus to evaluate the effect and action of taxifolin glycoside as a preventive compound in the disease process of atopic dermatitis.

Materials and Methods

Mice

Female C57BL/6 and BALB/c mice were purchased from Japan SLC, Inc. (Haruno production facility, Tokyo, Japan). Mice care was in accordance with the Declaration of Helsinki for the Care and Use Guide of Laboratory Animals and with the US National Institutes of Health guidelines for animal experiments. Mice were maintained under a 12 h light–dark cycle in a temperature-regulated (23 ± 1°C) animal room, with water and food continuously available.

Materials

Recombinant mouse (rm) granulocyte macrophage colony-stimulating factor (GM-CSF), rm interleukin (IL)-4, IL-1β and ELISA kits for IL-12 p70 and TNF-α were purchased from BD Pharmingen (San Diego, CA, USA). Lipopolysaccharide (from *Escherichia coli*), lipoteichoic acid (from *Staphylococcus aureus*), dichlorofluorescin diacetate ($DCFH_2$-DA), nitrate reductase (from *Aspergillus niger*) and other chemicals were purchased from Sigma-Aldrich Inc. (St Louis, MO, USA).

Extraction and isolation of taxifolin glycoside

Taxifolin 3-*O*-β-D-glucopyranoside (3′,4′,5′,7′ tetrahydroxy glavonol glucoside; Figure 1) was isolated from the root of *R. mucronulatum*. Desiccated roots (3.2 kg) were extracted with 80% aqueous acetone at room temperature for 3 days. After removal of acetone under vacuum, the aqueous solution was filtered through filter paper (Tokyo Roshi Kaisha Ltd, Japan). The filtered solution was concentrated and thin-layer chromatography performed with increasing concentrations of methanol (10 to 100%) from which eight fractions were separated. Fraction number 5 was applied to the column filled with MCl-Gel CHP 20P (75–150 μm, 5 × 60 cm; Mitsubishi Chemical Co., Tokyo, Japan) and the solution eluted with methanol (100% water to 100% methanol gradient). The solution was applied to a column filled with Sephadex LH-20 (25–100 μm, 3 × 70 cm; Pharmacia, Uppsala, Sweden) and the solution eluted with methanol (30% to 100% gradient). Finally, the eluted solution was applied to a column filled with YMC-Gel ODS-A (pore size 12 nm, particle size 15 μm, YMC Co. Ltd, Kyoto, Japan) and low-pressure liquid column chromatography was performed with increasing proportions of methanol (20% to 80%). The column was connected to a peristaltic pump (Gilson Minipuls 3) and Gilson 112 UV/VIS detector (254 nm), and operated using a Gilson 740 ProTech System Controller Software program (Gilson Inc., Middleton, WI, USA). From these procedures, taxifolin glycoside (5.7 g) was yielded, which was a white-yellow amorphous powder.

Negative LC-MS: *m/z* 475.5 [M-H]. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.67 (1H, s, 5-OH), 6.69~6.87 (3H in total, m, H-2′, H-5′ and H-6′), 5.93 (2H in total, s, H-6 and H-8), 5.39 (1H, d, J = 8.4 Hz, H-2), 4.73 (1H, d, J = 8.4 Hz, H-3), 4.68 (1H, d, J = 7.5 Hz, H-1). ^{13}C-NMR (75 MHz, DMSO-d_6): 81.3 (C-2), 75.2 (C-3), 192.7 (C-4), 163.5 (C-5), 95.9 (C-6), 167.3 (C-7), 95.1 (C-8), 161.7 (C-9), 100.9 (C-10), 126.4 (C-1′), 114.8 (C-2′), 144.5 (C-3′), 144.9 (C-4′), 115.3 (C-5′), 118.6 (C-6′), 102.8 (glc-1), 73.4 (glc-2), 76.8 (glc-3), 69.9 (glc-4), 76.7 (glc-5), 61.2 (glc-6).

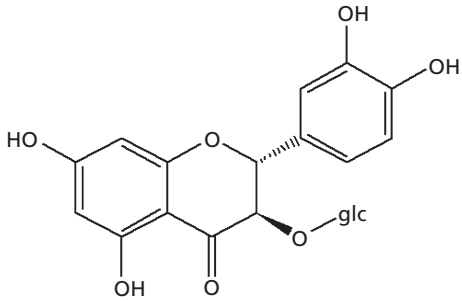

Figure 1 Chemical structure of taxifolin 3-*O*-β-D-glucopyranoside; glc = D-glucopyranoside.

Isolation and culture of dendritic cells

Bone marrow dendritic cells were prepared and cultured as described previously (Piercy et al 2006; Yoon et al 2006). Bone marrow was flushed from the tibiae and femurs of female C57BL/6 and BALB/c mice using RPMI 1640 with a syringe and 25-gauge needle. The tissue was suspended, passed through a nylon mesh (0.40 μm) to remove small pieces of bones and debris, and red blood cells were lysed with ammonium chloride. Cells were plated into six-well culture plates (10^6 cells mL^{-1}) in RPMI supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 50 μM 2-mercaptoethanol, 10 mM HEPES (pH 7.4), 20 ng mL^{-1} rmGM-CSF and 20 ng mL^{-1} rmIL-4 at 37°C and 5% CO_2. On day 3 of the culture, floating cells were gently removed, and fresh medium was added. On day 6 or 7, non-adherent cells and loosely adherent proliferating dendritic cell aggregates were harvested for analysis. On day 7, 80% or more of the non-adherent cells expressed CD11c.

Splenic dendritic cells were purified from spleens of female C57BL/6 mice (Fujita et al 2004; Yoon et al 2006). Spleens were minced and resuspended in RPMI 1640 medium, and the cell suspension filtered through a nylon mesh (0.40 μm).

Red blood cells were lysed and suspended in the RPMI supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 50 μM 2-mercaptoethanol and 10 mM HEPES, pH 7.4, for 2 h, and non-adherent cells were removed. The residual adherent cells were cultured in six-well culture plates in the above RPMI supplemented with 10 ng mL^{-1} rmGM-CSF and rmIL-4 at 37°C, 5% CO_2 for 6–7 days.

In order to obtain enriched and purified populations, dendritic cells were labelled with bead-conjugated anti-CD11c monoclonal antibody (mAb) (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) followed by positive selection through paramagnetic columns (LS columns; Miltenyi Biotec) according to the manufacturer's instructions. The purity of the selected cell fraction was greater than 90%.

Cytokine assay

Dendritic cells (5×10^5 cells per 200 μL) were treated with microbial products (lipopolysaccharide and lipoteichoic acid) or IL-1β in the presence of 1–100 μM taxifolin glycoside for 24 h at 37°C. After centrifugation at 412 *g* for 10 min, the amounts of IL-12 p70 and TNF-α in culture supernatants were measured using commerical ELISA kits according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader.

Measurement of reactive oxygen species and nitrite/nitrate production

The dye $DCFH_2$-DA, which is oxidized to fluorescent dichlorofluorescin (DCF) by hydroperoxides, was used to measure levels of cellular peroxides (Fu et al 1998). Dendritic cells (5×10^5 cells per 200 μL) were treated with microbial products (lipopolysaccharide and lipoteichoic acid) for 6 h at 37°C. Cells were washed with phosphate-buffered saline, suspended in RPMI, incubated with 50 μM dye for 30 min at 37°C, centrifuged at 412 *g* for 10 min and the medium removed. Cells were dissolved with 1% Triton X-100 and fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm using a fluorescence microplate reader (Spectrafluor, Tecan, Salzburg, Austria).

NO liberated from dendritic cells was measured by assaying the NO metabolites nitrite and nitrate (NO_x). After reaction, the nitrate in the medium was reduced to nitrite by incubation with nitrate reductase (500 munits mL^{-1}), 160 μM NADPH and 4 μM flavin adenine dinucleotide at room temperature for 2 h. The medium were mixed with an equal amount of Griess reagent (from Sigma Aldrich Inc.). Absorbance was measured at 540 nm and the amount of nitrite was determined using sodium nitrite as the standard. The results were expressed as total nitrite equivalents (NO_x).

Measurement of intracellular Ca^{2+} levels with the dye fura-2/AM

Dendritic cells were loaded with 2 mM fura-2/AM to give a concentration of 1 μM per 10^7 cells in Tyrode I solution (137 mM NaCl, 5 mM KCl, 5 mM glucose, 1 mM $MgCl_2$, 0.2% fetal bovine serum and 20 mM HEPES, pH 7.4) for 30 min at 37°C (Naganuma et al 1999). Cells were washed twice with Tyrode II solution (as Tyrode I but without fetal bovine serum) by centrifugation at 412 *g* for 10 min and suspended in the same solution. Loaded PC12 cells (4×10^6 mL^{-1}) were suspended in Tyrode II solution with 1mM $CaCl_2$. Fluorescence changes were measured in a luminescence spectrophotometer (Aminco-Bowman Series 2, Aminco-Bowman, Rochester, NY, USA) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm.

Changes in intracellular Ca^{2+} release were assessed in Ca^{2+}-free media (Parys et al 1993). Dendritic cells (4×10^6 mL^{-1}, fura-2 loaded) were suspended in Tyrode II solution with 1 mM EGTA. After 15 min' pre-incubation at 37°C, Ca^{2+} release was induced by the addition of 1 μg mL^{-1} lipopolysaccharide, and changes in intracellular Ca^{2+} levels were measured.

Statistical analysis

Data are expressed as the mean ± s.e.m. Statistical analysis was performed by one-way analysis of variance. When significance was detected, Duncan's test for multiple comparisons was used for post-hoc comparisons between the different groups. A P value below 0.05 was considered significant.

Results and Discussion

The levels of ILs and TNF-α are significantly increased in patients with atopic dermatitis, psoriasis and bronchial asthma compared with normal people (Antunez et al 2006; Numerof & Asadullah 2006). Dendritic cells respond to lipopolysaccharide and lipoteichoic acid by producing cytokines such as IL-6, -8, -10 and -12 and TNF-α (Verhasselt et al 1997; Yoon et al 2006; Kim et al 2007; Wan et al 2008). In this study, we assessed the inhibitory effect of taxifolin glycoside on cytokine production as one of the stimulated responses in dendritic cells exposed to microbial products. We measured the amounts of IL-12 and TNF-α in stimulated dendritic cells. In agreement with previous reports, bone marrow dendritic cells treated with 1 μg mL^{-1} lipopolysaccharide, 20 μg mL^{-1} lipoteichoic acid or 10 ng mL^{-1} IL-1β for 24 h produced 141.3, 52.9 and 78.5 pg mL^{-1} IL-12 p70, respectively. Bone marrow dendritic cells that were not treated with microbial products and cytokines liberated 25.6 pg mL^{-1} IL-12 p70. Taxifolin glycoside (1–100 μM) significantly inhibited the production of IL-12 p70 induced by lipopolysaccharide, lipoteichoic acid or IL-1β in bone marrow dendritic cells (Figure 2A). The inhibitory effect of taxifolin glycoside plateaued at 10 μM.

To confirm the inhibitory effect of taxifolin glycoside on dendritic cell response, we examined the effect of taxifolin on the microbial-product-stimulated responses in spleen-derived dendritic cells. When splenic dendritic cells were treated with 1 μg mL^{-1} lipopolysaccharide, 20 μg mL^{-1} lipoteichoic acid

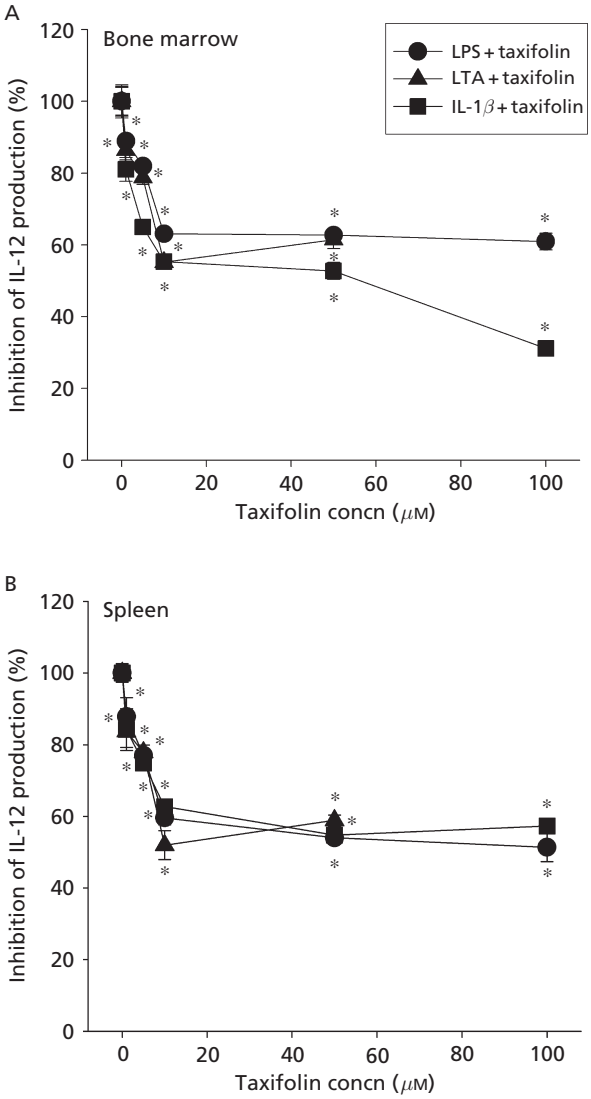

Figure 2 Inhibition of interleukin (IL)-12 p70 production in dendritic cells by taxifolin glycoside. Bone marrow dendritic cells (A) or splenic dendritic cells (B) were pre-treated with 1–100 μM taxifolin glycoside for 20 min, exposed to 1 μg mL^{-1} lipopolysaccharide (LPS), 20 μg mL^{-1} lipoteichoic acid (LTA) or 10 ng mL^{-1} IL-1β in combination with taxifolin glycoside for 24 h, and the amount of IL-12 p70 produced was measured. The amount of IL-12 p70 produced from stimulated dendritic cells was taken as 100%. Data are mean ± s.e.m. (n = 6). *$P < 0.05$ vs microbial products or IL-1β alone.

or 10 ng mL^{-1} IL-1β for 24 h, production of IL-12 p70 was 57, 51.2 and 87.1 pg mL^{-1}, respectively (Figure 2B). Intact splenic dendritic cells liberated 26.3 pg mL^{-1} IL-12 p70. Taxifolin glycoside (1–100 μM) significantly inhibited the lipopolysaccharide-, lipoteichoic acid- or IL-1β-induced production of IL-12 p70 in splenic dendritic cells; this inhibitory effect plateaued at 10 μM taxifolin glycoside.

IL-12 acts on natural killer cells and T cells to induce the production of cytokines, proliferation and increased cytotoxic activity, and exerts important pro-inflammatory functions (Trinchieri 1998). Therefore, taxifolin glycoside may prevent the IL-12-elicited cell responses and stimulated functions through inhibition of IL-12 production.

We also assessed whether taxifolin glycoside could inhibit production of another cytokine, TNF-α, which induces the activation of T cells and production of cutaneous T-cell-attracting chemokines in keratinocytes, and prolongs skin inflammation (Vestergaard et al 2005; Murakawa et al 2006). Bone marrow dendritic cells treated with 1 μg mL^{-1} lipopolysaccharide or 20 μg mL^{-1} lipoteichoic acid for 24 h produced 900.4 and 441.1 pg mL^{-1} TNF-α, respectively. Intact bone marrow dendritic cells liberated 109.9 pg mL^{-1} TNF-α. Taxifolin glycoside significantly inhibited the lipopolysaccharide- or lipoteichoic acid-induced production of TNF-α in bone marrow dendritic cells (Figure 3A).

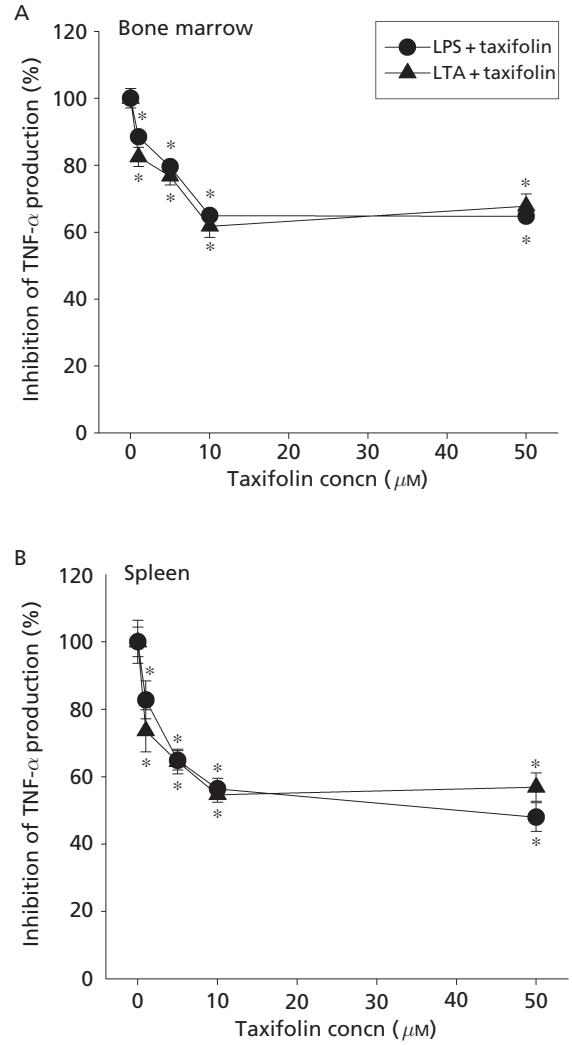

Figure 3 Inhibition of tumour necrosis factor (TNF)-α production in dendritic cells by taxifolin glycoside. Bone marrow dendritic cells (A) or splenic dendritic cells (B) were pretreated with 1–50 μM taxifolin glycoside for 20 min, exposed to 1 μg mL^{-1} lipopolysaccharide (LPS) or 20 μg mL^{-1} lipoteichoic acid (LTA) for 24 h, and the amount of TNF-α produced measured. The amount of TNF-α produced from stimulated dendritic cells was taken as 100%. Data are mean ± s.e.m. (n = 5). *$P < 0.05$ vs microbial products alone.

The inhibitory effect of 10 μM taxifolin glycoside was similar to or less than the effect of 50 μM.

We further investigated the inhibitory effect of taxifolin glycoside on the production of TNF-α by splenic dendritic cells. When splenic dendritic cells were treated with 1 μg mL^{-1} lipopolysaccharide or 20 μg mL^{-1} lipoteichoic acid for 24 h, production of TNF-α was 273.7 and 233.1 pg mL^{-1}, respectively. Intact splenic dendritic cells liberated 129.1 pg mL^{-1} TNF-α. In terms of cytokine production, the response of bone marrow dendritic cells to lipopolysaccharide or lipoteichoic acid seems to be greater than that of splenic dendritic cells. Taxifolin glycoside significantly inhibited the lipopolysaccharide- or lipoteichoic acid-induced production of TNF-α in splenic dendritic cells (Figure 3B). These effects were greater than the effect on cytokine production in bone marrow dendritic cells. Taxifolin glycoside exhibited a plateau inhibitory effect at 10 μM.

The findings suggest that taxifolin glycoside may prevent the TNF-α-elicited cell responses through inhibition of TNF-α production. The present results suggest that taxifolin glycoside seems to exert an inhibitory effect against the activation of dendritic cell response by external stimuli. This compound appears to inhibit the dendritic-cell-derived cytokine actions such as lymphocyte activation and inflammatory reaction.

In order to examine whether the inhibitory effect of taxifolin glycoside on stimulated dendritic cell response may be ascribed to a cytotoxic effect, we investigated the effect of taxifolin glycoside on cell viability in dendritic cells. When dendritic cells were treated with 1–100 μM taxifolin glycoside for 24 h, cell viability was not significantly altered (Figure 4); cell viability in dendritic cells treated with 100 μM taxifolin glycoside was similar to the control value. The result indicates that the inhibitory effect of taxifolin glycoside on cytokine production is not due to its cytotoxic effect.

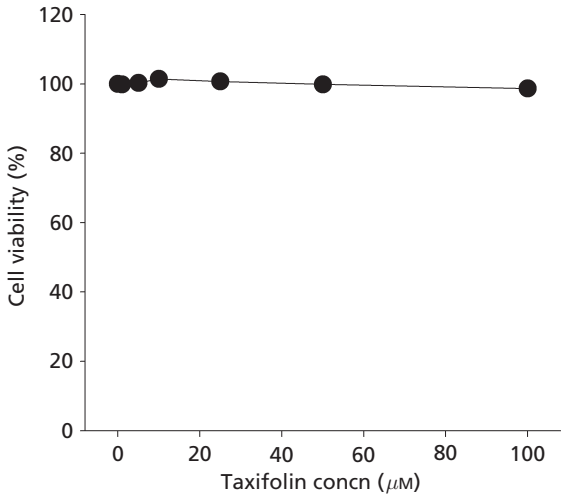

Figure 4 Effect of taxifolin glycoside on cell viability. Bone marrow dendritic cells were treated with 1–100 μM taxifolin glycoside for 24 h and cell viability determined using the MTT assay. Data rare mean ± s.e.m. (n = 6).

ROS formed during antigen presentation have been suggested to regulate communication between dendritic cells and T cells, and have been shown to be involved in the lipopolysaccharide-induced maturation of dendritic cells (Matsue et al 2003). Dendritic cells exposed to lipopolysaccharide liberate ROS, which may be involved in cytokine production (Yamada et al 2006). We therefore assessed the formation of ROS as one of the stimulated dendritic cell responses and the effect of taxifolin glycoside on this response. The formation of ROS within cells was determined by monitoring the conversion of $DCFH_2$-DA to DCF. Bone marrow dendritic cells treated with 1 μg mL^{-1} lipopolysaccharide or 20 μg mL^{-1} lipoteichoic acid for 6 h showed a significant increase in DCF fluorescence. We confirmed the formation of ROS induced by microbial products in dendritic cells by using radical scavengers. Treatment with 1 mM thiol compound *N*-acetylcysteine or 30 μM trolox (a scavenger of hydroxyl radicals and peroxynitrite) inhibited the lipopolysaccharide- or lipoteichoic acid-induced increase in DCF fluorescence (Figure 5A). Taxifolin glycoside inhibited the microbial-product-induced increase in DCF fluorescence in a concentration-dependent manner (Figure 5B). Our data suggest that taxifolin glycoside may prevent cell functions mediated by ROS formed during dendritic cell activation.

Nitrogen species, including NO, play a critical role in the physiological regulation of cellular functions and are involved in pathological conditions such as chronic inflammatory diseases and airway disease (Redington 2006; Pacher et al 2007). Nitrogen species provoke amplification of inflammatory processes in the airways and lung parenchyma, causing protein dysfunction and cell damage. We examined the production of NO in bone marrow dendritic cells treated with microbial products. Cells treated with 1 μg mL^{-1} lipopolysaccharide or 20 μg mL^{-1} lipoteichoic acid for 6 h liberated 8.44 ± 0.27 and 8.26 ± 0.94 μM NO_x, respectively (mean ± s.e.m., n = 5). Taxifolin glycoside significantly inhibited this lipopolysaccharide- or lipoteichoic acid-induced formation of NO_x in a concentration-dependent manner (Figure 5C). Therefore, taxifolin glycoside may interfere with cell functions mediated by NO formed during dendritic cell activation. The present results suggest that taxifolin glycoside seems to prevent the cell functions and damage mediated by ROS and NO formed during activation of dendritic cells.

Ca^{2+} signalling is suggested to be involved in the activation and maturation of dendritic cells (Bagley et al 2004). Ca^{2+}-calmodulin kinase II regulates critical stages of the maturation and antigen presentation capacity of dendrititc cells (Herrmann et al 2005). Elevation of intracellular Ca^{2+} levels causes the production of ROS and reactive nitrogen species (Ermak & Davies 2002). We therefore examined changes in intracellular Ca^{2+} levels in activated dendritic cells and assessed the role of intracellular Ca^{2+} in cell function. We assessed changes in intracellular Ca^{2+} levels using fura-2-loaded dendritic cells. Treatment of splenic dendritic cells with 1 μg mL^{-1} lipopolysaccharide or 20 μg mL^{-1} lipoteichoic acid resulted in an increase in intracellular Ca^{2+} levels; this was markedly inhibited by the addition of 80 μM dantrolene, a blocker of intracellular Ca^{2+} release (Figure 6).

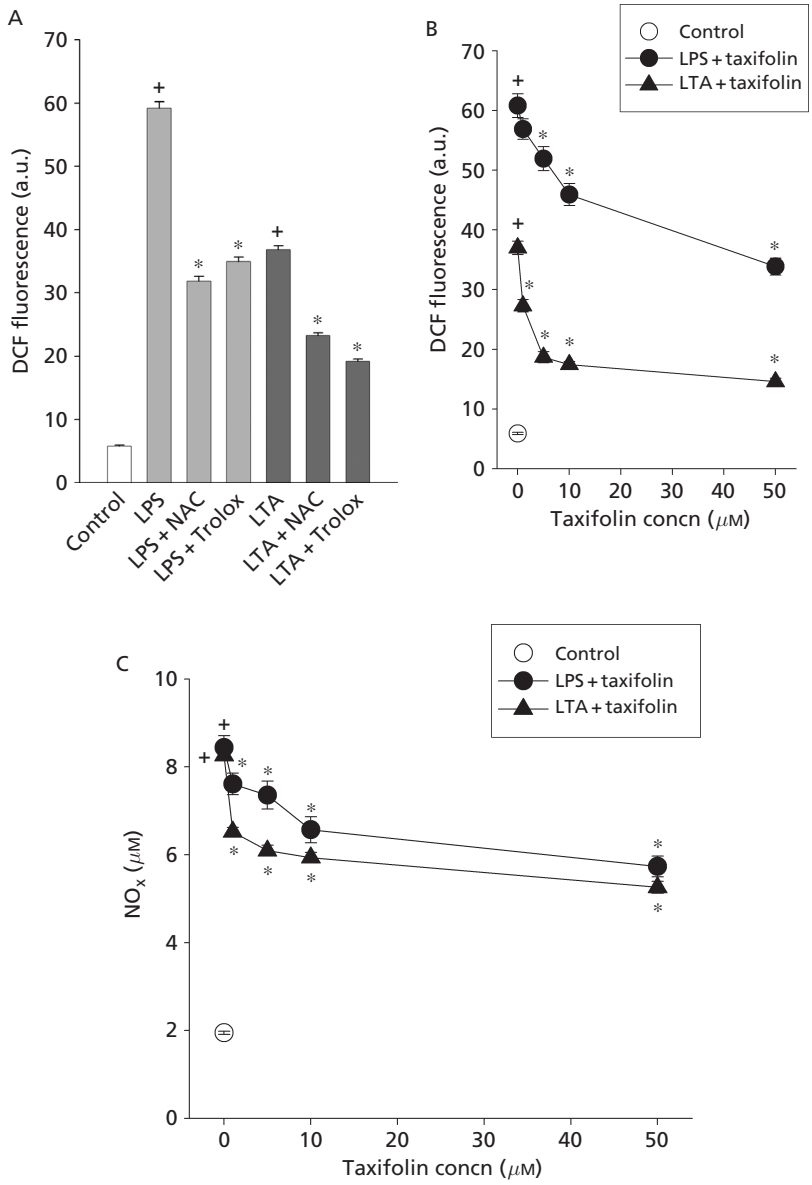

Figure 5 Inhibitory effect of taxifolin glycoside on microbial-product-induced formation of reactive oxygen species (ROS) and nitric oxide (NO). Bone marrow dendritic cells were pretreated with either scavengers [1 mM *N*-acetylcysteine (NAC) or 30 μM trolox] and 1–50 μM taxifolin glycoside for 20 min and exposed to 1 μg mL^{-1} lipopolysaccharide (LPS) or 20 μg mL^{-1} lipoteichoic acid (LTA) in combination with taxifolin glycoside for 6 h. Formation of ROS was measured as changes in DCF fluorescence changes (expressed as arbitrary units of fluorescence (a.u.)). NO production is represented as formation of NO_x (NO nitrite and nitrate metabolites). Values represent the mean ± s.e.m. (n = 5 or 6). $^{+}P < 0.05$ vs control; $^{*}P < 0.05$ vs microbial products alone.

The microbial-product-induced increase in intracellular Ca^{2+} levels was prevented by the addition of the Ca^{2+}-channel blocker nifedipine or taxifolin glycoside (10 μM each). Furthermore, in the Ca^{2+}-free medium containing 1 mM EGTA without addition of extracellular Ca^{2+}, the addition of lipopolysaccharide or lipoteichoic acid did not induce an increase in intracellular Ca^{2+} levels (Figure 6). This finding indicates that the microbial-product-induced elevation of intracellular Ca^{2+} levels may be accomplished by influx of Ca^{2+} from the extracellular milieu and by release of Ca^{2+} from intracellular Ca^{2+} storage sites. Ca^{2+} signalling plays a critical role in the function of dendritic cells (Bagley et al 2004; Herrmann et al 2005). The present results suggest that the inhibitory effect of taxifolin glycoside on the production of cytokines, ROS and NO may be associated with or come from the inhibitory effect on the increase in intracellular Ca^{2+} levels.

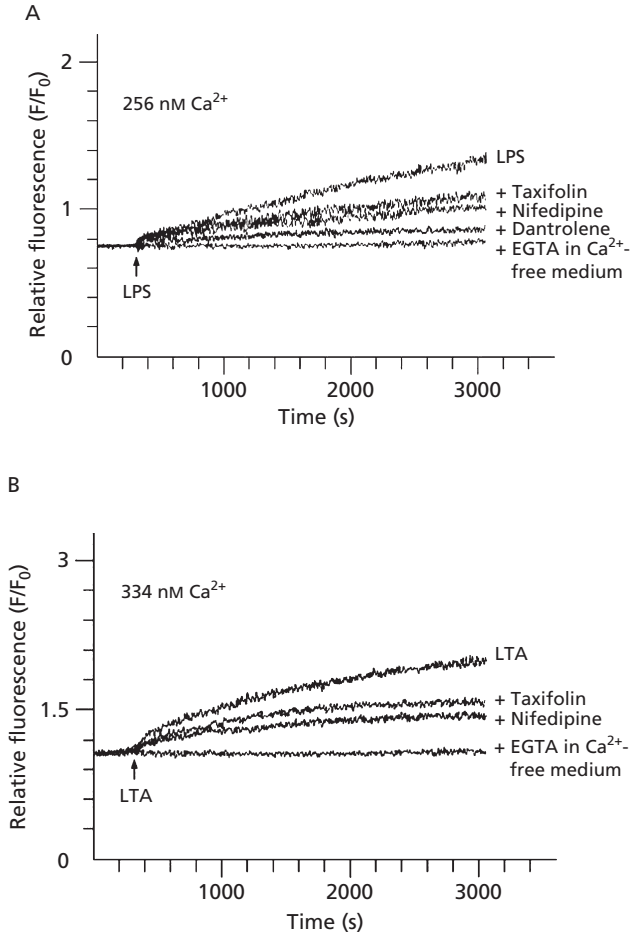

Figure 6 Inhibitory effect of taxifolin glycoside on elevation of intracellular Ca^{2+} levels in dendritic cells exposed to microbial products. After 20 min' incubation with compounds (10 μM taxifolin glycoside, 10 μM nifedipine or 80 μM dantrolene) at 37°C, 1 μg mL^{-1} lipopolysaccharide (LPS) (or 20 μg mL^{-1} lipoteichoic acid (LTA)) was added to the medium containing splenic dendritic cells (fura-2 loaded) at the arrow point; changes in the intracellular Ca^{2+} levels were measured in a luminescence spectrophotometer. Microbial products were added to the Ca^{2+}-free medium containing 1 mM EGTA and changes in the intracellular Ca^{2+} levels measured. The traces are representative of three different experiments.

Conclusions

Taxifolin glycoside inhibits the stimulated dendritic cell responses stimulated by exposure to microbial products or IL-1β and may therefore inhibit dendritic-cell-mediated immune responses. Taxifolin glycoside may provide a beneficial effect in the treatment of dendritic-cell-mediated atopic dermatitis, alone or in combination with drugs such as the immunosuppressant tacrolimus.

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