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Prostacyclin mimetics afford protection against lipopolysaccharide/D-galactosamine-induced acute liver injury in mice

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ABSTRACT

Prostacyclin (PGI_2) serves as a protective, anti-inflammatory mediator and PGI_2 mimetics may be useful as a hepatoprotective agent. We examined whether two PGI_2 mimetics, ONO-1301 and beraprost, are beneficial in acute liver injury and attempted to delineate the possible mechanism underlying the hepatoprotective effect. Acute liver injury was induced by lipopolysaccharide/D-galactosamine (LPS/GalN) in mice. Mice were given an intraperitoneal injection of PGI_2 mimetics 1 h before LPS/GalN challenge. Both ONO-1301 and beraprost significantly declined the LPS/GalN-induced increase in serum aminotransferase activity. ONO-1301 and, to a lesser extent, beraprost inhibited hepatic gene expression levels of pro-inflammatory cytokines, which were sharply elevated by LPS/GalN. The hepatoprotective effects of ONO-1301, to a lesser extent, of beraprost were also supported by liver histopathological examinations. The PGI_2 receptor antagonist CAY10441 abrogated their hepatoprotective effects. The mechanisms behind the benefit of PGI_2 mimetics in reducing LPS/GalN-induced liver injury involved, in part, their suppressive effects on increased generation of reactive oxygen species (ROS), since their ability to prevent LPS/GalN-induced hepatic apoptosis was mimicked by the antioxidant *N*-acetyl-L-cysteine. They significantly diminished LPS/GalN-induced activation of signal transducers and activators of transcription 3 (STAT3) in liver tissues, an effect which was highly associated with their hepatoprotective effects. We indicate that IP receptor activation with PGI_2 mimetics can rescue the damage in the liver induced by LPS/GalN by undermining activation of STAT3 and leading to a lower production of ROS. Our findings point to PGI_2 mimetics, especially ONO-1301, as a potential novel therapeutic modality for the treatment of acute liver injury.

1. Introduction

Acute liver failure (ALF) is a rare but life-threatening critical condition demanding urgent medical care that is marked by the sudden loss of hepatic function most often in patients without any past medical history of hepatic disease (Khan et al., 2006; Lee et al., 2008; Bernal and Wendon, 2013). There are multiple causes of ALF which include hepatitis viruses, drugs such as acetaminophen and alcohol, malperfusion, and toxins (Lee et al., 2008; Bernal and Wendon, 2013). Despite recent advances in intensive care in this field, the ALF management continues to be one of the truly challenging problems in clinical practice, and ultimately, liver transplantation is the only definitive treatment of fulminant hepatitis (Lai and Murphy, 2004; Bernal and Wendon, 2013). Liver injury induced by co-injection of lipopolysaccharide (LPS) and D-galactosamine (GalN) in rodents is a well-established experimental animal model of human liver injury (Silverstein,

Abbreviations: ALF, acute liver failure; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; c-Met, mesenchymal-epithelial transition factor; CREB, cyclic AMP response element binding protein; DHE, dihydroethidine hydrochloride; ERK1/2, extracellular signal-regulated protein kinase 1/2; GalN, D-galactosamine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GCS, glutamylcysteine synthetase; GSH, reduced glutathione; GSSG, glutathione disulfide; HB-EGF, heparin binding epidermal growth factor-like growth factor; HGF, hepatocyte growth factor; IL, interleukin; IP receptor, prostacyclin receptor; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; MPO, myeloperoxidase; NAC, *N*-acetyl-L-cysteine; NADPH, nicotinamide adenine dinucleotide phosphate; NQO1, NAD(P)H-quinone oxidoreductase; Nrf2, nuclear erythroid 2-related factor 2; OCT, optimum cutting temperature; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PGI_2, prostacyclin; PKA, protein kinase A; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TNF-α, tumor necrosis factor-α; TUNEL, terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling

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2004; Maes et al., 2016). This model has been widely used to understand the pathogenesis of human ALF and to develop novel potential liver-protective agents. Notably, hepatic inflammation is a common finding in the onset and development of various types of ALF, including drug-induced liver toxicity (Adams et al., 2010), and the LPS/GalN-based model is useful to study inflammation-mediated liver injury (Maes et al., 2016).

Prostacyclin (PGI_2) is a prostaglandin member of the eicosanoid family of bioactive lipids derived from arachidonic acid (Stitham et al., 2010). PGI_2 activates prostacyclin receptors (IP receptors) which signal through G_s-adenylate cyclase and cyclic AMP-protein kinase A (PKA) (Egan et al., 2004). PGI_2 was originally identified as a potent vasodilator and inhibitor of platelet aggregation. In addition to its vascular and anti-thrombotic effects, PGI_2 has anti-inflammatory properties. Thus, the anti-inflammatory actions of PGI_2 has been demonstrated in asthma models *in vivo* (Idzko et al., 2007; Jaffar et al., 2007), diabetic patients with vascular inflammation (Goya et al., 2003), critical limb ischemia patients (Di Renzo et al., 2005), and bacterial inflammation *in vitro* (Raychaudhuri et al., 2002; Zhou et al., 2007). Furthermore, PGI_2 analogues have been widely used as treatment of patients with pulmonary arterial hypertension (Hoeper et al., 2016), and evidence has been provided suggesting that they benefit those patients *via* anti-inflammatory actions (Hashimoto et al., 2004). Intriguingly, a recent report has shown the hepatoprotective effect of PGI_2 mimetic in carbon tetrachloride-induced liver injury (Xu et al., 2012).

In the present study, we investigated whether PGI_2 mimetics can protect mice from LPS/GalN-induced acute liver injury. We used ONO-1301 ((*E*)-[5-[2-[1-phenyl-1-(3-pyridyl)methylidene-aminooxy]ethyl]-7,8-.

dihydronaphthalene-1-yloxy]acetic acid) and beraprost, both of which show high affinities for IP receptors. While beraprost is a synthetic compound with a PGI_2-like structure, ONO-1301 has non-prostanoid structure with thromboxane A_2 synthase inhibitory activity (Kondo et al., 1995; Hayashi et al., 2010). It follows that ONO-1301 is chemically and biologically more stable than beraprost and may escape the desensitization of the action *in vivo* despite its less potency than beraprost in IP receptors. We found that both ONO-1301 and beraprost conferred significant protection against LPS/GalN-induced acute liver injury in mice, although ONO-1301 was more effective than beraprost in most of protective benefits. The goal of this study was to determine the signaling mechanisms through which PGI_2 mimetics would be beneficial in acute liver injury.

2. Materials and methods

2.1. Animals and treatment

Female C57BL/6J mice, 7 weeks of age, were quarantined in quiet, humidified, light-cycled rooms for at least 1 week before use. Mice were allowed *ad libitum* access to food and water throughout quarantine. To induce acute liver injury, mice were intraperitoneally injected with LPS (10 μg/kg; *E. coli* 055:B5; List Biological Laboratories, Campbell, CA, USA) and GalN (650 mg/kg; Wako Pure Chemical, Osaka, Japan). Then, the animals were returned to their cages and allowed food and water *ad libitum*. ONO-1301 (10 mg/kg) or beraprost (0.2 mg/kg) was administered with an intraperitoneal injection 1 h prior to LPS/GalN challenge. The doses of ONO-1301 and beraprost were chosen based on other reports using mice *in vivo* (Murata et al., 1989; Goya et al., 2003; Hayashi et al., 2010; Lee et al., 2013; Kashiwagi et al., 2015). Although lower doses of the two PGI_2 mimetics were tested in our pilot study, both ONO-1301 (1.1 and 3.3 mg/kg) and beraprost (0.02 and 0.067 mg/kg) were found to be ineffective in protecting against LPS/GalN-induced liver injury. When the IP receptor antagonist CAY10441 (10 mg/kg) was employed, it was intravenously given 15 min prior to ONO-1301 or beraprost. In some experiments, *N*-acetyl-L-cysteine (NAC; 200 mg/kg) and stattic (25 mg/kg) were intraperitoneally injected 60 and 30 min before LPS/GalN challenge, respectively, when applied to mice. Mice were sacrificed at 6 h after LPS/GalN challenge unless stated otherwise. The blood samples were collected for determining serum aminotransferases levels. Liver tissues were harvested and some of the lobes were fixed in formalin for morphological studies. Remaining liver lobes were kept at − 80 °C for subsequent analysis. All experimental procedures involving animals were conducted in accordance with the National Institute of Health Guidelines on the use of laboratory animal and with approval of the Care and Use Committee of the University of Toyama.

2.2. Serum aminotransferase measurement

Blood was collected in serum gel tubes (Sarsted, Nümbrecht, Germany), and serum was obtained and stored at − 80 °C. The quantitative determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum was made on Hitachi 7180 Biochemistry Automatic Analyzer (Hitachi High-Technologies, Tokyo, Japan). Assays were performed in duplicate.

2.3. RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was isolated from liver tissues with Sepazol-RNA I Super G (Nacalai Tesque, Kyoto, Japan). RNA was reverse-transcribed to cDNA by the use of PrimeScript RT Master Mix (Takara Bio, Ohtsu, Japan) or ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan), and real-time polymerase chain reaction (PCR) analyses were performed using SYBR Premix Ex Taq II (Tli RNaseH Plus), ROX plus (Takara Bio). The PCR program consisted of 95 °C for 60 s or 30 s for initial denaturation of DNA, followed by 40 cycles of 95 °C for 5 s or 15 s, 60 °C for 30 s for annealing of primers and elongation. Values were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) according to the manufacturer's protocol (MX3000P real-time PCR system; Agilent Technologies Inc., Santa Clara, CA). The PCR primers were designed as follows: forward 5′-GTTCTATGGCCCAGACCCTCAC-3′ and reverse 5′-GGCACCACTAG-TTGGTTGTCTTTG-3′ for tumor necrosis factor-α TNF-α, forward 5′-TCCAGGATGAGGACATGAGCAC-3′ and reverse 5′-GAACGTCACAC-ACCAGCAGGTTA-3′ for interleukin (IL)-1β, forward 5′-CCACTT-CACAAGTCGGAGGCTTA-3′ and reverse 5′-GCAAGTGCATCATCG-TTGTTCATAC-3′ for IL-6, forward 5′-CTCCAGCCTACTCATTGGGATCA-3′ and reverse 5′-GCATCCACGTGTTGGCTCA-3′ for monocyte chemotactic protein (MCP)-1, forward 5′-ATGTGGGGGACCAAACTTCTG-3′ and reverse 5′-GGATGGCGACATGAAGCAG-3′ for hepatocyte growth factor (HGF), forward 5′-GTGAACATGAAGTATCAGCTCCC-3′ and reverse 5′-TGTAGTTTGTGGCTCCGAGAT-3′ for mesenchymal-epithelial transition factor (c-Met), forward 5′-CTCAGCATGATGGACTTGGA-3′ and reverse 5′-TCTATGTCTTGCCTCCAAAGG-3′ for nuclear erythroid 2-related factor 2 (Nrf2), and 5′-CGGGGAGTGCAGATACCTG-3′ and reverse 5′-TTCTCCACTGGTAGAGTCAGC-3′ for heparin binding epidermal growth factor-like growth factor (HB-EGF), forward 5′-AGGATGGGAGGTACTC-3′ and reverse 5′-AGGCGTCCTTCCTTATATGCTA-3′ for NAD(P)H-quinone oxidoreductase (NQO1), forward 5′-AGGAGCTTCGGGACTGTATCC-3′ and reverse 5′-GGGACATGGTGCATTCCAAAA-3′ for glutamylcysteine synthetase (GCS), and forward 5′-TGTGTCCGTCGTGGATCTGA-3′ and reverse 5′-TTGCTGTTGAAGTCGCAGGAG-3′ for GAPDH.

2.4. Histologic examination

For routine histology, liver tissues were fixed in 4% paraformaldehyde and embedded in paraffin, and sliced into 4-μm-thick sections. After deparaffinization, slides were stained with hematoxylin and eosin using standard methods. Histopathologic changes were observed using an optical microscope (Olympus, Tokyo, Japan). All the histological studies were performed in a blinded fashion.