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Journal of Nutritional Biochemistry xx (2012) xxx–xxx

Journal of Nutritional Biochemistry

Lycopene suppresses proinflammatory response in lipopolysaccharide-stimulated macrophages by inhibiting ROS-induced trafficking of TLR4 to lipid raft-like domains☆

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Received 28 April 2012; received in revised form 12 August 2012; accepted 13 August 2012

Abstract

We recently showed that lycopene inhibited lipopolysaccharide (LPS)-induced productions of nitric oxide (NO) and interleukin-6 (IL-6) in murine RAW264.7 macrophages by mechanisms related to inhibition of ERK and nuclear factor-κB. Since the assembly of Toll-like receptor 4 (TLR4) in lipid rafts is a key element in LPS induced signaling, we investigated whether this process would be influenced by lycopene. We found that pretreatment of RAW264.7 cells with lycopene inhibited LPS-induced recruitment of TLR4 into fractions — enriched with lipid raft marker. By the methods of immunoprecipitation and immunoblotting, we also found that lycopene inhibited the subsequent formation of the complex of TLR4 with its adaptors including myeloid differentiation primary-response protein 88 and TIR domain–containing adaptor-inducing IFN-β. We also found that the lycopene induced inhibition was associated with reduced formation of reactive oxygen species (ROS), which was an upstream mechanism for the effects of lycopene, because treating the cells with the antioxidant *N*-acetyl-L-cysteine and NADPH oxidase inhibitor diphenyleneiodonium chloride significantly inhibited LPS-induced recruitment of TLR4 into lipid raft-like domains as well as the production of proinflammatory molecule NO and IL-6. Thus, our findings suggest that lycopene may prevent LPS-induced TLR4 assembly into lipid rafts through reducing intracellular ROS level.

Keywords: Lycopene; TLR4; ROS; LPS; RAW264.7 cells

1. Introduction

It is well known that chronic inflammation is one of the key etiological conditions for the development and progression of many chronic diseases including cancers and atherosclerosis [1,2]. Among many proinflammatory factors, lipopolysaccharide (LPS), the endotoxin derived from Gram-negative bacteria is an important one, which can activate macrophages, leading to production of nitric oxide (NO) and interleukin-6 (IL-6) [3]. The proinflammatory mediators trigger the multiple signal transduction pathways through Toll-like receptors (TLRs), a family of pattern-recognition receptors expressed in macrophages and other innate immune cells [4]. TLR4, a member of the TLRs family, possesses diverse functions in regulating immune inflammatory processes. TLR4 has been found in human atherosclerotic plaques as well as in murine models of atherosclerosis and may play important roles in development of atherosclerosis [5]. Upon stimulation, TLR4 is recruited to lipid rafts and subsequently interacts with its adaptor molecules, leading to activation of downstream targets, such as mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB), and production of proinflammatory cytokines in RAW264.7 cells [4,6]. This process occurs in an ROS-dependent manner because inhibition of NADPH oxidase suppresses TLR4 recruitment to lipid rafts [7]. Lipid rafts are membrane microdomains enriched in sphingolipids, cholesterol, and glycosylphosphatidylinositol-linked proteins. Recent studies with advanced microscope and spectroscope clearly confirmed that lipid rafts are physiological structures dynamically formed by assembling of specific lipids and proteins in a nanoscale, and they provide a platform to initiate multiple cellular responses to extracellular stimuli and affect intracellular trafficking [8]. Lipid rafts have been shown to be essential for TLR4-mediated signal transduction and target gene expression, and LPS-induced expression of inflammatory cytokines was significantly inhibited by lipid raft inhibitor methyl-β-cyclodextrin (MβCD) [9].

☆ Sources of Funding: This work was supported by the research grants from postdoctoral foundation of China (Grant No. 2011M501367), the Research Fund for the Doctoral Program of Higher Education of China (Grant No. 20110171120062) and by the National Natural Science Foundation of China (Grant No. 81130052).

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http://dx.doi.org/10.1016/j.jnutbio.2012.08.011

Lycopene is a fat soluble carotenoid found in red fruits including apricot, papaya, watermelon and tomatoes [10]. In the Western diets, lycopene is highly consumed, the total dietary intake of lycopene is approximately 2–5 mg/d, and the physiological concentration of lycopene amounts to 1–2 μmol/L [11,12]. Among various common carotenoids, lycopene is the most potent one with antioxidant properties [13]. After absorption in the intestine, the plasma concentrations of lycopene are negatively correlated with thiobarbituric acid reactive substrate. Epidemiological studies showed that dietary intakes of tomatoes and tomato-based products containing lycopene is associated with a decreased risk of atherosclerosis [14,15]. Cell culture studies also showed that lycopene has protective effect on endothelial cells and has antiproliferative effect on smooth muscle cells [16]. Apart from the antioxidant and antiatherosclerotic effects, lycopene also modulates redox sensitive molecular pathways involved in inflammation by activation of PPARγ signaling pathway and by inhibiting oxysterol-induced ROS production and NF-κB activation [17,18]. We have recently demonstrated that lycopene can prevent LPS-induced NO and IL-6 generation in murine RAW264.7 macrophages by mechanisms related to inhibition of MAPKs and NF-κB [19]. The present study was to examine whether lycopene may interfere the recruitment of TLR4 into lipid rafts by modulating intracellular ROS level and thus interrupts LPS-induced proinflammatory signaling.

2. Materials and methods

2.1. Materials

Lycopene, LPS (*Escherichia coli* 0111:B4), tetrahydrofuran (THF), MβCD, diphenyleneiodonium chloride (DPI), *N*-acetyl-L-cysteine (NAC) and the Griess reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Materials for cell culture were purchased from the Gibco (Grand Island, NY, USA). Antibodies for flotillin-1, myeloid differentiation primary-response protein 88(MyD88) and TIR domain–containing adaptor-inducing IFN-β (TRIF) were purchased from BD Biosciences (San Jose, CA, USA), eBioscience (San Diego, CA, USA) and Abcam (Cambridge, MA, USA), respectively. Protein A/G plus agarose and antibodies for TLR4 and NADPH oxidase-4(NOX-4) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). ELISA kit for IL-6 quantification was purchased from Biosource (Nivelles, Belgium).

2.2. Cell culture and treatment

RAW264.7 cells obtained from the American Type Culture Collection (Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in a 5% CO_2

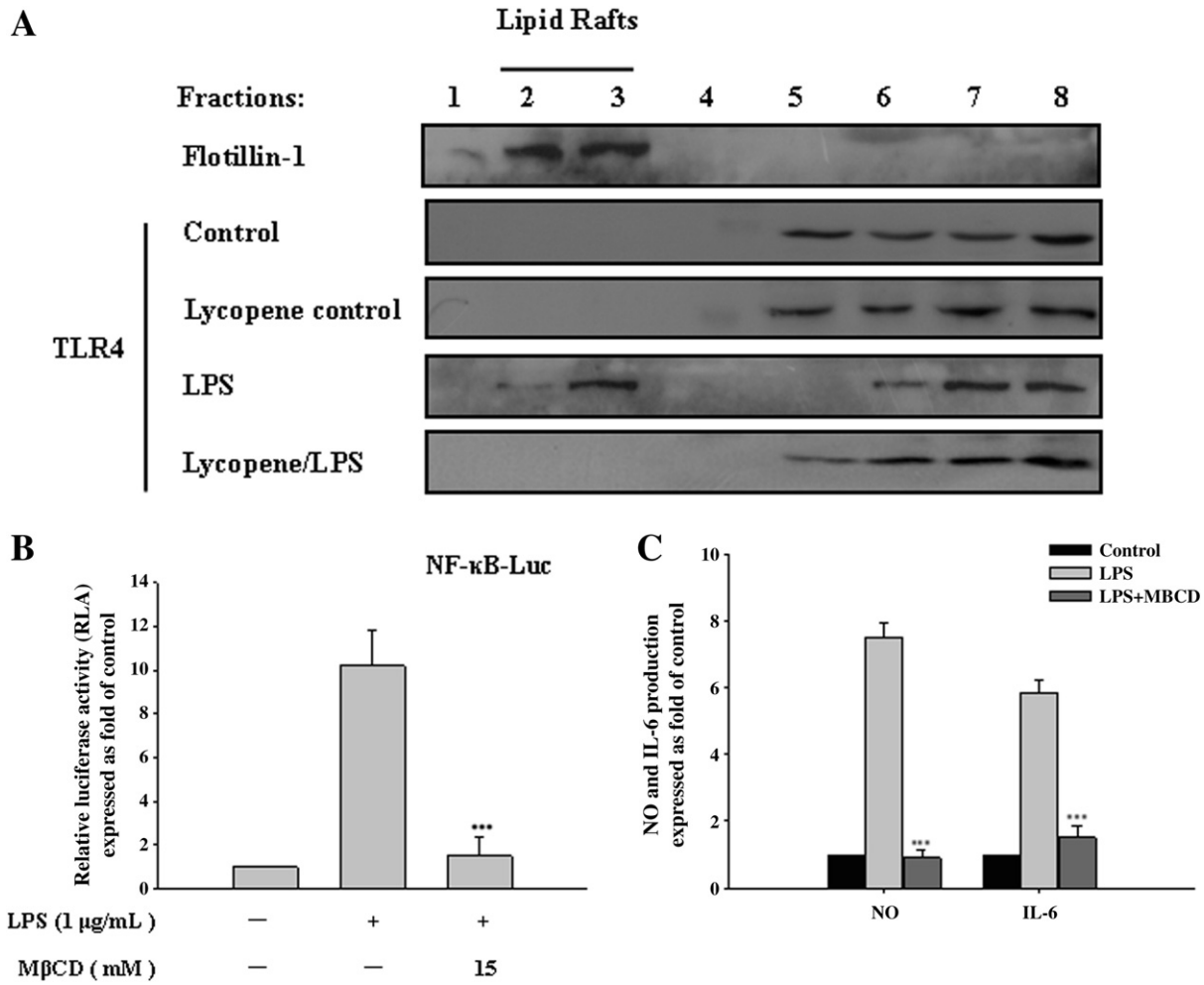

Fig. 1. Effects of lycopene on LPS-induced translocation of TLR4 receptor into lipid rafts in RAW264.7 cells. (A) RAW264.7 cells were pretreated with 10 μM lycopene for 1 h and stimulated with LPS (1 μg/mL) for 7 min. The cell lysates were fractionated by discontinuous sucrose density gradient ultracentrifugation, followed by fractionation to eight subfractions. The lipid rafts fraction and non-lipid rafts fraction were separated by immunoblotting for flotillin-1 and TLR4. The results are representative of three independent experiments. (B) For the analysis of NF-κB promoter activity, RAW264.7 cells transfected with NF-κB-luciferase construct were used. The cells were pretreated with 15 mM MβCD for 30 min, followed by stimulation with LPS for 6 h. The cells were lysed to determine the luciferase activity. The results were expressed as relative luciferase activity (RLA) against the value of the vehicle treatment. Data are the mean±S.E.M. of three independent experiments. $^{***}P<.001$ compared with LPS alone. (C) The cells were pretreated with 15 mM MβCD for 30 min and then stimulated by 1 μg/mL LPS for 24 h. The amounts of nitrite and IL-6 in the medium were measured as described in Materials and methods section. Data are the mean± S.E.M. of three independent experiments. $^{***}P<.001$ compared with LPS alone.