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Tetradecanol reduces EL-4 T cell growth by the down regulation of NF-κB mediated IL-2 secretion

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ABSTRACT

Tetradecanol is a straight-chain saturated fatty alcohol purified from *Dendropanax morbifera leaves*. We found that tetradecanol (30 μM) reduced specifically the growth of T cells such as EL-4 T cell and isolated murine $CD4^+$ T cells. In this study, we investigated the effects of tetradecanol on the regulation of interlukin-2 (IL-2), a potent T cell growth factor. Tetradecanol significantly inhibited IL-2 secretion in EL-4 T cells activated with phorbol 12-myristate 13-acetate (PMA) plus ionomycin (Io) and also in isolated murine $CD4^+$ T cells activated with anti-CD3 and anti-CD28 antibodies. Next, we examined the effect of tetradecanol on the transcriptional activity related to IL-2 production in T cells. Tetradecanol decreased PMA/Io-induced promoter activity of NF-κB in EL-4 T cells, but did not show any significant effects on the promoters of activator protein 1 (AP-1) and nuclear factor of activated T cells (NF-AT). Tetradecanol inhibited IκBα degradation and nuclear translocation of NF-κB subunit, p65 in PMA/Io–activated EL-4 T cells. These results suggest that tetradecanol might have immunosuppressive effects on T cell mediated disorders. Using a chronic allergic contact dermatitis model induced by repeated application of oxazolone, we showed that tetradecanol reduced ear thickness induced by oxazolone.

1. Introduction

Immune-related diseases are increased these days and T lymphocytes have important roles on immunomodulation. When naïve T cells encounter antigens presented by antigen-presenting cells, interleukin-2 (IL-2) is synthesized and secreted by the stimulation of the T-cell receptor (TCR)/CD3 complex with the CD28 costimulatory signal. IL-2 drives clonal expansion of the activated T cells and prompts cell differentiation to acquire effector functions (Smith, 1992). Hence, the appropriate production of IL-2 is an important determinant of the magnitude of T cell-dependent immune responses. The production of IL-2 is mainly regulated at the transcriptional level through multiple transcription factors. The nuclear factor of activated T cells (NF-AT) associated with activator protein-1 (AP-1) has been reported to bind several motifs within the IL-2 promoter (Rooney et al., 1995). The binding of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in the IL-2 promoter activates a canonical site for NF-κB and transcription of the CD28 response element (Hoyos et al., 1989). Cyclosporine A and tacrolimus inhibit IL-2 production and represent the mainstay of immunosuppressive therapy in transplantation (Kalli et al., 1998; Marienfeld et al., 1997). The mechanism of action is the inactivation of the Ca/calmodulin-dependent serine-threonine phosphatase, calcineurin, leading to the inactivation of NF-AT, a transcription factor that is required for the expression of IL-2, interferon-γ (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) genes (Ferraccioli et al., 2005).

Immunomodulatory herb plants have been studied to treat inflammation and immune-related diseases. *Dendropanax morbifera* Leveille (Araliaceae) is an endemic species growing in the southern part of Korea (Han et al., 1998). Several studies have revealed *Dendropanax morbifera* to have diverse therapeutic potential such as anti-cancer, anti-thrombotic, anti-diabetic, anti-oxidant, and anti-inflammatory activities (Akram et al., 2016; Hyun et al., 2013; Kim et al., 2015, 2014; Lee et al., 2013; Moon, 2011; Yu et al., 2012a). Active

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ingredients such as rutin, polyacetylenes, or oleifoliosides purified from *Dendropanax morbifera* have been reported. Rutin, a bioflavonoid protects against thromboembolism and neuronal injury (Choi et al., 2015; Park et al., 2014). Petylenes ((3S)-falcarinol, (3S,8S)-falcarindiol, and (3S)-diynene) were reported to inhibit complement activity (Park et al., 2004). Oleifoliosides (triterpenoids) attenuate macrophage-mediated inflammation and show anticancer effect through inducing cell death (Jin et al., 2013; Yu et al., 2012a). Recently, tetradecanol, a straight-chain saturated fatty alcohol, was isolated from *Dendropanax morbifera* and its effects have been reported on skin whitening and moisturizing (Lee et al., 2015b). There are some reports about therapeutic actions in experimental periodontitis of 1-tetradecanol complex (Hasturk et al., 2009, 2007). We found that tetradecanol reduced T cell growth and the production of IL-2, a potent T cell growth factor. In this study, we also studied the underlying molecular mechanisms of tetradecanol.

2. Material and methods

2.1. Animals

Female BALB/c mice and male C57BL/6 mice were purchased from Damool Science (Daejeon, Korea). The mice were maintained in pathogen limited conditions and kept at a regulated room temperature and under a 12 h light/dark cycle (lights on at 09:00 h) with water and commercial food given ad libitum. All animal procedures were carried out in accordance with the guidelines of the animal care and use committee of Chonnam national university.

2.2. Chemicals and reagents

Tetradecanol (purity > 99%) was isolated from the n-hexane fraction of the water extract of *Dendropanax morbifera* (Lee et al., 2015a). Phorbol 12-myristate 13-acetate (PMA) (PubChem CID: 27924), Ionomycin (Io) (PubChem CID: 6912226), dexamethasone (PubChem CID: 5743), and dimethylsulfoxide (DMSO) (PubChem CID: 679) were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). PMA and Io were dissolved in DMSO. Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute (RPMI) 1640 were purchased from Welgene (Daegu, Korea). Iscove's modified Dulbecco's medium (IMDM) was supplied from Gibco BRL (Rockville, MD, USA). Penicillin-streptomycin and fetal bovine serum (FBS) were purchased from Gibco BRL. Other chemicals used are analytical reagent grade.

2.3. Cell cultures

EL-4 T and HeLa human cervix epithelial cells were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). RAW264.7 mouse macrophage cells were purchased from Korea Cell Line Bank (Seoul, Korea). EL-4 T cells were cultured in RPMI1640 media supplemented with 1% penicillin/streptomycin and 10% FBS under an atmosphere of 5% CO_2 in a humidified 37 °C incubator. HeLa and RAW264.7 cells were cultured in DMEM supplemented with 1% penicillin/streptomycin and 10% FBS. L929 cells were cultured in IMDM containing 10% FBS, 1% sodium pyruvate, 1% MEM non-essential amino acids, and 1% penicillin/streptomycin in a 5% CO2 incubator at 37 °C.

2.4. Preparation of $CD4^+$ T cells and bone marrow derived macrophages (BMDMs)

$CD4^+$ T cells from lymph nodes and spleens of BALB/c were purified using magnetic activated cell sorting $CD4^+$(L3T4) microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). The $CD4^+$ T cells (purity > 90%) were resuspended in complete medium (RPMI 1640 with 10% FBS, 1% penicillin–streptomycin, 2-mercaptoethanol, and L-glutamine) and activated with plate-bound anti-CD3 and anti-CD28 antibodies (1 μg/ml) (Lee et al., 2015a).

Macrophages from bone marrow (BMDMs) of C57BL/6 mouse were prepared as previously described (Celada et al., 1984; Lutz et al., 1999). Briefly, the femur and tibia by cutting the knee joint were separated and all muscle tissue from bones were removed. The bone marrow cells obtained by syringe passages were cultured in IMDM containing 30% L929 cell culture supernatant, 10% FBS, 1% sodium pyruvate, 1% MEM non-essential amino acids, and 1% penicillin/streptomycin (complete IMDM) for 5 days.

2.5. Assay for cell viability

Tetradecanol (10–1000 μM) was treated to each cells for 48 h. The viability of $CD4^+$ T cells or EL-4 T cells was assayed by 2-(2-methoxy-4-nitrophenyl)–3-(4-nitrophenyl)–5-(2,4-disulfophenyl)–2H-tetrazolium, monosodium salt (CCK-8, Dojindo, Rockville, MD, USA). The viability of BMDMs (2×10^4 cells/well) was assessed by 3-(4,5-Dimethylthiazol-2-yl)–2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich Chemical Co, St. Louis, MO, USA). The cell viability of RAW264.7 and HeLa cells was assayed by using was 3-(4, 5-dimethylthiazol-2-yl)–5-(3-carboxymethoxyphenyl)–2-(4-sulfophenyl)–2H-tetrazolium (MTS, Promega, Madison, WI, USA). Absorbance was read with an ELISA microplate reader (ELx808, BioTek Instruments, Inc., Winooski, VT, USA).

2.6. Enzyme-linked immunosorbent assay (ELISA)

EL-4 T cells (1×10^5 cells/well) were treated with tetradecanol (30 μM) for 4 h, followed by addition with PMA (1 ng/ml)/Io (10 nM) for 48 h. In addition, murine $CD4^+$ T cells isolated from lymph nodes and spleens of BALB/c mice as described above were treated with tetradecanol (30 μM) for 48 h in complete medium with plate-bound anti-CD3 antibody (1 μg/ml) and anti-CD28 antibody (1 μg/ml) (Lee et al., 2015a).

IL-2 and IL-4 cytokines in the cell culture supernatants were measured by using ELISA kits following the manufacturer's experimental protocols (R & D system, Minneapolis, MN, USA). The assay was performed at room temperature and the optical absorbance was measured at 450 nm using an ELISA microplate reader (ELx808, BioTek Instruments, Inc., Winooski, VT, USA) within 30 min.

2.7. DNA transfection and luciferase assays

The murine IL-2 promoter fragment from −319 to −7 was generated by PCR and cloned into pGL3-basic vector (Choi et al., 2009). The NF-κB, NF-AT, or AP-1-driven plasmids pNF-κB-Luc, pNF-AT-Luc, or pAP-1-Luc were purchased from Promega (Madison, WI, USA). EL-4 T cells seeded in a 6-well plate (5×10^5 cells/well) were transfected with the indicated firefly luciferase reporter DNA (3–5 μg) using Lipofectamine 3000 (Invitrogen, Carlsbad, MA, USA). After 48 h incubation, the cells were treated with tetradecanol (30 μM) for 4 h, followed by treatment with PMA (1 ng/ml)/Io (10 nM) for 24 h. Luciferase activity in the cell lysates was measured with the luciferase reporter system (Promega, Madison, WI, USA).

2.8. Effect of tetradecanol on NF-κB translocation into nucleus

EL-4 T cells were preincubated with tetradecanol (100 μM) 4 h before the treatment of PMA (80 nM) /Io (1 μM) for 6 h. Cyclosporine A (PubChem CID: 5284373) was used as a control for the NF-κB inhibition. The cells were fixed using 4% paraformaldehyde (Molecular Probes, Inc., Eugene, OR, USA) and permeabilized with 0.1% triton for 15 min. The EL-4 T cells were immunostained with a polyclonal anti-NF-κB p65 antibody (Invitrogen, Carlsbad, MA, USA) and Alexa Fluor