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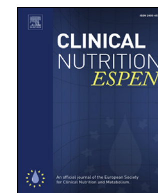

Original article

Oral tributyrin prevents endotoxin-induced lipid metabolism disorder

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S U M M A R Y

Background & aims: Sepsis leads to dysregulation of lipid and lipoprotein metabolism. Butyrate increases peroxisome proliferator-activated receptors (PPARs), which are key nuclear hormone receptors to induce fatty acid oxidation and synthesis. Oral administration of tributyrin, a prodrug of butyrate contained in dairy products, suppresses lipopolysaccharide (LPS)-induced liver injury through attenuating nuclear factor-κB activity with an increased hepatoportal butyrate level. In this study, we elucidated the protective effect of oral administration of tributyrin against LPS-mediated lipid metabolism disorder in rats.
Methods: Male Wistar rats were randomly divided and were administered tributyrin or vehicle orally 1 h before LPS injection and then sacrificed at 0, 1.5, 6, and 24 h after LPS. Liver tissue expressions of nuclear hormone receptors, enzymes associated with fatty acid metabolism, and histone acetylation were analyzed by real-time polymerase chain reaction or western blotting. Plasma lipids levels were measured.
Results: Tributyrin enhanced expression of PPARs and histone H3 in the liver at basal levels. Tributyrin suppressed LPS-induced repression of PPARs fatty acid oxidation-associated enzymes: fatty acid transport protein and fatty acid binding protein, and fatty acid synthesis-associated enzyme: sterol regulatory element binding protein-1c. Tributyrin reduced the increase in plasma triglyceride, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels at 24 h after LPS injection.
Conclusions: Oral tributyrin administration prevented elevation of plasma triglyceride, TC, and LDL-C levels through improved fatty acid oxidation in endotoxemic rats.
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1. Introduction

Sepsis is a complex acute syndrome with both inflammatory and metabolic complications [1]. The acute-phase responses in sepsis induce dyslipidemia with increased plasma triglyceride (TG) and decreased high-density lipoprotein cholesterol (HDL-C) levels [2]. The effects of infection on TG metabolism are common in all species and lipopolysaccharide (LPS) similarly induces hypertriglyceridemia in rodents [3,4]. Failure of fatty acid oxidation is a key risk factor for dyslipidemia associated with sepsis [1,5,6].

The nuclear hormone receptors, especially peroxisome proliferator-activated receptors (PPARs) and liver X receptor (LXR)-α, play important roles in lipid metabolism during infection and inflammation, and these liposensors heterodimerize with retinoid X receptors (RXRs) for efficient gene regulation [2]. In the liver, PPARs mediate for a number of enzymes that are associated both with fatty acid oxidation and with fatty acid synthesis [2,7]. LXR-α, a target gene of PPAR-γ, promotes lipid biosynthesis by inducing expression of lipogenic enzymes [7].

Butyrate, the end product of anaerobic bacterial fermentation of carbohydrates in the colon, plays important roles in the biology of colonocytes and effects on different cells except colonocytes. Our previous *in vitro* acute inflammatory studies indicated that butyrate suppresses tumor necrosis factor (TNF)-α production from peripheral blood mononuclear cells, induces neutrophil apoptosis, and attenuates inflammation and lipolysis generated by the

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interaction of adipocytes and macrophages [8–10]. Butyrate blocks histone deacetylase 3, which is associated with PPARs and nuclear factor (NF)-κB activation [11–15]. Several *in vitro* studies have shown that butyrate upregulates the expression of PPARs in various cell lines, with increased PPAR-γ gene expression in intestinal cells and increased PPAR-α gene expression via histone acetylation in breast cancer cell lines [16,17].

Oral administration of tributyrin, a prodrug of butyrate which is present in milk fat, suppresses liver injury and plasma TNF-α level through attenuated hepatic expression of TNF-α mRNA via inhibition of NF-κB activation in endotoxemic rats [18]. A recent report indicated that repeated long-term administration of tributyrin alleviated hypertriglyceridemia and hypercholesterolemia induced by a high-fat diet in mice [19]. However, the effect of oral administration of tributyrin on lipid metabolism in acute inflammation, such as endotoxemia, has not been elucidated.

Based on these studies, we hypothesized that tributyrin administration improves lipid metabolism abnormality in endotoxemia. To test our hypothesis, we investigated whether oral administration of tributyrin alleviates lipid metabolism abnormality and upregulates expression of nuclear hormone receptors, fatty acid oxidation enzymes, and fatty acid synthesis enzymes in the liver during rat endotoxemia.

2. Materials and methods

2.1. Experimental design

Seventy-six male Wistar rats (CLEA Japan, Tokyo, Japan), aged 7–8 weeks, were kept at 22 °C under a 12-h light–dark cycle with food and water *ad libitum* and were randomly divided into 2 groups, tributyrin and vehicle. Preparation of tributyrin solution and the doses of administration of tributyrin and LPS were as in our previous study [18]. Rats were provided with water only for 12 h until they were sacrificed. Rats were administrated 1 g/kg tributyrin (Sigma–Aldrich, St Louis, MO, USA) orally, and after 1 h, were injected with 10 mg/kg LPS (*Escherichia coli* O111:B4; Sigma–Aldrich) intraperitoneally. Lipid emulsion and saline were used as the vehicles for tributyrin and LPS, respectively. Lipid emulsion (Intralipos® 10%; Otsuka Pharmaceutical Factory, Tokushima, Japan) includes 10% purified soybean oil, 1.2% yolk lecithin, and 2.2% glycerol. Rats were sacrificed at 0, 1.5, 6, and 24 h after LPS injection under diethylether anesthesia ($n = 8$ at 0 h and $n = 10$ at 1.5, 6, 24 h). All animal experiments were performed in accordance with the Guidelines for Animal Experimentation at Kobe University.

2.2. Biochemical analysis

Blood samples were collected in heparin-coated tubes from the inferior vena cava at 24 h after LPS injection. The survival rate at 24 h was 87.5% in the vehicle group and 88.2% in the tributyrin group in our previous study [18]. The laboratory data for plasma levels of TG, free fatty acid (FFA), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, and glucose were determined using an outside clinical laboratory testing facility (SRL. Inc., Tokyo, Japan).

2.3. Total RNA extraction, real-time polymerase chain reaction (PCR)

Liver tissues were harvested at 0, 1.5, and 6 h and were stored at −80 °C until used. Total RNA was extracted from the rat livers by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and 1 μg of total RNA extracted was reverse transcribed to yield single-stranded cDNA by using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). Real-time quantitative PCR analysis (SYBR Green Real time Master Mix; Toyobo, Osaka, Japan) was carried out with MyiQ (Bio-Rad). The expression of nuclear hormone receptors (PPARs, LXR-α, and RXR-α), fatty acid oxidation enzymes (fatty acid transport protein: FATP, fatty acid binding protein: FABP, carnitine palmitoyltransferase: CPT), and fatty acid synthesis enzymes (sterol regulatory element binding protein −1: SREBP-1, acetyl-CoA carboxylase: ACC, fatty acid synthase: FAS) were detected. The real-time PCR conditions and primer sequences are listed in Table 1. Relative expression of genes was calculated using the ΔΔCt method after normalization to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) level and expressed as a ratio based on the vehicle group at 0 h.

2.4. Western blot analysis

The liver tissues were homogenized in PRO-PREP (iNtRON Biotechnology Inc., Gyeonggi-do, Korea). Extracted proteins (20 μg), using pooled samples due to insufficient volume for measurement in each rat, were separated by 10% acrylamide gels and transferred to polyvinylidene difluoride membranes (GE Healthcare, Chalfont St Giles, UK). After blocking with Tris-buffered saline-Tween 20 containing 20 g/L skimmed milk, the membranes were probed with a polyclonal rabbit anti-PPAR-α antibody (diluted 1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), polyclonal rabbit anti-PPAR-γ antibody (diluted 1:500, Santa Cruz Biotechnology), polyclonal rabbit anti-acetyl-histone H3 antibody (diluted 1:1000, Cell Signaling Technology, Danvers, MA, USA), or β-actin (diluted 1:1000, Sigma–Aldrich), followed by a horseradish-peroxidase-conjugated secondary antibody when appropriate. Blots were subsequently developed using the ECL-plus Western Blotting Detection System (GE Healthcare) and exposed to Hyperfilm (GE Healthcare).

Table 1
Primer sequences used for real-time PCR.

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)	Annealing temperature (°C)
Peroxisome proliferator-activated receptor-α	ACTATGGAGTCCACGCATGTGA	TTGTCGTACGCCAGCTTTAGC	59
Peroxisome proliferator-activated receptor-γ	TCAACCCTTTACCACGGTT	CAGGCTCTACTTTGATCGCA	59
Liver X receptor-α	TCAGCATCTTCTCTGCAGACCGG	TCATTAGCATCCGTGGGAACA	61
Retinoid X receptor-α	CCTGCCGTGACAACAAGGA	CACTTCTGGTATCGGCAGTACTG	59
Fatty acid transport protein	TCAAGGTGTGCTCAACAGCC	AGGATAAAACACACCAACTGT	59
Fatty acid binding protein	CAAGTCGGTCGTGACACTGG	CCTGCCCGTCCCACTTC	59
Carnitine palmitoyltransferase-1a	TCTTGCAGTCGACTCACCTT	TCCACAGGACACATAGTCAGG	59
Sterol regulatory element binding protein-1c	GCAACACTGGCAGAGATCTACGT	TGGCGGGCACTACTTAGGAA	65
Acetyl-CoA carboxylase	AGGAAGATGGTGTCCCGCTCTG	GGGGAGATGTGCTGGGTCAT	65
Fatty acid synthase	ATTGGGTGCCGATTACAACC	GCCCTCCCGTACACTCACTC	61
Glyceraldehyde-3-phosphate dehydrogenase	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA	59

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