Kobe J. Med. Sci., Vol. 66, No. 4, pp. E129-E138, 2020

Effect of Oral Tributyrin Treatment on Lipid Mediator Profiles in Endotoxin-Induced Hepatic Injury

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Received 26 June 2020/ Accepted 21 October 2020

Keywords: lipid mediator; lipidomics; sepsis; lipopolysaccharide

Eicosanoid modulation by butyrate has been reported in various cells and conditions. Recently, comprehensive analyses of lipid mediators using liquid chromatography/tandem mass spectrometry has been reported. We hypothesized that tributyrin, a prodrug of butyrate, may attenuate LPS-induced liver injury in rats by suppressing the production of pro-inflammatory lipid mediators and/or by inducing anti-inflammatory specialized proresolving mediators. To test this, groups of Wistar rats were orally administered tributyrin (1 g/kg body weight) or vehicle 1 h before intraperitoneal injection of LPS. The livers were collected at 0, 1.5, 6, and 24 h later and analyzed: lipid mediators were profiled by liquid chromatography/tandem mass spectrometry; expression of cyclooxygenase-2, 5-lipoxygenase (LOX), 12/15-LOX, and leukotriene (LT) A_4 hydrolase, and nuclear translocation of 5-LOX were evaluated by western blot analysis; and induction of liver injury was assessed by immunostaining for 8-hydroxy-2′-deoxyguanosine, an indicator of oxidative DNA damage. We found that tributyrin treatment attenuated LPS-induced production of pro-inflammatory LTB_4 ($p < 0.05$) and decreased oxidative stress levels in the liver. Tributyrin also attenuated the nuclear translocation of 5-LOX in response to LPS, suggesting a possible mechanism for the LTB_4 reduction. LPS-induced changes in other lipid mediators were not significantly affected by tributyrin treatment up to 24 h after LPS injection. Our results suggest that oral tributyrin administration protects against endotoxemia-associated liver damage by reducing production of the pro-inflammatory eicosanoid LTB_4.

INTRODUCTION

Butyrate is a short-chain fatty acid produced by anaerobic fermentation of dietary fiber by colonic bacteria that has direct effects on colonic epithelial function and mucosal immunity. Recent studies indicate that butyrate also has multiple systemic effects on a variety of cell types, including inhibition of cell cycle progression, induction of programmed cell death and cellular differentiation, and modulation of immunity (1, 9, 19, 30). The immunomodulatory effects of butyrate on various immune cell populations have recently been reviewed (6).

At the cellular level, butyrate has been reported to have effects on the production of a number of eicosanoids, a class of molecules derived from polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA). For example, butyrate increases secretion of prostaglandin (PG) E_2, leukotriene (LT) B_4, 15d-PGJ_2, and thromboxane (Tx) B_2 by human monocytes exposed to bacterial lipopolysaccharide (LPS), and additionally increases PGE_2 secretion in co-cultures of adipocytes and macrophages (7, 10, 22). We previously showed that butyrate inhibits production of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) by increasing PGE_2 secretion and suppressing activation of the transcription factor nuclear factor-kappa B (NF-κB) in LPS-treated human peripheral mononuclear cells (28). In addition, butyrate increases transcription of the eicosanoid metabolic enzymes 5-lipoxygenase (LOX), 12-LOX, and 15-LOX and increases tight junction permeability in monolayers of the colorectal epithelial cell line Caco-2 (21).

Lipid mediator levels in cells and tissues can be comprehensively profiled using liquid chromatography/tandem mass spectrometry (LC-MS/MS) (2). This approach enables quantitation of the entire lipidome under physiological and pathological conditions, and it has been successfully used to analyze changes in lipid mediators in experimental and clinical sepsis (8, 26). LC-MS/MS can identify multiple classes of lipid mediators, including the so-called specialized proresolving mediators (SPM) that serve to counterbalance the activity of pro-inflammatory eicosanoids and thus promote the timely resolution of inflammation. SPMs include eicosapentaenoic acid (EPA)

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derived E-series resolvins (Rv), docosahexaenoic acid (DHA)-derived D-series Rvs, AA-derived lipoxins (LXs), and DHA-derived maresins and protectins (2). In the past, the effects of butyrate on lipid mediators has most often been analyzed using isolated cells rather than whole tissues (10, 16) and, as far as we are aware, there have been no studies examining the effect of butyrate on SPM production.

In the present study, we hypothesized that tributyrin may affect the production of pro-inflammatory lipid mediators and/or SPM in rats with LPS-induced liver injury, a commonly used animal model of sepsis. Up to 80% of intraperitoneally injected LPS accumulates in the liver; therefore, we focused our analysis on the effects of orally administered tributyrin on LPS-induced changes in the liver lipidome for up to 24 h after LPS injection. In our series of experiments, oral administration of tributyrin, a prodrug of butyrate present in milk fat, increases the plasma butyrate level in the portal vein up to 2.4 mM. It also suppresses acute severe liver injury induced by LPS and decreasese plasma TNF-α level through inhibition of hepatic NF-κB activation by enhanced histone H3 acetylation as an effect of the histone deacetylase (HDAC) inhibitor action of butyrate (17, 18).

MATERIALS AND METHODS

Animals

Male Wistar rats (CLEA Japan, Tokyo, Japan), aged 8 weeks and weighing 140–160 g, were used in all experiments. Rats were kept at 22°C on a 12-h light/dark cycle and were provided with food and water *ad libitum.* Food was withdrawn from the day before the experiment until sacrifice, but water was allowed *ad libitum*. Rats were divided into two groups and orally administered either tributyrin (1 g/kg; Sigma-Aldrich, St Louis, MO, USA) or vehicle (Intralipos® 10% lipid emulsion; Otsuka Pharmaceutical Factory, Tokushima, Japan). One hour later, the vehicle- or tributyrin-treated rats were injected intraperitoneally with 10 mg/kg body weight of LPS (*Escherichia coli* O111:B4; Sigma-Aldrich) as previously reported (18). At 0, 1.5, 6, and 24 h after LPS injection, rats were euthanized with domitor 1 mg/mL (Nippon Zenyaku Kogyo, Fukushima, Japan), dormicum 5 mg/mL (Astellas Pharma, Tokyo, Japan) and vetorphale 5 mg/mL (Meiji Seika Pharma, Tokyo, Japan), and the livers were collected and stored at −80°C until analyzed. To clarify the effect of tributyrin administration itself on hepatic lipid mediators, the liver was also collected 25 h after tributyrin administration, which corresponded to 24 h after LPS injection. This study was approved by the Institutional Animal Care and Use Committee and performed according to the Kobe University Animal Experimentation Regulations (P101208).

Immunostaining analysis

Oxidative DNA damage was evaluated by immunohistochemical staining of liver sections with a monoclonal antibody to 8-hydroxy-2′-deoxyguanosine (8-OHdG) (N45.1, 10 mg/mL; Japan Institute for the Control of Aging, Shizuoka, Japan). Briefly, livers were deparaffinized and antigen retrieval was achieved by incubation in citrate buffer solution (pH 7.0) at high heat and pressure for 10 min. The sections were blocked with 5% bovine serum albumin for 5 min and then incubated with anti-8-OHdG antibody (diluted 1:2000) overnight at room temperature in a humidified chamber. The sections were washed, incubated with a secondary antibody for 1 h, and then incubated with the colorimetric substrate 3, 3′-diaminobenzidine (Dako, Tokyo, Japan) for 3 min at room temperature. Sections were counterstained with hematoxylin and visualized using a bright-field microscope. Cells in five randomly chosen areas per slide were counted. The results are expressed as the mean percentage of positively stained cells/total cells.

Lipid mediator metabololipidomics

LC-MS/MS was performed as previously described (5). Deuterium-labeled internal standards were added to facilitate quantification of sample recovery. All samples were extracted using C18 solid phase extraction columns before LC-MS/MS. To monitor and quantify the levels of targeted lipid mediators, a multiple reaction monitoring method (MRM) was devised with signature ion fragments for each molecule. Quantification was carried out based on the peak area of the MRM transition and linear calibration curves were obtained with an authentic standard for each compound.

RNA extraction, complementary DNA synthesis, and reverse-transcription polymerase chain reaction assay

RNA was extracted from frozen liver tissues by homogenization at 4°C in TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA concentration and purity were determined by absorbance at 260 and 280 nm, and 1 μg total RNA per sample was reverse transcribed using an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Quantitative PCR was performed in duplicate as previously reported (20) using SYBR Green Real-Time Master Mix (Toyobo, Osaka, Japan). Primer sequences were: rat cyclooxygenase-2 (COX-2*)* forward primer 5′-CTCTGCGATGCTCTTCCGAG-3′ and reverse primer 5′-

AAGGATTTGCTGCATGGCTG-3′; rat 5-LOX forward primer 5′-TGGCATCTAGGTGCAGTGTG-3′ and reverse primer 5′-CCTCCAGGTTCTTGCGGAAT-3′; rat 12/15-LOX forward primer 5′-GATGGGTGTCTACCGCATCC-3′ and reverse primer 5′-CCTCTCCATGCTGTCCAACC-3′; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer 5′-GGCACAGTCAAGGCTGAGAATG-3′ and reverse primer 5′-ATGGTGGTGAAGACGCCAGTA-3′. Reactions were analyzed using a MyiQ Real-Time PCR system (Bio-Rad). Relative gene expression was calculated using the ΔΔCt method after normalization to GAPDH mRNA. Results are expressed as the fold-difference compared with the vehicle group at 0 h.

Western blot analysis

Liver tissue samples were homogenized in PRO-PREP solution (iNtRON Biotechnology, Seoul, Korea) or were fractionated into cytoplasmic, nuclear, and membrane fractions using a CNM Compartmental Protein Extraction Kit (BioChain Institute, Newark, CA, USA). Extracted proteins (20 μg per sample) were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes (GE Healthcare, Chalfont St Giles, UK). The membranes were blocked with Tris-buffered saline/Tween 20 containing 50 mg/mL skimmed milk, and then incubated with the following primary antibodies: rabbit polyclonal anti-COX-2 (diluted 1:1000; Abcam, Cambridge, UK), rabbit polyclonal anti-5-LOX (1:500; Abcam), rabbit polyclonal anti-ALOX15 (1:1000; Origene Technologies, Rockville, MD, USA), rabbit monoclonal anti-LTA_4 hydrolase (LTA_4H) (1:70,000; Abcam), mouse monoclonal anti-β-actin (1:50,000, Sigma-Aldrich), mouse monoclonal anti-lamin A/C (1:2000, Cell Signaling Technology, Danvers, MA, USA), or rabbit polyclonal anti-Na, K-ATPase (1:800, Cell Signaling Technology) for overnight at 4°C. After washing, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody as appropriate. Blots were developed using the Luminata Forte Western HRP substrate (Millipore, Darmstadt, Germany).

Statistical analyses

Statistical analyses were performed using Statcel2 software (OMS Publishing, Saitama, Japan). Differences between values at 0 and 25 h after tributyrin administration were assessed using a t-test. For all other experiments, two-factor analysis of variance was performed. The results are expressed as the mean ± standard error (SEM). A p value <0.05 was considered statistically significant.

RESULTS

Oxidative stress in the liver

To assess the effect of LPS on liver function, we first examined evidence of oxidative stress. Groups of rats were orally administered vehicle or tributyrin for 1 h, injected 1 h later with LPS (referred to 0 h), and sacrificed at 0, 6, and 24 h for liver sectioning and immunostaining for 8-OHdG, a marker of oxidative DNA damage. As shown in Figure 1, tributyrin treatment alone for 1 h had no significant effect on the percentage of liver cells positively stained for 8-OHdG (mean ± SEM 15.9 ± 6.5% and 27.0 ± 10.8% in the vehicle and tributyrin groups, respectively). Injection of LPS alone induced a significant increase in the proportion of 8-OHdG-positive liver cells at both 6 h and 24 h ($p < 0.01$–0.05, Figure 1). However, treatment with tributyrin significantly reduced the LPS-induced increase in 8-OHdG-positive cells from 95.0 ± 2.2% to 40.9 ± 2.7% ($p < 0.01$) at 6 h and from 86.2 ± 4.4% to 62.6 ± 4.5% ($p < 0.05$) at 24 h (Figure 1). Thus, tributyrin treatment reduces LPS-induced oxidative stress in the liver.

Changes in lipid mediator production

Next, we analyzed LPS and tributyrin effects on lipid mediator levels in the liver by LC-MS/MS. LPS injection significantly increased the levels of LTB_4 and LXA_4, which are produced by 5-LOX-catalyzed metabolism of AA (Figure 2A, $p < 0.01$–0.05). Tributyrin administration significantly attenuated the LPS-induced increase of LTB_4 at 24 h ($p < 0.05$), but had no effect on LXA_4 levels (Figure 2). Strikingly, other 5-LOX-derived mediators, including LXB_4, 5-hydroxyeicosatetraenoic acid (HETE), and 15-HETE, were unaffected by either LPS or tributyrin.

Analysis of EPA- and DHA-derived mediators and intermediates indicated that LPS significantly decreased the levels of EPA-derived RvE3 and 5-hydroxyeicosapentaenoic acid (5-HEPE) in the liver (Figure 2B, $p < 0.01$–0.05), but it had no effect on DHA-derived eicosanoids or intermediates (Figure 2C). Other SPMs, such as EPA-derived RvE1 and RvE2 and DHA-derived RvD1 and RvD5, did not change after LPS injection. Tributyrin treatment significantly decreased 5-HEPE and increased 4-hydroxydocosahexaenoic acid (4-HDHA) at 6 h after LPS injection (Figure 2B, C, $p < 0.01$). However, tributyrin treatment had no significant effect on the LPS-induced changes in either 5-HEPE or 4-HDHA.

The LC-MS/MS analysis demonstrated that tributyrin tended to further augment LPS-induced changes in several 12/15-LOX pathway intermediates; namely, AA-derived 12-HETE, EPA-derived 12-HEPE and 15-HEPE, and DHA-derived 14-HDHA and 17-HDHA, at 6 h after LPS injection. However, those trends did not reach the level of statistically significant differences (Figure 2).

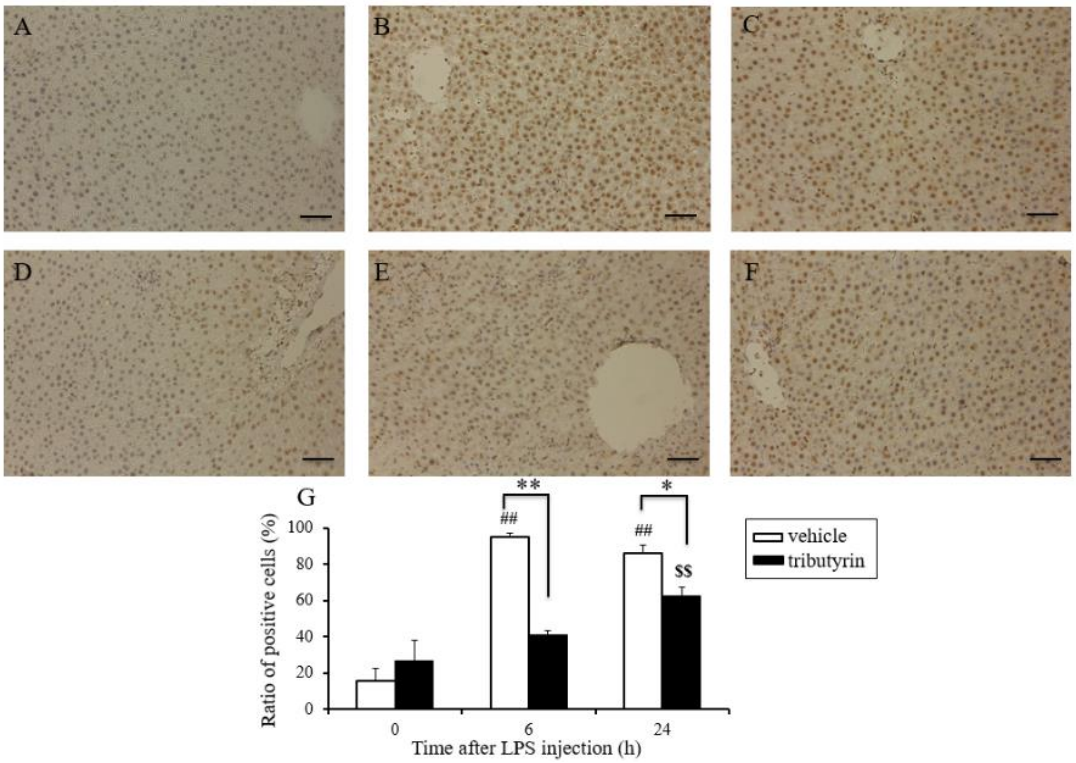

Figure 1. Effect of tributyrin on oxidative stress in the liver at 0, 6, and 24 h after LPS injection.
(A–F) Immunostaining of 8-OHdG in liver sections of rats pretreated with vehicle (A–C) or tributyrin (D–F) for 1 h and injected with LPS for 0 h (A, D), 6 h (B, E), and 24 h (C, F). Magnification 200 ×, scale bars = 100 μm. (G) Quantification of 8-OHdG-positive cells in liver sections from the animals described in (A–F). Values represent the mean ± SEM of 5–7 animals per group. $^{\#\#}p < 0.01$ versus vehicle at 0 h, $^{\$\$}p < 0.01$ versus tributyrin at 0 h, $^{*}p < 0.05$, $^{**}p < 0.01$ versus vehicle at the same time point. 8-OHdG, 8-oxo-2′-deoxyguanosine; LPS, lipopolysaccharide.

Expression of COX-2, 5-LOX, 12/15-LOX, and LTA_4H in the liver

To investigate the mechanism by which LPS and tributyrin modulated lipid mediator levels, we examined expression of several PUFA-oxidizing enzymes in the liver at the mRNA (data not shown) and protein (Figure 3) levels. We found that COX-2, 5-LOX, and 12/15-LOX mRNA expression was elevated at 1.5 and 6 h after LPS injection; however, tributyrin had no effect on these LPS-induced changes (data not shown). Similarly, western blot analysis revealed that LPS significantly increased COX-2 and 5-LOX protein expression ($p < 0.05$) and slightly, but insignificantly, increased 12/15-LOX protein expression at 6 h after LPS injection (Figure 3). Here, too, we found that tributyrin did not inhibit the LPS-induced changes in COX-2, 5-LOX, and 12/15-LOX protein expression. Neither LPS nor tributyrin treatment had an effect on liver levels of LTA_4H, which generates LTB_4.

To determine the effects of LPS and/or tributyrin on translocation of 5-LOX from the cytoplasm to the nucleus, which promotes LTB_4 biosynthesis, we prepared cytoplasmic, nuclear, and membrane fractions of liver and analyzed the subcellular distribution of 5-LOX by western blot analysis (Figure 4). LPS injection increased the appearance of 5-LOX in the nuclear and membrane fractions ($p < 0.01$), but not in the cytoplasmic fraction, at 6 h. Although tributyrin attenuated the LPS-induced increase in 5-LOX nuclear expression at 6 h, the effect was not significant.

Effect of tributyrin alone on lipid mediators in the liver

Because the preceding experiments were all performed on LPS-injected rats, we also assessed the effect on lipid mediator levels of treatment with tributyrin alone for 25 h (i.e., the total duration of the tributyrin + LPS experiments). In the absence of LPS, tributyrin significantly ($p < 0.01$) increased the production of PGD_2 and TxB_2, which are generated by COX-2-mediated metabolism of AA (Table I).

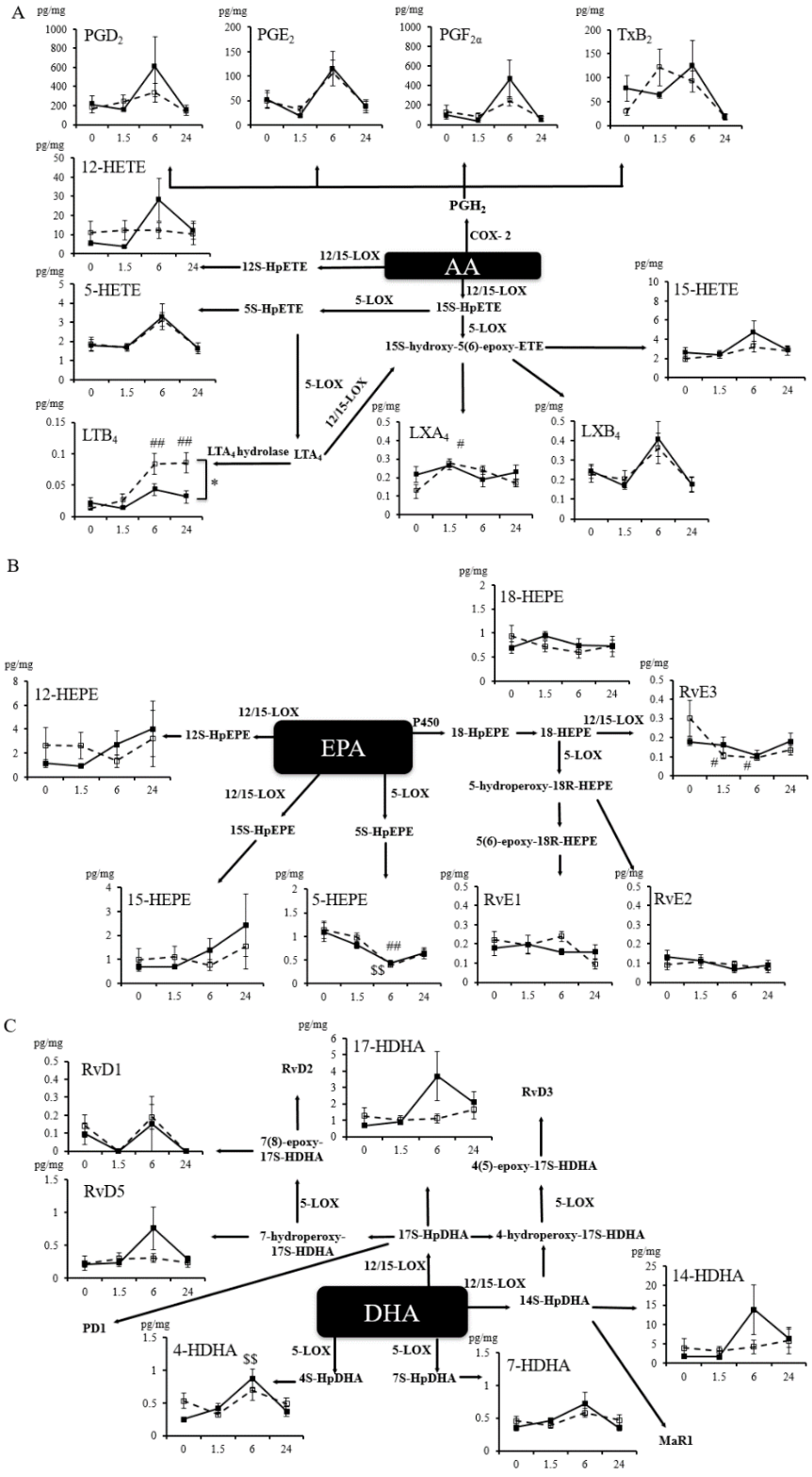

Figure 2. Effect of tributyrin on PUFA-derived lipid mediators in rat liver at 0, 1.5, 6, and 24 h after LPS injection. (A) AA-, (B) EPA-, and (C) DHA-derived lipid mediators and intermediates are presented as pg per mg liver tissue. Vehicle and tributyrin groups are indicated by the dashed and solid lines, respectively. Values represent the mean ± SEM of 5–7 animals per group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus vehicle 0 h, $^{*}p < 0.05$ versus vehicle at the same time point, $^{\$\$}p < 0.01$ versus tributyrin 0 h. LPS, lipopolysaccharide; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid; COX, cyclooxygenase; LOX, lipoxygenase; PG, prostaglandin; Tx, thromboxane; LT, leukotriene; HETE, hydroxyeicosatetraenoic acid; LX, lipoxin; HEPE, hydroxyeicosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid; Rv, resolvin.

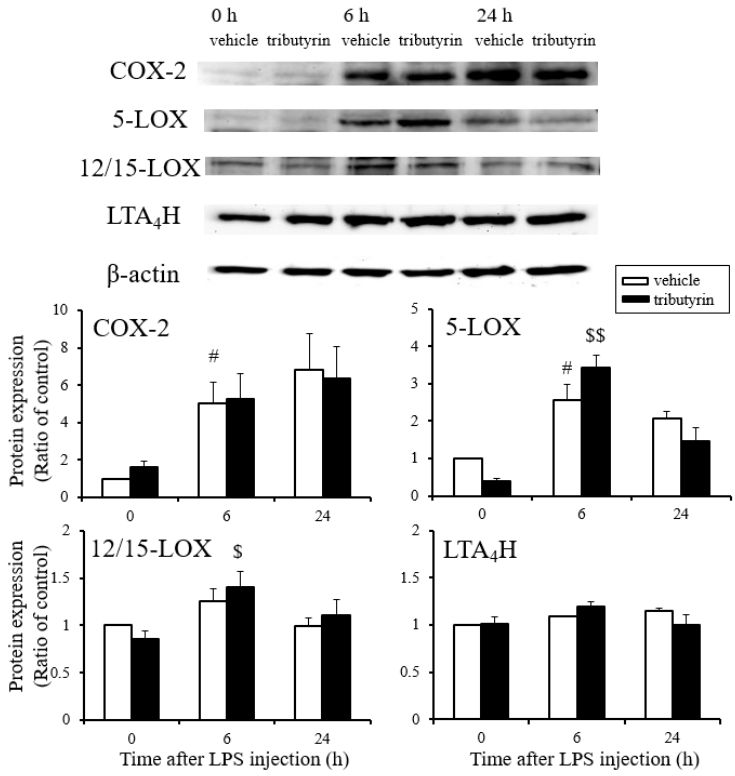

Figure 3. Expression of COX-2, 5-LOX, 12/15-LOX, and LTA_4H protein in rat liver at 0, 6, and 24 h after LPS injection. Western blot analysis (top) and densitometric quantification (bottom) of liver proteins prepared at the indicated times after LPS injection. Values represent the mean ± SEM of 5–7 animals per group. $^{\#}p < 0.05$ versus vehicle 0 h, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus tributyrin 0 h. LPS, lipopolysaccharide; COX, cyclooxygenase; LOX, lipoxygenase; LTA_4H, leukotriene A_4 hydrolase.

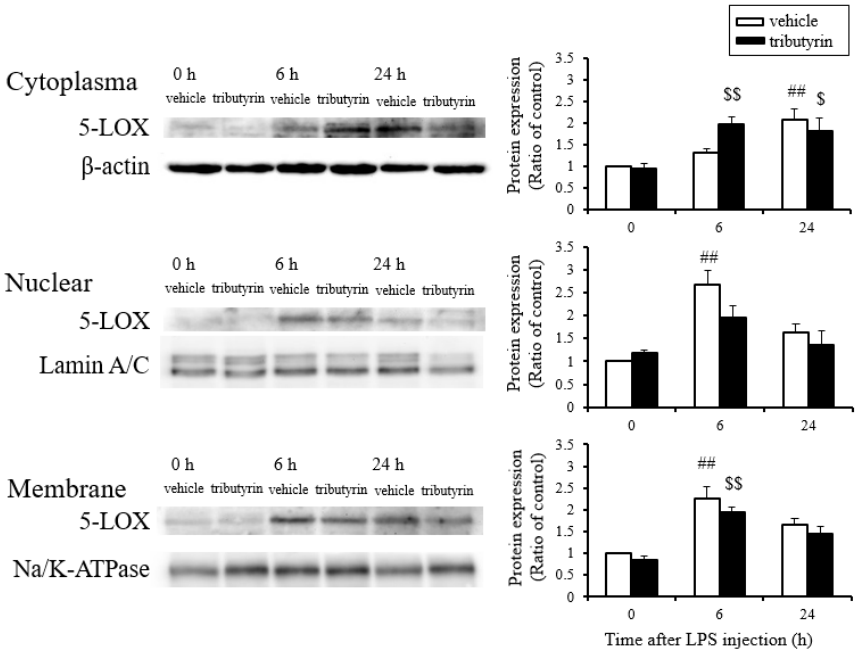

Figure 4. Subcellular expression of 5-LOX protein in the liver at 0, 6, and 24 h after LPS injection.
Western blot analysis (left) and densitometric quantification of proteins (right) in the cytoplasmic, nuclear, and membrane protein fractions from rat liver. β-actin, Lamin A/C, and Na, K-ATPase were used as loading controls and markers of the cytoplasmic, nuclear, and membrane fractions, respectively. Values represent the mean ± SEM of 5–7 animals per group. $^{\#\#}p < 0.01$ versus vehicle 0 h, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus tributyrin 0 h. LPS, lipopolysaccharide; 5-LOX, 5-lipoxygenase.

Table I. Effect of oral tributyrin administration on lipid mediators and pathway markers.

pg/mg tissue	0 h	25 h	*p*
PGE_2	49.68 ± 15.93	91.20 ± 43.31	0.30
PGD_2	167.64 ± 45.66	625.88 ± 169.61	0.01
$PGF_{2\alpha}$	128.95 ± 74.03	257.53 ± 130.98	0.38
TxB_2	28.32 ± 6.86	95.85 ± 27.04	0.01
LTB_4	0.013 ± 0.003	0.020 ± 0.008	0.36
LXA_4	0.13 ± 0.04	0.18 ± 0.02	0.36
LXB_4	0.23 ± 0.04	0.31 ± 0.05	0.28
RvE1	0.22 ± 0.04	0.21 ± 0.08	0.91
RvE2	0.09 ± 0.02	0.15 ± 0.04	0.20
RvE3	0.30 ± 0.09	0.12 ± 0.01	0.11
RvD1	0.14 ± 0.06	0.22 ± 0.13	0.56
RvD5	0.23 ± 0.11	0.53 ± 0.11	0.12
5-HETE	1.87 ± 0.35	2.34 ± 0.21	0.40
12-HETE	11.02 ± 5.94	14.07 ± 2.24	0.74
15-HETE	1.93 ± 0.29	2.45 ± 0.60	0.40
5-HEPE	1.14 ± 0.18	0.83 ± 0.18	0.32
12-HEPE	2.65 ± 1.47	5.42 ± 1.34	0.27
15-HEPE	0.99 ± 0.48	2.66 ± 0.80	0.10
18-HEPE	0.94 ± 0.22	1.13 ± 0.22	0.60
4-HDHA	0.53 ± 0.11	0.40 ± 0.05	0.47
7-HDHA	0.46 ± 0.07	0.51 ± 0.07	0.68
14-HDHA	3.88 ± 2.38	6.68 ± 1.51	0.46
17-HDHA	1.26 ± 0.52	1.88 ± 0.27	0.46

Values represent the mean ± SEM of 3–6 animals per group. Livers were examined before (0 h) and 25 h after tributyrin administration. PG, prostaglandin; Tx, thromboxane; LT, leukotriene; LX, lipoxin; Rv, resolvin; HETE, hydroxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid.

DISCUSSION

To the best of our knowledge, this is the first report of a comprehensive investigation of the effects of tributyrin on LPS-induced changes in lipid mediator content in whole livers. We showed that tributyrin suppresses LPS-induced increases in oxidative stress and LTB_4 production, and additionally attenuates the nuclear translocation of 5-LOX in the liver. Tributyrin administration alone significantly increased the production of PGD_2 and TxB_2 in the liver.

Changes in hepatic lipid mediators, especially LTs, have been reported in a rat model of endotoxin shock (34) and a mouse model of cecal ligation and puncture-induced sepsis (11). The source of lipid mediators in the liver is unclear, but Kupffer cells have been suggested because Kupffer and hepato-sinusoidal cells are early responders in the defense against porto-systemic infections, including bacterial (23). Our finding that both 5-LOX activity and LTB_4 levels were increased by LPS injection are consistent with the findings of Yashaswini et al., who used the same LPS-induced peritonitis model as in the present study (34). Li et al. also reported increased LTB_4 levels in the liver of D-galactosamine/LPS-treated rats (11). Bitto et al. showed that hepatic 5-LOX and serum LTB_4 levels increased in a mouse model of sepsis (3), which are similar results to those obtained here. High plasma LTB_4 levels, but not other traditional clinical indices, were associated with poor outcomes for patients admitted to intensive care units with sepsis (8).

Butyrate is known to inhibit HDACs (17, 18). Our result of an attenuated LPS-induced hepatic LTB_4 level by tributyrin is in accordance with Lu's report. In their study of LPS-induced lung injury in mice, Lu et al. reported two HDAC inhibitors, suberanilohydroxamic acid and its analogue, were effective inhibitors of LTA_4H and inhibited an increase in LTB_4 levels in bronchoalveolar lavage fluid (14). Although we did not see an effect of tributyrin on LTA_4H activity in the present study, butyrate was reported to rescue LPS-induced suppression of LTA_4H gene expression in cultured monocytes (10). To determine the mechanism by which tributyrin reduced 5-LOX activity, we examined both the expression and subcellular distribution of 5-LOX protein, as reported previously (15, 24, 35). A nuclear shift of 5-LOX has been more important. 5-LOX can accumulate in either the cytoplasm or the nucleoplasm, upon cell stimulation, and translocates to membranes to initiate leukotriene synthesis. The action of 5-LOX is a key point of regulation in LTB_4 synthesis (15, 24, 35). We found that tributyrin treatment attenuated the LPS-induced increase in nuclear 5-LOX expression, suggesting a potential mechanism by which LTB_4 levels are reduced by tributyrin. Our results are in accordance with a report that pretreatment of selective 5-LOX inhibitor AA861 attenuated acute liver injury and suppressed hepatic LTB_4 levels in a D-galactosamine/LPS-treated rats (11).

Several mechanisms may explain the beneficial effect of tributyrin-mediated downregulation of LTB_4 in our sepsis model. LTB_4 binding to its cell surface receptor BLT1 is known to amplify expression of myeloid differentiation factor 88 (MyD88), a crucial adaptor protein for Toll-like receptor (TLR)- and IL-1β receptor-dependent signal transduction and NF-κB activation (25, 35). Activation of NF-κB through the LTB_4–BLT1 pathway is a pivotal contributor to the host innate immune and inflammatory responses in various pathological conditions, including sepsis, peritonitis, bronchial asthma, atherosclerosis, bone homeostasis, arthritis, and atopic dermatitis (35). In addition to signaling *via* BLT1, LTB_4 can also bind to and activate peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ, two members of the nuclear receptor family of transcription factors (31). PPARs interfere the activity of NF-κB through protein–protein interactions (29, 31). Using the same endotoxemia model in rats as in the present study, we previously showed that tributyrin administration decreased TLR2/TLR4-induced translocation of NF-κB p65 into the nucleus and increased PPAR-α and PPAR-γ expression (17, 18), those can be explained by suppressing LTB_4 production.

We found that administration of tributyrin alone to rats increased PGD_2 and TxB_2 levels in the liver. This result is consistent with the finding by Kovarik et al. that COX-2 gene expression is upregulated by butyrate treatment of human monocytes *in vitro*; however, in that study, the effect of butyrate on lipid mediator was only observed on LPS-stimulated cells (10). In the present study, tributyrin in the absence of LPS had no effect on liver 5-LOX activity, which contrasts with our previous results using cultured Caco-2 cells (21). The latter result is likely to reflect differences between the *in vitro* model with cultured intestinal cells and the *in vivo* model examining whole liver.

As noted earlier, changes in SPM levels are important in counterbalancing the activity of pro-inflammatory lipid mediators. To our knowledge, this study is the first to report that LPS-induced liver injury is accompanied by a decrease in the production of EPA-derived RvE3 and 5-HEPE. RvE3, which is produced from EPA *via* the 12/15-LOX pathway, has been shown to attenuate metabolic syndrome-related liver inflammation (13). Similarly, increased plasma levels of 5-HEPE, which is generated from EPA *via* the 5-LOX pathway, have been reported in mice after omega-3 PUFA supplementation, a treatment that reduces liver injury resulting from a high fat diet (32). However, we did not detect changes in EPA- or DHA-derived SPMs by LPS or tributyrin in the present study.

The study has several limitations. First, the mechanism by which tributyrin altered the translocation of 5-LOX protein remains unknown. Second, *in vivo* studies may be limited in their ability to detect small changes in lipid mediators from producing cells because these molecules have very short half-lives and are rapidly inactivated. Finally, we administered tributyrin to the animals only once, and further studies will be needed to evaluate the effects of repeated or continuous tributyrin administration.

It is now clear that intestinal flora can produce numerous short-chain fatty acids, including butyrate, that act as hormones and have effects on organs remote from the intestine (4, 9, 33). Those studies support a role for butyrate in the control of various diseases, including metabolic syndrome, obesity, atherosclerosis, cancer, central nervous system diseases, and intestinal diseases. Modulation of LTB_4 and various other lipid mediators by butyrate may therefore be a potential therapeutic strategy for such disorders (12, 24, 35). Indeed, butyrate was recently reported to modulate DHA metabolism in cancer cells using an *in vivo* tumor model (27), supporting the possibility that butyrate might alter DHA- and/or EPA-derived SPM levels.

Conclusion

Oral tributyrin administration reduces hepatic production of pro-inflammatory LTB_4 in an endotoxemia model in rats.

ACKNOWLEDGEMENTS

This work was partially supported by JSPS KAKENHI (Grant-in-aid for Scientific Research, number 25450450). The authors have no conflicts of interest to declare.

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