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Peroxidized unsaturated fatty acids stimulate Toll-like receptor 4 signaling in endothelial cells

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ABSTRACT

Aim: Although unsaturated fatty acids are assumed to be protective against inflammatory disorders that include a pathway involving Toll-like receptor 4 (TLR4) activation, they might actually be toxic because of their high susceptibility to lipid peroxidation. Here we studied the effects of peroxidized unsaturated fatty acids on the TLR4–nuclear factor (NF)-κB pathway in endothelial cells.
Main methods: Confluent cultured endothelial cells from bovine aorta were incubated for 1 h with fatty acids integrated into phosphatidylcholine vesicles. Lipopolysaccharide (LPS) or phosphatidylcholine vesicles without fatty acids were also applied as a positive control or a control for fatty acid groups, respectively. Activation of TLR4 and downstream signaling was assessed by membrane fractionation and Western blotting or immunofluorescent staining.
Key findings: In the same way as LPS, application of sufficiently peroxidized unsaturated fatty acids like oleic acid or docosahexaenoic acid, acutely caused TLR4 translocation to caveolae/raft membranes, leading to activation of NF-κB signaling in endothelial cells. In contrast, saturated fatty acids did not show such effects. Applying well-peroxidized unsaturated fatty acids, but not saturated fatty acids, acutely activates the TLR4/NF-κB pathway.
Significance: Peroxidation of unsaturated fatty acid is essential for the acute activation of TLR4 by the fatty acids that follow the same pathway as the activation by LPS. Unsaturated fatty acids have been assumed to be protective against inflammatory disorders, and drugs containing unsaturated fatty acids are now developed and provided. Our result suggests that, for inflammatory disorders involving TLR4 signaling, using unsaturated fatty acids as anti-inflammatory drugs may cause contrary effects.

Introduction

Since 1970, when studies were conducted in an Eskimo population (Bang et al., 1971; Kromann and Green, 1980), possible benefits of intake of polyunsaturated fatty acids (PUFAs) for the prevention of atherosclerotic cardiovascular diseases have been a focus of research. In fact, a series of randomized clinical trials did show that PUFA intake reduces cardiovascular event risk, albeit modestly (Burr et al., 1989; Marchioli et al., 2002; Yokoyama et al., 2007). Saturated fatty acids (SFAs), on the other hand, may have pro-atherogenic and pro-diabetic effects through activation of Toll-like receptor 4 (TLR4) signaling which is a crucial pathway in innate immunity, to bacterial infection, but which also may promote progression of atherosclerosis and insulin resistance by causing chronic inflammation through the activation of the transcriptional factors such as NF-κB resulting in secretion of pro-inflammatory cytokines and chemokines (Holland et al., 2011; Kim et al., 2007; Shi et al., 2006; Suganami et al., 2007). In contrast, PUFAs reportedly suppress such TLR4-mediated inflammatory reactions (Lee et al., 2001, 2003). However, it is noteworthy that PUFAs may also have toxic effects, as they are highly susceptible to lipid peroxidation, which is implicated in the pathogenesis of various diseases (Guillen and Goicoechea, 2008; Serini et al., 2011). And indeed, there is reported experimental and clinical evidence that suggests an association of lipid peroxides from PUFAs with atherosclerosis (Esterbauer et al., 1992; Glavind et al., 1952; Jira et al., 1998; Stringer et al., 1989). Thus, the aim of the present study was to determine whether peroxidized unsaturated fatty acids stimulate TLR4 signaling.

Materials and methods

Cell culture

Fetal bovine aortic endothelial cells (ECs) were purchased from Japanese Collection of Research Bioresources (JCRB, Osaka, Japan) or were isolated from bovine fetuses, as previously described (Mutoh et al., 2008). ECs were cultured in Medium 199 (GIBCO,

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Life technologies Japan Ltd., Tokyo, Japan) supplemented with 20% complement-depleted fetal bovine serum (FBS) (Nichirei Biosciences Inc. Tokyo, Japan) in a humidified CO_2 incubator at 37 °C, were passaged before full confluence, and were used before passage 15 for all experiments. Normal cell functions (eNOS phosphorylation and nitric oxide production) of cultured ECs isolated from bovine fetuses were confirmed at passage 20, and therefore it was decided that it was reasonable to use ECs up to passage 15 in the current study.

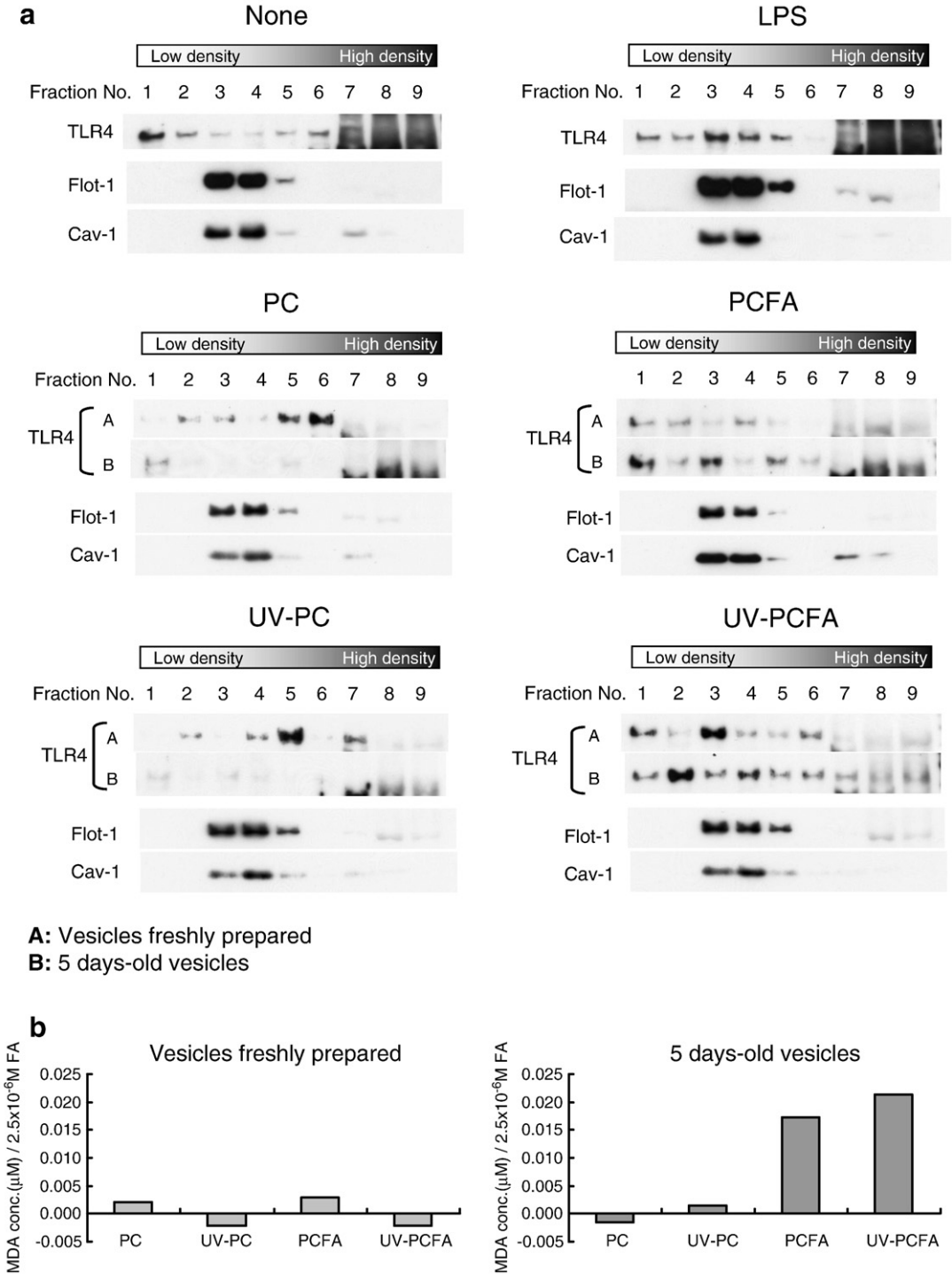

Fig. 1. Effect of fatty acid peroxidation on TLR4/NF-κB signaling in endothelial cells. Experiments presented in a, b and c were performed simultaneously, twice (using the same vesicle preparation sets divided into halves), immediately following preparation and after aging, 5 days later. ECs were incubated with no stimulation (None), 10 ng/ml LPS, PC, PCFA (2.5×10^{-6} M as fatty acids), or UV-irradiated PC or PCFA (UV-PC, UV-PCFA), respectively for 1 h. (a) Distribution of TLR4 in plasma membranes determined by sucrose ultracentrifugation. The fractions are along the lines of sucrose gradient that start from lowest density (fraction No. 1; 5% sucrose) to highest density (No. 9; 42.5% sucrose). Caveolae/raft membranes, detected by Flot-1 and Cav-1, found in fractions 3 and 4. TLR4 blots showed ECs incubated with freshly prepared (A) or 5-day-old (B) vesicle samples. (b) MDA concentration in vesicles used in a and c analyzed by the TBARS method. (c) Expression of IκB-α in EC whole cell lysates by Western blotting in duplicate. The same vesicles were used in both panels, with the difference being the freshness (indicating the level of peroxidation of fatty acids), as showed in a and b.