Biochemical and Biophysical Research Communications 446 (2014) 1225–1230

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Influence of olive-derived hydroxytyrosol on the toll-like receptor 4-dependent inflammatory response of mouse peritoneal macrophages

Yohei Takeda [a,1], Vuong N. Bui [a], Kenta Iwasaki [b], Takaaki Kobayashi [b,c], Haruko Ogawa [a,*], Kunitoshi Imai [a]

[a] Research Center for Animal Hygiene and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
[b] Department of Transplant Immunology, Nagoya University School of Medicine, Nagoya, Aichi 466-8550, Japan
[c] Department of Surgery II, Nagoya University School of Medicine, Nagoya, Aichi 466-8550, Japan

ARTICLE INFO

Article history:
Received 17 March 2014
Available online 27 March 2014

Keywords:
Hydroxytyrosol
Macrophage
Inflammation
Toll-like receptor 4
Lipopolysaccharide

ABSTRACT

Macrophages play important roles in the host innate immune response and are involved in the onset of diseases caused by inflammation. Toll-like receptor 4 (TLR4)-mediated inflammatory responses of macrophages may be associated with diseases such as diabetes and diseases of the cardiovascular system. Hydroxytyrosol (HT) exerts strong antioxidant and anti-inflammatory effects and may be applied in the treatment of inflammatory diseases. In the present study conducted *in vitro*, we investigated the effects of the TLR4-dependent anti-inflammatory effect of HT on peritoneal macrophage of BALB/c mice. We show here that the elevated levels of iNOS gene expression and nitric oxide production induced by lipopolysaccharide (LPS) (0.25 μg/ml) were suppressed by HT (12.5 μg/ml). LPS-dependent NF-κB gene expression and phosphorylation of NF-κB were not affected by HT under these conditions. In contrast, the expression of TNF-α was significantly increased in the presence of LPS and HT. These results suggest that HT suppressed nitric oxide production by decreasing iNOS gene expression through a mechanism independent of the NF-κB signaling pathway. These novel findings suggest that the modulation by HT of the expression of genes involved in inflammation may involve multiple mechanisms.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Macrophages play important roles in innate immune responses by quickly responding to invading pathogens, leading to their elimination through phagocytosis and the inflammatory response. Recent studies reveal the functional diversities of macrophages, which include two main phenotypes designated M1 and M2 and further emphasize the role of macrophages in the host defense system and the maintenance of homeostasis. In contrast, macrophages are involved in the progression of inflammation-related diseases through a mechanism involving their penetration into tissues and induction of the production of proinflammatory cytokines and nitric oxide (NO) [1].

Toll-like receptor 4 (TLR4), which is a pattern-recognition receptor, recognizes a variety of molecules such as lipopolysaccharide (LPS), which is a component of the outer membrane of Gram-negative bacteria, as well as viral envelope proteins and internal ligands such as intracellular proteins and extracellular matrix components [2]. TLR4 initiates the inflammatory responses of macrophages. Chronic inflammation of adipose tissues and liver is caused by the infiltration of inflammatory macrophages activated by the TLR4 signaling pathway in a mouse model of obesity. Chronic inflammation may induce insulin resistance and dysfunction of islet beta cells, which lead to type 2 diabetes [3,4] and indirectly lead to cardiovascular disorders and inflammatory disorders of the central nervous system [5,6].

$CD14^{+}TLR4^{+}$ macrophages infiltrating the intestinal tract are involved in the pathogenesis of chronic intestinal inflammation in Crohn disease [7]. Moreover, an elevated level of high mobility group box protein-1, an internal ligand of TLR4, induces the production of inflammatory cytokines in a macrophage cell line as well as in the alveolar macrophages of patients with acute lung injury, which indicates an association between the TLR4-dependent production of inflammatory cytokines and pathogenesis [8]. Thus, regulating the excessive inflammatory responses in macrophages through the TLR4 signaling may facilitate to control of the onset of such diseases.

Mediterranean foods, which contain a large amount of olive oil, are associated with the reduction of risk factors for coronary heart

* Corresponding author. Address: Research Center for Animal Hygiene and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada, Obihiro, Hokkaido 080-8555, Japan. Fax: +81 155 49 5893.
E-mail address: hogawa@obihiro.ac.jp (H. Ogawa).
[1] Present address: Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido 060-8638, Japan.

http://dx.doi.org/10.1016/j.bbrc.2014.03.094
0006-291X/© 2014 Elsevier Inc. All rights reserved.

diseases and stroke, and the results of epidemiological studies show the inverse association between olive oil consumption and the risk of cardiovascular disease [9,10]. Among the small molecules that comprise 2% of the total weight of olive oil, phenolic compounds with antioxidant activity may account for the reduction of risk of cardiovascular diseases [11,12]. Hydroxytyrosol (HT) is one of the small phenolic molecules in olive oil that exerts strong antioxidant activity and acts as an anti-inflammatory, antithrombotic, antitumor, and antimicrobial agent [13]. HT lacks toxicity in rodent studies [14], and it is commercially available in foods and nutritional supplements, which possess anti-inflammatory and antioxidant activities [15]. Elucidating the efficacy of the anti-inflammatory effect of HT may lead to its use to treat diseases caused by inflammation. The present study aimed to evaluate the anti-inflammatory effect of HT in the TLR4-dependent inflammatory response in macrophages, which occurs in a number of diseases.

2. Materials and methods

2.1. Hydroxytyrosol (HT)

HT (>98% purity: Tokyo Chemical Co., Ltd., Tokyo, Japan) was dissolved in ultrapure water (milliQ; Millipore, Billerica, MA) to 500 μg/ml and was stored at −80 °C.

2.2. In vitro culture of mouse peritoneal macrophages and cytotoxicity test

Female BALB/c mice (8–14 weeks of age) were intraperitoneally administered 2 ml of a 4.05% solution of Thioglycolate (Becton Dickinson and company, Franklin Lakes, USA). Four days later, the mice were euthanized, and the peritoneal macrophages were collected in cold phosphate-buffered saline (PBS). The cells were cultured in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. To obtain sufficient numbers of cells, macrophages were collected from 2–3 mice and pooled for some experiments. All studies of mice were conducted in compliance with the institutional rules for the care and use of laboratory animals.

The cells were added to 96-well plates (6×10^5 cells per well). Two hours later, the medium was replaced with fresh medium to remove nonadherent cells. Fresh medium containing serially diluted HT (0–12.5 μg/ml) was added to the wells, and the cells were cultured at 37 °C for 24 h. The cytotoxicity of HT was determined by measuring the lactate dehydrogenase released from the cells using a Cytotoxicity Detection kit PLUS (Roche Applied Science, Penzberg, Germany), and the percentage cytotoxicity was calculated.

2.3. Effect of HT on mouse macrophages stimulated with LPS

Mouse peritoneal macrophages were added to 24-well plates (3–6×10^6 per well), and nonadherent cells were removed 2 h later. Adherent cells were cultured in medium containing HT (3.13–12.5 μg/ml) with LPS (0.125–0.5 μg/ml) at 37 °C for 24 or 48 h. The cells cultured in the absence of HT, LPS, or both served as controls. The cultured cells were harvested, and the real-time reverse transcription polymerase chain reaction (RRT-PCR) was used to measure the levels of mRNAs encoding proteins involved in inflammation. The NO concentrations of culture supernatants harvested following a 24-h incubation were determined using a Nitric Oxide (total) detection kit (Enzo Life Sciences, New York, USA). For western blotting analysis, the cells were harvested after 15 min to analyze the expression of inhibitor-κBα (IκBα) and phosphorylated nuclear factor-κB (pNF-κB).

2.4. RRT-PCR analysis of expression of the genes encoding inflammation-related proteins and antiviral cytokines

Total RNA was extracted from cells using Isogen II (Nippon Gene, Tokyo, Japan) and were reverse-transcribed using random primers (Invitrogen, Carlsbad, CA) and M-MLV reverse transcriptase (Invitrogen) as follows: 25 °C for 10 min, 37 °C for 60 min, and 60 °C for 10 min. RRT-PCR was performed using the cDNAs and EagleTaq Master Mix with ROX (Roche Applied Science, Germany) with an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, USA). The primers and probes (Taqman Gene Expression Assays, Applied Biosystems) are listed in Table 1. The PCR reactions were conducted as follows: 95 °C for 15-s to 10 min, 45 cycles of 95 °C for 15-s, 60 °C for 1 min. The threshold cycle (C_t) was defined as the number of cycles required for the intensity of fluorescence to rise above the threshold value. The C_t values were normalized to 18S rRNA and calculated as the difference relative to the RNA of untreated control cells. The C_t value for samples that were undetectable using RRT-PCR was defined as 45. The results are expressed as the difference in gene expression (relative quantity of template) using the comparative *C*(T) method [16].

2.5. Western blotting analysis

Lysates of the cells harvested after 24 h were electrophoresed through a 10% sodium dodecyl sulfate polyacrylamide gel, and the proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). The membranes were blocked with PBS containing 5% skim milk and then incubated with the antibodies provided in the NF-κB Pathway Sampler Kit (Cell Signaling Technology, Danvers, USA). Antigen–antibody complexes bound to the membrane were detected using the ECL Western blotting analysis system (GE healthcare, Buckinghamshire, UK) and analyzed with an LAS-3000 (Fujifilm). Chemiluminescence intensity was measured using Science Lab 2005 Multi Gauge ver. 3.0 (Fujifilm, Tokyo, Japan). As an internal control, β-actin was detected using the anti-β-Actin HRP-DirecT kit (Medical & Biological Laboratories, Aichi, Japan), and the relative ratios of the levels of NF-κB pathway proteins were calculated.

2.6. Statistical analysis

Results are presented as the mean ± standard error of the mean (S.E). Statistical analysis was performed using the Student *t* test. Statistical values of $P < 0.05$ were considered significantly different.

Table 1
Probe and primer sets (Taqman MGB, Applied Biosystems) used in real-time RT-PCR.

Gene name	Product number
iNOS (nitric oxide synthase 2, inducible)	Mm00440502_m1
IL-10 (interleukin 10)	Mm00439614_m1
Ptgs2 (prostaglandin-endoperoxide synthase 2) (COX-2)	Mm00478374_m1
TNF-α (tumor necrosis factor alpha)	Mm00443258_m1
IL-6 (interleukin 6)	Mm00446190_m1
IL-12 (interleukin 12 alpha)	Mm00434165_m1
NF-κB (Nuclear factor of kappa light polypeptide gene enhancer in B-cells)	Mm00476361_m1
Eukaryotic 18S rRNA (Internal control)	ABI433860F

Download English Version:

https://daneshyari.com/en/article/10755694

Download Persian Version:

https://daneshyari.com/article/10755694

Daneshyari.com