Assessment of hepatic prostaglandin E₂ level in carbamazepine induced liver injury

Ken-ichi OBA^{1,*}, Hiroaki SHIMADA^{1,*}, Ryota HASHIMOTO¹, Atsushi KAWASE¹, Takeo NAKANISHI², Masahiro IWAKI^{1,3,4}

¹Department of Pharmacy, Faculty of Pharmacy, Kindai University, Osaka, Japan; ²Department of Pharmacy, Faculty of Pharmacy, Takasaki University of Health and Welfare, Takasaki, Japan; ³Pharmaceutical Research and Technology Institute, Kindai University, Osaka, Japan; ⁴Antiaging Center, Kindai University, Osaka, Japan

E-mail: iwaki@phar.kindai.ac.jp

*These authors contributed equally to this work.

Objective. Carbamazepine (CBZ), a widely used antiepileptic drug, is one major cause of the idiosyncratic liver injury along with immune reactions. Conversely, prostaglandin E₂ (PGE₂) demonstrates a hepatoprotective effect by regulating immune reactions and promoting liver repair in various types of liver injury. However, the amount of hepatic PGE₂ during CBZ-induced liver injury remains elusive. In this study, we aimed to evaluate the hepatic PGE₂ levels during CBZ-induced liver injury using a mouse model.

Methods. Mice were orally administered with CBZ at a dose of 400 mg/kg for 4 days, and 800 mg/kg on the 5th day.

Results. Plasma alanine transaminase (ALT) level increased in some of mice 24 h after the last CBZ administration. Although median value of hepatic PGE₂ amount in the CBZ-treated mice showed same extent as vehicle-treated control mice, it exhibited significant elevated level in mice with severe liver injury presented by a plasma ALT level >1000 IU/L. According to these results, mice had a plasma ALT level >1000 IU/L were defined as responders and the others as non-responders in this study. Even though, the hepatic PGE₂ levels increased in responders, the hepatic expression and enzyme activity related to PGE₂ production were not upregulated when compared with vehicle-treated control mice. However, the hepatic 15-hydroxyprostaglandin dehydrogenase (15-PGDH) expression and activity decreased significantly in responders when compared with control mice.

Conclusions. These results indicate that elevated hepatic PGE₂ levels can be attributed to the downregulation of 15-PGDH expression under CBZ-induced liver injury.

Key words: liver injury, prostaglandin E₂, carbamazepine, 15-PGDH

Drug-induced liver injury (DILI) is the primary cause of the discontinuation of clinical studies and the withdrawal of new drugs from the market. Although the underlying mechanism of DILI remains unclear, it has been suggested that idiosyncratic DILI may be triggered by reactive metabolites generated by cytochrome P450 (CYP) and uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronosyltransferase, UGT).

Carbamazepine (CBZ), a primary antiepileptic agent, induces severe idiosyncratic hepatotoxicity unrelated to serum concentrations (Bjornsson 2008). In humans, CBZ is mainly metabolized to stable CBZ-10,11-epoxide by CYP3A4, which is before excreting further metabolized by hydroxylation (Lertratanangkoon and Horning 1982; Bertilsson and Tomson 1986). Additionally, CBZ is metabolized to reactive metabo-

lites, including 2,3-epoxide, 2-hydroxy (OH) and 3-OH CBZ, which might be involved in the development of idiosyncratic liver injury via covalent binding with proteins and production of reactive oxygen species (Pirmohamed et al. 1992b; Lu and Uetrecht 2008; Iida et al. 2015). Moreover, it has been speculated that immunological mechanisms are involved in CBZ-induced liver injury. In a previous study, autoantibody directed against liver microsomal protein has been detected in patients with CBZ-induced hepatotoxicity, presenting fever, lymph node swelling, and leukocytosis (Pirmohamed et al. 1992a). To clarify the involvement of metabolic activation and immune reactions in CBZ-induced liver injury, Higuchi et al. (2012) have established a mouse model of CBZ-induced hepatotoxicity. Reportedly, exogenous administration of prostaglandin (PG) E₁, an analog compound of PGE₂ that is almost not biosynthesized, attenuates the hepatic injury induced by CBZ in mice.

PGE₂ is a widely known lipid mediator synthesized by the cyclooxygenase (COX/PTGS)/PGE synthase (PGES/PTGES) pathway, demonstrating a broad effect on the body regulating homeostasis. Notably, PGE₂ exhibits protective effects against liver injury by promoting liver regeneration and regulating cytokine production. PGE₂ can enhance the proliferation of hepatocytes and regulate the production of cytokines, including tumor necrosis factor- α , interleukin-1 β , and interferon- γ , in various types of liver injury (Tsujii et al. 1993; Kuzumoto et al. 2005). Moreover, chemical inhibition or genetic deletion of PGE₂ synthetic enzymes, including COX and PGES, exacerbates liver injury induced by several hepatotoxic agents (Reilly et al. 2001; Cavar et al. 2010). Therefore, the hepatic level of endogenous PGE₂ is likely to play an important role in the attenuation of the liver injury.

PGE₂ is converted into an inactivated PGE₂ metabolite, 15-ketoPGE₂, by 15-prostaglandin dehydrogenase (15-PGDH/HPGD). 15-PGDH is a rate-limiting enzyme for PGE₂ inactivation, mainly expressed in the lungs, liver, intestines, and stomach (Matsuo et al. 1996). Reportedly, genetic deletion and chemical inhibition of 15-PGDH promote liver regeneration along with the escalation of hepatic PGE₂ levels (Zhang et al. 2015). Furthermore, the liver can efficiently remove 89–95% of circulating PGE₁ in a single passage (Dawson et al. 1970). Thus, the liver is considered a major organ for PGE₂ inactivation. Under pathological conditions, the PGE₂ level in tissues is increased by the induction of inducible enzymes related to PGE₂ synthesis, including COX2 and microsomal PGES (mPGES)-1. However, the hepatic 15-PGDH expression is downregulated by LPS-induced inflammation in rats (Ivanov et al. 2003).

Moreover, we recently have reported that hepatic PGE₂ levels are regulated by downregulation of 15-PGDH-mediated PGE₂ inactivation in a carbon tetrachloride (CCl₄)-induced liver injury murine model (Shimada et al. 2020). Accordingly, we hypothesize that as the synthesized PGE₂ is rapidly inactivated by 15-PGDH in the liver, hepatic PGE₂ levels are regulated by altered 15-PGDH activity under pathological conditions.

Although endogenous PGE₂ seems to play an important role in the suppression and repair of liver damage, it remains unclear how hepatic PGE₂ is processed during CBZ-induced liver injury. In the present study, we aimed to clarify the regulatory mechanism of hepatic PGE₂ levels during CBZ-induced liver injury in a mouse model.

Materials and methods

Materials. CBZ and Transaminase CII-Test-Wako kit were purchased from Wako Pure Chemical Industries (Osaka, Japan). Fast SYBR Green Master Mix and Pierce™ BCA Protein Assay Kit were obtained from Thermo Fisher Scientific (Waltham, MA, USA). All other compounds and reagents were of reagent grade.

Animals. In this study, we used male BALB/cCrSlc mice (weighing 20–22 g, aged 8–9 weeks old). All animals were allowed free access to food and water, maintained under a standard 12-h light/12-h dark cycle in a temperature-controlled room (24±2 °C). Animals were acclimatized for one week before use. All animal experiments were performed in accordance with the requirements of the Committee for the Care and Use of Laboratory Animals of the School of Pharmacy of Kindai University (Approved number; KAPS-2020-029).

CBZ administration. Mice were administered with CBZ as previously described (Higuchi et al. 2012). In brief, CBZ was suspended in corn oil and orally administered at a dose of 400 mg/kg for 4 days and 800 mg/kg on the 5th day. Control mice were orally administered with the same volume of corn oil. Then, 24 h after the last administration, whole blood and the liver were collected under anesthesia. The degree of liver injury was evaluated by measuring serum alanine aminotransferase levels using the Transaminase CII-Test-Wako kit. Liver samples were immediately frozen in liquid nitrogen and maintained at –80 °C. The degree of liver injury was also evaluated by hematoxylin and eosin (H&E) staining.

Measurement of liver PGE₂ level. Liver samples were homogenized with a mechanical homogenizer (Histocolon NS-50, Microtech Nichion, Chiba, Japan) and sonicated using Branson Sonifier 250 (Emerson

Electric, St. Louis, MO, USA) in a homogenization buffer consisting of 0.1 M sodium phosphate buffer, containing 100 μ M indomethacin and 1 mM EDTA. The homogenates were vortexed and centrifuged at 8000 $\times$ g at 4°C for 10 min. PGE₂ levels in the resultant supernatant were measured using a prostaglandin E₂ Express ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Quantitation of liver mRNA expression. Total RNA was extracted from the liver using Sepasol[®] RNA I Super G (Nacalai Tesque, Kyoto, Japan) and reverse transcribed to cDNA with a ReverTra Ace-first strand cDNA Synthesis Kit (Toyobo, Osaka, Japan). mRNA expression levels of PGE₂-related genes were quantified using the StepOne Plus Real-Time PCR system (Thermo Fisher Scientific), normalized to that of hypoxanthine phosphoribosyltransferase (*Hprt*), followed by analysis using the 2^{- $\Delta\Delta$ CT} method. The primer sequences are listed in Table 1.

Western blot analysis. Western blot analysis was performed as previously described with some modifications (Shimada et al. 2015). Liver samples were homogenized in RIPA buffer (Santa Cruz Biotechnology, Dallas, TX, USA) and stirred at 4°C for 2 h. Homogenates were centrifuged at 10000 $\times$ g for 10 min at 4°C. The protein concentration in the resultant supernatant was determined using the Pierce[™] BCA Protein Assay Kit. A 100 μ g aliquot of the total cell lysate was separated on an SDS polyacrylamide gel and electrotransferred onto a polyvinylidene difluoride membrane (GE Healthcare, Little Chalfont, UK). The membrane was incubated for 1 h at 4°C with 2% bovine serum albumin (BSA)/TBS-T (Tween 20-containing Tris-buffered saline). The membrane was incubated with primary antibodies against Cox2 (1:250 dilution, Cayman Chemical) and β -actin (1:2000, Abcam, Cambridge, UK) for 1 h at room temperature. After washing with TBS-T, the membrane was treated with HRP-conjugated secondary antibody (1:10000, Abcam) for 1 h at room temperature, and bands were detected with ECL Prime Western

Blotting Detection Reagent (GE Healthcare). Western blots were analyzed using a CS analyzer (ATTO, Tokyo, Japan) to quantify band intensity; data were normalized to control conditions.

PGE₂ formation assay. Liver samples were homogenized with 1% Triton X-100/PBS (v/v) and centrifuged at 12000 $\times$ g for 3 min at 4°C. The supernatant was recovered as a liver homogenate and the protein concentration was measured using the Pierce[™] BCA Protein Assay Kit. PGE₂ formation was evaluated using 1 mg protein/mL liver homogenates containing 0.1 M Tris-HCl (pH8.0) and 1 mM arachidonic acid. The reaction was initiated by adding arachidonic acid and terminated by adding acetonitrile 5 min after initiation. Samples were centrifuged at 10000 $\times$ g for 10 min at 4°C. The formed PGE₂ in the supernatant were extracted using ethyl acetate and quantified by liquid chromatography with tandem mass spectrometry (LC-MS/MS) as described previously (Shimada et al. 2020).

15-PGDH activity assay. 15-PGDH activity in liver homogenates was determined using a PicoProbe[™] 15-PGDH Activity Assay Kit (Biovision, Milpitas, CA, USA). Approximately 10 mg of mouse liver was homogenized with 100 μ L ice-cold 15-PGDH Assay Buffer. Then, 200 μ L saturated ammonium sulfate was added to liver homogenates and maintained on ice for 30 min. Samples were centrifuged at 10000 $\times$ g at 4°C for 10 min and the pellet was resuspended in 100 μ L ice-cold 15-PGDH Assay Buffer. In liver homogenates, the protein concentration was measured with the BCA protein assay kit (Thermo Fisher Scientific). Liver homogenates were diluted using 15-PGDH Assay Buffer to attain a final concentration of 1 mg/mL in the reaction medium. The reaction was initiated by adding the 15-PGDH substrate, and fluorescence was immediately measured in kinetic mode for 30 min using a fluorescence microplate reader (SH-9000, Corona Electric Co., Ibaraki, Japan), at excitation and emission wavelengths of 485 and 535 nm, respectively. In this study, 15-PGDH activity was exhibited as the reduction rate of nicotin-

Table 1
Sequence of primers for qPCR

Target	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>Ptgs1</i>	CATTGCACATCCATCCACTC	CCAAAGCGGACACAGACAC
<i>Ptgs2</i>	GTTTCATCCCTGACCCCAAG	GCGCAGTTTATGTTGTCTGTCC
<i>Ptges1</i>	GCACACTGCTGGTCATCAAG	ATGAGTACACGAAGCCGAGG
<i>Ptges2</i>	GCTGGGGCTGTACCACAC	GATTCACCTCCACCAC
<i>Hpgd</i>	CAACAATGCAGGCGTGAACA	GCGTGTGAATCCGATGATGC
<i>Hprt</i>	CGCAGTCCCAGCGTCGTGATT	CTTGAGCACACAGAGGGCCACAA

amide adenine dinucleotide, a byproduct of 15-PGDH-mediated oxidation of PGE₂ into 15-ketoPGE₂.

Statistical analysis. Data are presented as individual values, and statistical analysis was performed using Student's t-test or Dunnett's test after analysis of variance, with $p < 0.05$ as the significance criterion. GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) was used to perform Dunnett's test.

Results

Elevation of plasma ALT and hepatic PGE₂ levels in CBZ-treated mice. Individual plasma ALT values and hepatic PGE₂ levels were investigated 24 h after the last CBZ administration (Figure 1). Severe liver injury that presented increased plasma ALT levels up to 1000 IU/L were observed in 24% of CBZ-treated mice (Figure 1A). Although hepatic PGE₂ levels varied in CBZ-treated mice, median value of CBZ-treated mice was same extent as that of vehicle-treated mice (Figure 1B). However, the mice exhibited severe liver injury had significantly higher hepatic PGE₂ amounts than the others after the CBZ treatment (Figure 1C). These results suggest that hepatic PGE₂ levels increase

with the development of CBZ-induced liver injury. From these results, we defined the mice with plasma ALT levels higher than 1000 IU/L as responders, and less than 1000 IU/L as non-responders in this study. In H&E staining (Figure 1D), loss of hepatocytes around the central vein was observed in the responders, while no significant differences were observed between vehicle-treated controls and non-responders.

Alteration of PGE₂-related gene expression in CBZ-treated mice. To elucidate the mechanism of hepatic PGE₂ regulation under CBZ-induced liver injury, altered gene expression of enzymes related to PGE₂ synthesis and inactivation was evaluated by quantitative polymerase chain reaction (qPCR) and western blotting (Figure 2 and Figure 3). *Ptgs2* mRNA expression was not detected up to 35 cycles by qPCR in vehicle-treated mice, non-responders, and responders. mRNA expressions of *Ptgs1*, *Ptges1*, *Ptges2*, as well as protein expression of COX2, were not significantly increased in non-responders and responders (Figures 2A–D). In non-responders, *Ptgs1* and *Ptges2* mRNA expression decreased significantly when compared with the vehicle-treated control (Figures 2A, C). PGE₂ synthesis was not increased in liver homogenates obtained from

Figure 1. Individual plasma ALT activities and hepatic PGE₂ levels 24 h after last CBZ administration. (A) Plasma ALT values were evaluated 24 h after the last CBZ administration (n=17). (B) Hepatic PGE₂ levels were quantified 24 h after the last CBZ administration. (C) Relationship between plasma ALT values and hepatic PGE₂ levels in CBZ-treated mice was analyzed. (D) H&E staining of liver cryosections. White circles and black diamonds represent vehicle-treated control and CBZ-treated mice, respectively. Bars indicate individual and median values. Abbreviations: ALT – alanine transaminase; CBZ – carbamazepine; CV – central vein; PV – portal vein; PGE₂ – prostaglandin E₂.

responders and non-responders, tending to decrease in liver homogenates when compared with the vehicle-treated control (Figure 2E). Conversely, hepatic *Hpgd* mRNA expression and 15-PGDH activity were significantly downregulated in responders (Figures 3A, B). Non-responders failed to demonstrate significant changes in *Hpgd* mRNA expression and activity. Since expression levels of *Hpgd* mRNA showed good correlation with 15-PGDH activity (Figure 3C), it seems that hepatic 15-PGDH activity was downregulated by means of suppressing the expression level under CBZ-induced liver-injury condition.

Although no correlation was observed between PGE₂ synthesis and hepatic PGE₂ levels (Figure 4A), 15-PGDH activity was well correlated with hepatic PGE₂ levels in vehicle-treated control and CBZ-treated mice (Figure 4B), suggesting that the downregulation of hepatic 15-PGDH expression could be a dominant factor in determining hepatic PGE₂ levels in CBZ-induced liver injury.

Discussion

CBZ is known to cause severe liver injury. A previous study has indicated that exogenous PGE₁ administration can attenuate CBZ-induced liver injury by regulating hepatic cytokine production (Higuchi et al. 2012), however, it remains unclear how endogenous hepatic PGE₂ levels are modulated. In the present study, we investigated the regulatory mechanism of hepatic PGE₂ levels during CBZ induced liver injury.

Previously, it has been reported a significant elevation of plasma ALT levels 24 h after the last CBZ administration in approximately 75% of male BALB/c mice (Higuchi et al. 2012). In the present study, plasma ALT levels were significantly increased only in 24% of CBZ-treated mice at 24 h after the last CBZ administration (Figure 1A). The incidence rate showed lower values than the previous study, however, we successfully established the CBZ-induced liver injury model. It is not clear that the reason why CBZ showed lower

Figure 2. Alteration of hepatic activities and expression of enzymes related to PGE₂ synthesis 24 h after the last CBZ administration. Hepatic mRNA expression of (A) *Ptgs1*, (B) *Ptges1*, and (C) *Ptges2* and protein expression of (D) COX2 were evaluated 24 h after the last CBZ administration. (E) Hepatic PGE₂ synthetic activities were investigated 24 h after the last CBZ administration. * indicates a significant difference from vehicle-treated control determined by (A–C and E) Dunnett's test, with $p < 0.05$. White circles, gray and black diamonds represent vehicle-treated control, non-responders, and responders, respectively. Bars indicate median values. In mRNA expression of *Ptges1* (B) and *Ptges2* (C), 3 and 4 out of 11 non-responders could not have detected them up to 35 cycles, respectively. Abbreviations: CBZ – carbamazepine; COX2 – cyclooxygenase-2; PGE₂ – prostaglandin E₂; *Ptgs1* – prostaglandin-endoperoxide synthase 1; *Ptges2* – prostaglandin-endoperoxide synthase 2.

Figure 3. Alteration of hepatic activities and expression of 15-PGDH 24 h after the last CBZ administration. (A) Hepatic mRNA expression of *Hpgd* and (B) activities of 15-PGDH were investigated 24 h after the last CBZ administration. (C) Correlation between hepatic mRNA expression of *Hpgd* represented as ΔCt value and 15-PGDH activities was analyzed. * indicates a significant difference from vehicle-treated control determined by Dunnett's test, with $p < 0.05$. White circles, gray and black diamonds represent vehicle-treated control, non-responders, and responders, respectively. Bars indicate median values. Abbreviations: CBZ – carbamazepine; *Hpgd* – 15-hydroxyprostaglandin dehydrogenase gene; 15-PGDH – 15-hydroxyprostaglandin dehydrogenase.

Figure 4. Relationship between individual PGE₂ levels and PGE₂ synthesis or 15-PGDH activities in liver. Correlation between individual PGE₂ synthesis (A), 15-PGDH activities (B) and PGE₂ levels in the liver was analyzed. White circles, gray and black diamonds represent vehicle-treated control, non-responders, and responders, respectively. Abbreviations: PGE₂ – prostaglandin E₂; 15-PGDH – 15-hydroxyprostaglandin dehydrogenase.

toxicity in this study than previous report, even though the rearing environments and the way of CBZ administration were adjusted to previous report as possible (Higuchi et al. 2012). To evaluate the altered hepatic PGE₂ levels following CBZ administration, we quantified hepatic PGE₂ levels (Figure 1B). Reportedly, long-term CBZ administration in rats led to the down-regulation of COX activity in the brain (Ghelardoni et al. 2004), with a decrease in PGE₂ levels in the hypothalamus (Nassar et al. 2016). No significant differences in median values of hepatic PGE₂ levels were observed between vehicle-treated control and CBZ-treated mice. However, a significant increase in hepatic PGE₂ levels was observed in the mice exhibiting severe liver injury after CBZ administration (Figure 1C). These results suggest that hepatic PGE₂ levels may be

elevated in CBZ-induced liver injury. Hepatic PGE₂ levels increased only 2-fold in maximum in mice exhibited severe liver injury after CBZ administration. The slight increase of hepatic PGE₂ levels may be caused by using whole liver homogenate to evaluate PGE₂ levels in liver. Since it is possible that PGE₂ levels significantly elevate in locally within neighborhood of their receptors, we have to evaluate local PGE₂ distribution in liver. Further investigation for local PGE₂ distribution in liver using imaging MS may help us to understand an impact of elevated hepatic PGE₂ levels on liver injury. To investigate the mechanism of elevation of hepatic PGE₂ levels in CBZ-induced liver injury, we divided the mice treated CBZ into responders and non-responders that presented plasma ALT levels higher and less than 1 000 IU/L, respectively.

The liver is considered as one of the major organs for removing circulating PGE₂, and 15-PGDH is a major enzyme for PGE₂ inactivation in the liver (Dawson et al. 1970; Zhang et al. 2015). In our previous study, hepatic PGE₂ levels were mainly increased following the downregulation of hepatic 15-PGDH expression in CCl₄-induced liver injury mice (Shimada et al. 2020). Although PGE₂ is generally increased by the upregulation of inducible enzymes related to PGE₂ synthesis, including COX2 and mPGES-1, under various pathological conditions, induction of their hepatic expression could not be detected in CCl₄-induced liver injury mice. Furthermore, genetic deletion or chemical inhibition of constitutive COX1 could exacerbate CCl₄-induced acute liver injury (Xiao et al. 2015). Conversely, no effect was observed following inducible COX2 inhibition (Ekor et al. 2013). Thus, 15-PGDH-mediated PGE₂ inactivation appears to be crucial for the regulation of the hepatic PGE₂ levels during CCl₄-induced liver injury. Additionally, despite significantly increased hepatic PGE₂ levels in responders (Figure 1C), hepatic expression of enzymes related to PGE₂ synthesis was not induced (Figure 2). Conversely, hepatic 15-PGDH expression and activity were downregulated in responders (Figure 3). These results indicate that the downregulation of 15-PGDH-mediated PGE₂ inactivation may be a critical factor for the regulation of hepatic PGE₂ levels following injury, irrespective of the hepatotoxicant. An increase in PGE₂ levels induced by 15-PGDH downregulation has been reported in several types of cancer cells or tissues presenting minimal COX2 expression (Hughes et al. 2008; Thiel et al. 2009; Zidar et al. 2009). As we were unable to detect *Ptgs2* mRNA expression in this study, the liver also revealed minimal COX2 expression. Therefore, PGE₂ levels in the tissue possess poor PGE₂ synthetic ability, which could be elevated by downregulating PGE₂ inactivation. As the volume of circulating PGE₂-containing blood flow into the liver is relatively higher than that in other tissues, the induction of PGE₂ synthesis may not be necessary to increase hepatic PGE₂ levels. Conversely, immunohistochemical studies have indicated that COX2 protein can be detected in some hepatocytes, and rarely in epithelial cells of intra-hepatic bile ducts in healthy humans (Zidar et al. 2009). Previous studies have indicated that adult hepatocytes fail to induce COX2 expression regardless of the pro-inflammatory factors used; only Kupffer and stellate cells retain the ability to express COX2 (Casado et al. 2007). It has been reported that mPGES-1 expression is mainly observed in migrated neutrophils in ischemic/reperfusion-induced liver injury mice (Nishizawa et al. 2018). As hepatocytes occupy most of the liver as

parenchymal cells, there exists a possibility a marginal increase in PGE₂ synthesis in non-parenchymal cells could not be detected by assessing the activity and expression of enzymes related to PGE₂ synthesis using whole liver homogenates from CBZ-induced liver injury mice. Thus, accurate analysis for identifying whether PGE₂ synthesis enzymes are upregulated in non-parenchymal cells during CBZ-induced liver injury can be performed using a cell sorter.

In this study, an increase in hepatic PGE₂ levels along with 15-PGDH downregulation was observed in responders, but not in non-responders. It is impossible to determine whether mice that originally downregulate 15-PGDH expression undergo CBZ-induced liver injury, or whether the CBZ-induced liver injury downregulates 15-PGDH expression. However, PGE₂ generally prevents or disrupts liver damage (Tsujii et al. 1993; Kuzumoto et al. 2005). Thus, 15-PGDH expression seems to be downregulated only in mice presenting CBZ-induced liver injury. If downregulation of 15-PGDH causes the increase in hepatic PGE₂ content in responders, the hepatic concentration of 15-ketoPGE₂ should be decreased. To prove this, we attempted to measure 15-ketoPGE₂ in the liver by LC-MS/MS, but we could not detect it. 15-ketoPGE₂ seems to be converted quickly into further metabolites. Therefore, we believe that further studies are needed to prove that the increase in hepatic PGE₂ levels in responders is due to a decrease in 15-PGDH expression.

In addition to 15-PGDH-mediated degradation, PGE₂ uptake from plasma may be one of the regulatory factors for hepatic PGE₂ level. Although we did not measure plasma PGE₂ levels in this study, we cannot ignore the possibility that the increase in hepatic PGE₂ levels is related to the increase in plasma PGE₂ levels in the responders. Recent studies, including ours, have indicated that transporters are involved in the regulation of PGE₂ disposition in several tissues. Notably, genetic deletion of organic anion transporting polypeptide (OATP) 2A1 alters PGE₂ disposition in the brain and lungs (Nakanishi et al. 2015; Nakamura et al. 2018). OATP2A1 contributes to both PGE₂ secretion and uptake in various cells, including macrophages and endothelial cells (Shimada et al. 2015; Kasai et al. 2016; Nakanishi et al. 2017). Although OATP2A1 knockout demonstrates no significant effect on hepatic PGE₂ levels in lipopolysaccharide-induced inflammatory model mice (Gose et al. 2016), OATP2A1 is expressed in Kupffer and endothelial cells in the liver (Shimada et al. 2020). To comprehensively clarify the PGE₂ regulatory mechanism in the liver, the role of OATP2A1 in the regulation of hepatic PGE₂ disposition warrants further investigation.

In conclusion, downregulation of 15-PGDH expression seems to be involved in the elevation of hepatic PGE₂ levels in CBZ-induced liver injury. As hepatic PGE₂ possesses a hepatoprotective effect, individual variation in 15-PGDH activity may impact the incidence of drug-induced idiosyncratic liver injury. Further investigation is necessary to clarify the underlying mechanism of development of CBZ-induced liver injury and whether alteration of endogenous hepatic PGE₂ amount contributes to the process of it.

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