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Inhibition of interferon-γ-induced nitric oxide production by 10-hydroxy-*trans*-2-decenoic acid through inhibition of interferon regulatory factor-8 induction

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ABSTRACT

10-Hydroxy-*trans*-2-decenoic acid (10H2DA) is a major lipid component of royal jelly, a honey bee secretion used to nourish the queen bee and young larvae. In this study, we examined the effect of 10H2DA on interferon (IFN)-γ-induced nitric oxide (NO) production. IFN-γ-induced NO production and activation of the inducible NO synthase promoter were significantly inhibited by 10H2DA. IFN-γ-induced phosphorylation of signal transducer and activator of transcription-1 was not affected by 10H2DA. In contrast, IFN-γ-induced tumor necrosis factor (TNF)-α production and nuclear factor (NF)-κB activation were inhibited by 10H2DA. IFN-γ-mediated induction of interferon regulatory factor (IRF)-8, but not IRF-1, was also inhibited by 10H2DA. IFN-γ-induced TNF-α production followed by activation of NF-κB is known to be essential for NO production. Together, 10H2DA inhibited IFN-γ-induced NO production by inhibiting IRF-8 induction and TNF-α production. 10H2DA might modulate IFN-γ-mediated cellular responses by inhibiting the induction of IRF-8 and IRF-8-dependent genes.

1. Introduction

Nitric oxide (NO) exhibits a wide range of functions, including vascular homeostasis, neurotransmission and antimicrobial defense [1]. NO production is mediated by NO synthase (NOS), which converts L-arginine to L-citrulline and produces NO. There are currently three known isoforms of the enzyme: two (neuronal NOS and endothelial NOS) are constitutively expressed and their enzymatic functions are Ca^{2+}-dependent, while the third isoform (inducible NOS (iNOS)) functions independently of Ca^{2+}. Some types of immune and inflammatory cells such as macrophages and neutrophils can produce iNOS in response to various stimuli [2]. NO, especially that produced by macrophages, plays an important role in host defense against bacterial, viral, and parasitic infection [1]. However, inappropriate or excessive NO production is detrimental to normal tissues and is involved in the pathogenesis of many autoimmune and chronic inflammatory diseases including multiple sclerosis, hypercholesterolemia, atherosclerosis, rheumatoid arthritis, systemic lupus erythematosus, and asthma, [2–4]. NO may also be involved in the onset and progression of inflammatory bowel diseases (IBDs) [5]. Several reports have shown that NOS inhibitors attenuate experimental IBDs [6–10]. These findings suggest that inhibition of NO production may alleviate autoimmune and inflammatory diseases.

Among cytokines that stimulate NO production, interferon (IFN)-γ is known to be one of the strongest inducers of iNOS transcription in macrophages. Binding of IFN-γ to its receptor recruits two types of Janus kinase (JAK-1 and JAK-2), which phosphorylate signal transducer and activator of transcription (STAT)1 [11]. The resultant dimer of this transcription factor is called IFN-γ-activated factor (GAF), and migrates to the nucleus. GAF binds to the IFN-γ-activated site (GAS) on promoters of various genes and induces their transcription. Some interferon regulatory factors (IRFs), including IRF-1 and IRF-8, are upregulated as a result GAS activation [12,13]. The iNOS promoter contains the IFN-stimulated response element (ISRE) [14], which responds to IFN-stimulated gene factor 3 and IRFs. ISRE is activated by IFN-γ stimulation followed by IRF-1 induction [15].

Royal jelly is a product secreted from the hypopharyngeal and mandibular glands of worker honeybees, and is fed to all larvae

Abbreviations: 10H2DA, 10-hydroxy-*trans*-2-decenoic acid; GAF, IFN-γ-activated factor; GAS, IFN-γ-activated site; HPRT, hypoxanthine phosphoribosyltransferase; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; IRF, IFN regulatory factor; ISRE, IFN-stimulated response element; JAK, Janus kinase; NF, nuclear factor; NO, nitric oxide; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor.

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in the colony [16]. Larvae consuming copious amounts of royal jelly become queen bees. Royal jelly consists of a complex mixture of proteins, amino acids, sterols, phenols, sugars, minerals and other components. Many pharmacological activities have been reported for royal jelly, including anti-inflammatory, anti-tumor and anti-bacterial actions [17–19]. Kohno et al. demonstrated that royal jelly inhibits lipopolysaccharide and IFN-γ-induced cytokine production [17]. They also demonstrated that a <5 kDa molecular weight substance was one of the major anti-inflammatory components in royal jelly.

10-Hydroxy-*trans*-2-decenoic acid (10H2DA) is a unique component contained at high levels in royal jelly, and it is unsurprising that it exhibits some similar therapeutic properties to those shown by holistic royal jelly. Indeed, 10H2DA has been reported to have anti-tumor and anti-bacterial activity [20–22], and estrogenic, neurogenic and angiogenic activities have also been reported recently [23–25].

In this study, we examined the inhibitory effect of 10H2DA on IFN-γ-induced NO production in murine macrophage cells. We further examined the mechanisms by which 10H2DA inhibits IFN-γ-induced iNOS induction at the transcriptional level. The impact of 10H2DA inhibition of IFN-γ-stimulated IRF-8 induction is also discussed.

2. Materials and methods

2.1. Reagents

10H2DA with a purity of above 98% (analyzed by HPLC) was kindly gifted from Api Co., Ltd. (Gifu, Japan). IFN-γ was purchased from PBL Interferon Source (Piscataway, NJ, USA). Antibodies against STAT1 and phospho-Tyr701 STAT1 (Sigma, St. Louis, MO USA), IRF-1 and IRF-8 (Santa Cruz Biotechnology, Inc., CA, USA) and Akt (Cell Signaling Technology, Inc. Danvers, MA, USA) were used.

2.2. Plasmids

The cloned plasmid of the iNOS promoter region [26] was kindly gifted from Dr. Sang-Gi Paik (Chungnam National Univ., Korea). A luciferase reporter gene cassette of pTA-luc (Clontech, Palo Alto, CA, USA) was inserted downstream of the iNOS promoter sequence in the plasmid to create piNOS-luc1974. Nuclear factor (NF)-κB and GAS reporter gene plasmids, pNF-κB-TA-luc and pGAS-TA-luc, were purchased from Clontech. A transfection control reporter plasmid, pRL-TK, was from Promega (Madison, WI, USA). Plasmids were extracted with GenElute HP endotoxin-free plasmid midiprep kit (Sigma–Aldrich, Inc.) for transfection into cultured cell lines.

2.3. Culture of RAW264 cells, 10H2DA treatment and stimulation

The RAW264 murine macrophage cell line was obtained from RIKEN Bioresource Center (Tokyo, Japan) and maintained in RPMI-1640 medium containing 5% heat-inactivated fetal bovine serum. Cells were seeded in a 96-well plate at 2×10^5 cells/well for NO and cytokine assays and 5×10^4 cells/well for the reporter gene assay. Cells were pre-incubated with or without the indicated concentration of 10H2DA for 30 min, and then stimulated by adding IFN-γ (100 U/ml) for the indicated periods.

2.4. Measurement of NO production

Nitrite, the end product of NO metabolism, in the culture supernatant was determined as an indicator of NO production as described previously [27]. Briefly, the supernatant was mixed with 100 μl of Griess reagent [28], and the nitrite concentration was measured at an absorbance of 540 nm. Data are expressed as the mean ± SD of triplicate points within a representative data set from at least two independent experiments.

2.5. RT-PCR analysis for mRNA expression

RAW264 cells were cultured on 6-well plate and stimulated with IFN-γ (100 U/ml). The cells were harvested and the total RNA was extracted using the RNeasy mini kit (Qiagen Inc., Valencia, CA, USA) as recommended by the manufacturer. The RNA was reverse-transcribed with ReverTra-Plus- (Toyobo, Tokyo, Japan). Complementary DNA of tumor necrosis factor (TNF)-α, iNOS, IRF-1 or IRF-8 was amplified with specific primers for each gene, listed in Table 1, by Quick Taq HS DyeMix (Toyobo). Amplified PCR products were analyzed by agarose gel electrophoresis followed by ethidium bromide staining. Real-time PCR analysis was also performed with ThunderBird SYBR Green Master Mix (Toyobo).

2.6. Cytokine analysis

TNF-α concentration in culture supernatant was analyzed by conventional sandwich ELISA. Antibodies and recombinant cytokines for ELISA were purchased from e-Bioscience (San Diego, CA, USA).

2.7. Reporter gene assay

The cells seeded in wells of a 96-well plate were transfected with one of the reporter gene constructs and pRL-TK plasmid using FuGENE-HD (Roche, Indianapolis, IN, USA) as recommended by the manufacturer, and incubated for 24 h. The cells were stimulated with IFN-γ (100 U/ml) for a further 24 h, then lysed with Passive Lysis Buffer (Promega). The luciferase activity in the cell lysate was determined with the Dual-Luciferase Reporter assay system (Promega).

2.8. Immunoblotting

RAW264 cells were pre-treated with 10H2DA (5 mM) for 30 min, and then cultured with IFN-γ (100 U/ml). The cells were harvested and lysed in a lysis buffer (50 mM Tris–HCl, pH 7.5, containing 0.5% Nonidet P-40, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride and phosphatase inhibitor cocktail II (Sigma–Aldrich, Inc.). After boiling for 5 min at 100 °C, cell lysates containing equal amounts of protein (20 μg/lane) were loaded onto an 8% polyacrylamide gel, run under reducing conditions and transferred onto the Immobilon Transfer Membrane (Millipore Corporation, Bedford, MA, USA).

Table 1
Primers used for RT-PCR analysis in this study.

Gene (Product; bp)	Primer sequence	Reference
iNOS (807)	CTG CAG GTC TTT GAC GCT CG GTG GAA CAC AGG GGT GAT GC	[27]
TNF-α (374)	TTG ACC TCA GCG CTG TTG CCT GTA GCC CAC GTC GTA GC	[45]
IRF-1 (407)	TCT TGC CCT CCT GAG TGA GT TCT AGG GCC AGT GCT ATG CT	This study
IRF-8 (111)	AAG GTC ACC GTG GTC CTT AG GGA AAG CCT TAC CTG CTG AC	This study
HPRT (177)	GTA ATG ATC GTC AAC GGG GGA C CCA GCA AGC TTG CAA CCT TAA C	[46]

iNOS, inducible nitric oxide synthase; TNF, tumor necrosis factor; IRF, interferon regulatory factor; HPRT, hypoxanthine phosphoribosyltransferase.