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TLR4 mediates high-fat diet induced physiological changes in mice via attenuating PPARγ/ABCG1 signaling pathway

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ABSTRACT

High-fat diet (HFD) is known to promote atherosclerosis which accelerates the development of atherosclerotic cardiovascular diseases. Vascular dysfunction characterized by inflammation and lipid accumulation is common in atherosclerosis caused by HFD. The specific effects of HFD on blood vessels and the underlying mechanisms need to be further clarified. Toll-like receptor 4 (TLR4) is a key contributing factor in atherosclerosis and TLR4 deficiency protects vascular smooth muscle cells against inflammatory responses and lipid accumulation *in vitro*. However, the physiological significance of TLR4 signaling in HFD-induced changes is unknown. In this study, we observed that HFD feeding increased body weight, circulating inflammatory cytokines and lipid accumulation in the aorta of wild-type mice but apart from increasing body weight, did not affect the TLR4 knockout mice. TLR4 expression increased significantly in the arterial walls after receiving HFD treatment, while that of the co-localizing PPARγ and ABCG1 markedly decreased. TLR4 deficiency reversed the HFD-induced attenuation of PPARγ and ABCG1. In conclusion, TLR4 mediates HFD induced increase in body weight, inflammation and aortic lipid accumulation through, at least partly, the PPARγ/ABCG1 signaling pathway. Therefore, interfering with TLR4 signaling is a viable therapeutic option in diet induced atherosclerosis.

1. Introduction

Atherosclerotic cardiovascular diseases (ASCVD), which include coronary heart disease, stroke and other atherosclerotic vascular diseases, are currently the leading cause of death around the world. The pathological basis of ASCVD is atherosclerosis, an inflammatory process that culminates with lipid accumulation in the artery walls [1]. Vascular dysfunction is a major complication of metabolic disorder, especially high-fat diet (HFD)-induced hyperlipidemia or obesity. HFD is known to induce atherosclerosis which accelerates the development of ASCVD [2,3]. However, the specific effects of HFD on blood vessels and the underlying mechanisms are yet to be elucidated.

Toll-like receptors (TLRs) play key roles in the innate and adaptive immune systems [4]. Increasing evidence suggests that TLRs, in particular TLR4, also contributes to the initiation and progression of atherosclerotic disease [5,6]. In previous studies from our research group showed that TLR4-mediated inflammation promotes foam cell formation in vascular smooth muscle cells (VSMCs), a crucial event in the development of atherosclerosis, by dysregulating cholesterol metabolism [7,8]. Furthermore, we also found that TLR4 deficiency protected VSMCs against inflammatory responses and lipid accumulation *in vitro* [9]. However, whether TLR4 signaling is the link between HFD-induced obesity, vascular inflammation, and lipid dysfunction *in vivo* is still unknown. In a recent study, specific knockdown of TLR4 in the hypothalamic arcuate nucleus reversed dysregulated glucose and lipid metabolism during the course of diet-induced obesity in rats [10]. Therefore, we hypothesized that TLR4 is also a pivotal mediator in

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HFD induced vascular dysfunction.

Cholesterol homeostasis is a key metabolic process associated with atherosclerosis. Cholesterol metabolism requires the activation of peroxisome proliferator-activated receptor gamma (PPARγ) and the upregulation of liver X receptor alpha (LXRα) [11], which in turn regulate ATP-binding cassette (ABC) transporter A/G1 (ABCA1/ABCG1) expression and cholesterol efflux [12]. PPARγ, as a fatty acid sensor, plays a critical role in protecting cerebral vessels against endothelial dysfunction in response to HFD [13]. We demonstrated in a previous study that PPARγ expression was impaired in HFD fed mice, suggesting the involvement of PPARγ in cholesterol metabolism [7].

Several studies have indicated a crosstalk between PPARγ and TLR4-mediated inflammation. Lipopolysaccharide (LPS), a bacterial toxin which activates TLR4, reduces PPARγ expression and function and activates NF-κB signaling in macrophages [14]. PPARγ inhibits VSMC phenotypic transformation and foam cell formation by suppressing TLR4-mediated inflammation [7,15]. These findings suggest that PPARγ may also participate in TLR4-mediated vascular dysfunction induced by HFD. There are reports that LPS downregulates the expression of ABCG1 in macrophages [16], and we were able to show that TLR4 downregulated ABCG1 expression through PPARγ/LXRα signaling in VSMCs *in vitro* [9]. The present study was undertaken to determine whether TLR4 mediates HFD induced vascular dysfunction, especially vascular inflammation and lipid accumulation, to ascertain whether the effects of TLR4 are facilitated by the PPARγ/ABCG1 signaling pathway.

2. Materials and methods

2.1. Animal treatments

Male C57BL/6J wild-type (WT) and C57BL/10ScNJ TLR4 knockout (KO) mice weighing 15−25 g at 6−8 weeks of age were used in the present study. All mice were purchased from the Jackson Laboratory. Both WT and TLR4 KO mice were randomly divided into two groups (n = 8 mice per group) and fed with the high-fat diet (HFD, protein 18.1%, fat 61.6%, carbohydrates 20.3%, D12492, Research Diets, USA) and the normal diet (ND, protein 18.3%, fat 10.2%, carbohydrates 71.5%, D12450B, Research Diets, USA) for 12 weeks, respectively. Body weight (g) was measured every week. After 12 weeks, these animals were sacrificed for subsequent experiments. Animal procedures were approved by the Experimental Animal Ethics Committee of the Chengdu Military General Hospital and the Third Military Medical University prior to performing this study, and conformed to the Guide for the Care and Use of Laboratory Animals (NIH, 2011).

2.2. ELISA analysis

WT and TLR4 KO mice were fed with the HFD or ND for 12 weeks. Then, plasma was harvested for measuring the concentrations of cytokines including tumor necrosis factor (TNF) α, interleukin (IL)-6, IL-1β and monocyte chemoattractant protein (MCP)-1 by using Enzyme-linked Immunosorbent Assay (ELISA) Kits (BioSource, Camarillo, CA). All assays were performed in accordance with the manufacturer's instructions.

2.3. BODIPY staining

10 μm-thick consecutive frozen sections were cut from the fixed mice aortas, mounted on glass slides, and oven-dried at 60 °C for 2 h (h). Then, the aortic sections were washed 3 times in phosphate buffered saline (PBS), permeabilized with 0.3% Triton X-100 for 5 min (min) and stained with BODIPY 493/503 dye (1 μg/mL, Molecular Probes, Eugene, OR, USA) for 30 min at room temperature to detect lipid accumulation. In the meantime, a negative control was performed using PBS instead of BODIPY 493/503 dye. Lipid content was quantified by fluorescence intensity using confocal microscope (ZEISS LSM 780, GER) as we performed previously [9].

2.4. Immunofluorescence

The thoracic aortas were dissected out of the mice under anesthesia by urethane and then fixed with 4% paraformaldehyde. Frozen serial sections of the aortas were washed 3 times in PBS and then permeabilized with 0.3% Triton X-100 for 30min. Nonspecific proteins were blocked with normal goat serum (working solution) for 1 h. After blocking, the sections were incubated with primary antibodies (mouse anti-mouse TLR4, 1:50, Abcam, CA, USA; rabbit anti-mouse PPARγ, 1:50, Abcam, CA, USA; rabbit anti-mouse ABCG1, 1:50, Thermo Fisher Scientific, Waltham, MA, USA) at 4 °C for 24 h, followed by incubation with fluorescently conjugated secondary antibodies (Alexa Fluor® 555 donkey anti-mouse and Alexa Fluor® 647 goat anti-rabbit, 1:200, Life Technology, Carlsbad, CA, USA) at room temperature for 1 h. Tissue sections were counterstained with 4, 6-diamidino-2-phenylindole (DAPI, Life Technology, Carlsbad, CA, USA) to label the cell nuclei for 5 min at room temperature. The specimens were analyzed using a confocal microscope (ZEISS LSM 700, GER).

2.5. Statistical analysis

Data are presented as the mean ± SEM, and each experiment was performed a minimum of 3 times. Two-group comparisons were performed using *t*-test for independent samples. Comparisons of the data between multiple groups were performed using one-way ANOVA followed by Tukey's multiple comparison procedure. Statistics were calculated with the GraphPad Prism 5 software package (La Jolla, CA, USA). Significant differences were established at $P < 0.05$.

3. Results

3.1. HFD increased body weight, circulating inflammatory cytokines and lipid accumulation in the aortic root of mice

WT mice were fed for 12 weeks with either ND (fat content 10.2%) or HFD (fat content 61.6%). As shown in Fig. 1A, body weight of mice increased over time, being significantly higher in the HFD group compared to the ND group from the 2 nd week. After 12 weeks, the average body weight of HFD fed mice was approximately 46.62g and that of ND fed mice was approximately 28.33g (Fig. 1B). These data, consistent with previous results in the latest study [17], shown that HFD contributed to the increase in body weight of mice. After 12 weeks, the animals were sacrificed for subsequent experiments. Plasma levels of cytokines including TNFα, IL-6, IL-1β and MCP-1 were measured using ELISA. HFD feeding significantly increased the circulating levels of pro-inflammatory cytokines. TNFα, IL-6, IL-1β and MCP-1 levels in the HFD group were 2.78, 3.31, 2.60 and 1.93 times the respective levels in the ND group (Fig. 1C). Lipid accumulation in the aortic roots was detected though BODIPY 493/503 staining of lipid droplets previously described [8]. Vascular lipids were detected in the aortic roots of all mice, and the lipid content, as quantified by fluorescence intensity, was approximately 1.88 times in the HFD group compared to the ND group (Fig. 1D−F). Taken together, the data demonstrated that HFD feeding increased body weight, circulating inflammatory cytokines and lipid accumulation in the aortic root of mice.

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