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Cytochrome P450 2E1 is responsible for the initiation of 1,2-dichloropropane-induced liver damage

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Abstract

1,2-Dichloropropane (1,2-DCP), a solvent, which is the main component of the cleaner used in the offset printing companies in Japan, is suspected to be the causative agent of bile duct cancer, which has been recently reported at high incidence in those offset printing workplaces. While there are some reports about the acute toxicity of 1,2-DCP, no information about its metabolism related to toxicity in animals is available. As part of our efforts toward clarifying the role of 1,2-DCP in the development of cancer, we studied the metabolic pathways and the hepatotoxic effect of 1,2-DCP in mice with or without cytochrome P450 2E1 (CYP2E1) activity. In an *in vitro* reaction system containing liver homogenate, 1,2-DCP was only metabolized by liver tissue of wild-type mice but not by that of *cyp2e1*-null mice. Furthermore, the kinetics of the solvent in mice revealed a great difference between the two genotypes; 1,2-DCP administration resulted in dose-dependent hepatic damage, as shown biochemically and pathologically, but this effect was only observed in wild-type mice. The nuclear factor κB p52 pathway was involved in the liver response to 1,2-DCP. Our results clearly indicate that the oxidative metabolism of 1,2-DCP in mice is exclusively catalyzed by CYP2E1, and this step is indispensable for the manifestation of the hepatotoxic effect of the solvent.

Keywords

1,2-Dichloropropane; CYP2E1; metabolism; hepatotoxicity; DNA damage

Introduction

1,2-Dichloropropane (1,2-DCP), also known as propylene dichloride, is a chemical intermediate that is used as a solvent in industry and as an insecticidal fumigant in

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agriculture (Bruckner et al., 1989). Acute exposure to 1,2-DCP can cause damage to the liver and kidneys following accidental uptake in humans and by a variety of routes in animals (Fiaccadori et al., 2003; Ministry of Health, Labour and Welfare, Japan, 2013a). A long-term inhalation experiment with 1,2-DCP resulted in the development of lung cancer in rats (Umeda et al., 2010), and 2 years of exposure to 1,2-DCP significantly increased the combined incidence of bronchioloalveolar adenomas and carcinomas in female mice and the incidence of Harderian gland adenomas in male mice (Matsumoto et al., 2013).

High incidence of bile duct cancer (cholangiocarcinoma) was recently reported among employees of an offset printing company in Osaka, Japan (Kumagai et al., 2013). In a later nationwide survey sponsored by the government, more cancer cases were found in this as well as other offset printing factories (Ministry of Health, Labour and Welfare, Japan, 2013a). The extremely high incidence, especially among young workers (25–45 years old), suggested that some occupational factors in the workplaces may play an important role in the increased occurrences of this type of cancer.

From employee interviews and supplier certificates, 1,2-DCP was found to be used as a cleaner for inks in large amounts in the factory in Osaka as well as other offset printing factories. Other solvents, such as dichloromethane and 1,1,1-trichloroethane, were also components used in the cleaner at different periods of time. However, 1,2-DCP was the only chemical that coincided with the occurrence of all cholangiocarcinoma cases, either as a single solvent or in a mixture with dichloromethane or 1,1,1-trichloroethane (Kumagai, 2014). The large quantities of 1,2-DCP used and the high rate of evaporation of 1,2-DCP, as well as the poor ventilation in the facilities, resulted in high concentrations of this solvent in workplaces, as demonstrated in an experiment that reproduced the working environment of the proof-printing room in that factory (Ministry of Health, Labour and Welfare, Japan, 2012, 2013b). Furthermore, many of the employees diagnosed with or without cholangiocarcinoma had medical records of acute hepatitis, as indicated by high levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the blood during the period they were working in the factory (Kumagai et al., 2013). This is thought to be mainly due to their exposure to high levels of 1,2-DCP.

There is little information available regarding the relationship between the metabolic routes and the toxicity of 1,2-DCP *in vivo*. With reconstituted systems containing purified human cytochrome P450 2E1 (CYP2E1), Guengerich et al. (1991a) found that 1,2-DCP was metabolized to a product that could be trapped as a glutathione (GSH) conjugate. Imberti et al. (1990) reported that oral administration of 1,2-DCP in rats caused a loss of hepatic GSH and an increase in the degree of liver damage, concluding that GSH plays a critical role in modulating the toxicity of the solvent. The CYP2E1-mediated oxidative pathway and a GSH conjugation pathway affect the metabolism of 1,2-DCP, although it is still unclear whether they occur simultaneously and/or in sequence.

In an effort to clarify the role of 1,2-DCP in the induction and development of cholangiocarcinoma, we studied the metabolic pathways of 1,2-DCP, the hepatic damage induced by this chemical as well as the genotoxic effect of 1,2-DCP in the liver of mice of wild-type and *cyp2e1* gene knockout mice. Our results clearly indicate that the oxidative

metabolism of 1,2-DCP in mice is exclusively catalyzed by CYP2E1 and that this step is indispensable for the manifestation of the hepatotoxic effect of the solvent.

Materials and methods

Animals and reagents

The animal experiments were conducted following the guidelines for animal experiments of the National Institute of Occupational Safety and Health. Male *cyp2e1*$^{-/-}$ mice were obtained from a colony developed in the laboratory of Dr Gonzalez (Lee et al., 1996) at the National Cancer Institute (Bethesda, Maryland, USA). Male *cyp2e1*$^{+/+}$ mice were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). Both mouse strains were from a 129Sv background. Male B6C3F1 mice were purchased from Charles River Japan (Yokohama, Japan). Mice were housed in a controlled environment with a 12-h light–12-h dark cycle and provided with tap water and CE-2 pellets (CLEA Japan, Inc., Osaka, Japan) *ad libitum*. Animals were allowed to acclimatize for at least 1 week prior to experimentation. 1,2-DCP (>98.5% in purity) was purchased from Tokyo Chemical Industries Co. Ltd (Tokyo, Japan).

Animal treatments

1,2-DCP was administered to 8–9-week-old *cyp2e1*$^{-/-}$ and *cyp2e1*$^{+/+}$ mice intraperitoneally (i.p.) at the doses of 100, 200, and 300 mg/kg body weight (BW) (low, medium, and high dose, respectively). Control mice were administered corn oil. Six mice were included in each group. Animals were killed under anesthesia 16 h after the treatment. Blood samples were collected and the liver was harvested immediately for biochemical and histological analyses.

Hematological and biochemical examinations

All blood samples obtained were mixed with 15% ethylenediaminetetraacetic acid (EDTA)–dipotassium anhydrate before hematological examination was conducted with the Celltac α automatic blood cell counter (Nihon Kohden Corporation, Tokyo, Japan). Plasma was separated from the blood, and AST and ALT levels were determined with an automatic analyzer (LABOSPECT 003, Hitachi Ltd, Tokyo, Japan).

Histopathological examination

A slice from each liver sample was fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at a thickness of 4 μm. Sections were stained with hematoxylin–eosin for microscopic examination.

Alkali comet assay

Approximately 25 mg of liver tissue from each mouse was cut and minced with scissors to release the cells in 1 ml of mincing buffer (20 mM EDTA–disodium anhydrate and 10% dimethyl sulfoxide in Hanks' balanced salt solution (calcium ion and magnesium ion free, pH 7.5) and stored at –80°C until analysis. An alkali comet assay was conducted as described previously (Weng et al., 2012). At least 100 cells were scored per sample. Tail intensity (TI) was measured for each nucleus scored as an index of DNA damage.

In vitro *metabolism assay*

Untreated 8–9-week-old *cyp2e1*$^{+/+}$ and *cyp2e1*$^{-/-}$ mice (n = 5 per strain) were killed, with a portion of their liver homogenized (10% w/v) with 1.15% potassium chloride in a Teflon-glass homogenizer. The homogenate was then centrifuged at 10,000g for 10 min and 50 μl of the resultant supernatant was added to a reaction system containing cofactors (2 mM nicotinamide adenine dinucleotide (NAD^+), 2 mM nicotinamide adenine dinucleotide phosphate ($NADP^+$), 2 mM GSH, 4 mM glucose-6-phosphate, and 10 mM magnesium chloride in 0.08 M phosphate buffer, pH 7.4). After a 5-min preincubation at 37°C, 10μl 1,2-DCP solution (20 μl/dl) containing the internal standard (tetrahydrofuran (THF)) was added to the system to a final volume of 0.26 ml. The reaction was run for up to 10 min at 37°C and then stopped by heating. The reaction times and protein content used were within the linear range of the reaction rate. Vials with reaction times of 2, 4, 6, 8, and 10 min were analyzed for decrease in 1,2-DCP using the headspace gas chromatography method as reported previously (Weng et al., 2013). Protein concentration in the liver homogenate was measured with a Pierce bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Waltham, Massachusetts, USA) and used for correction of the metabolic rate.

In vivo *metabolism analysis*

1,2-DCP at a dose of 300 mg/kg BW was administered i.p. to *cyp2e1*$^{+/+}$ and *cyp2e1*$^{-/-}$ mice (n = 5 per group). Blood samples obtained from the tail vein were collected in the headspace vials at 10, 20, 30, 60, 90, 120, 180, and 240 min after 1,2-DCP administration. THF solution was added to the vials, which were then tightly capped. 1,2-DCP levels were determined by headspace gas chromatography as mentioned above. The terminal half-life ($t_{1/2}$) was calculated from the peak time to 240 min, while the area under the curve (AUC) of the blood 1,2-DCP concentration from 10 min to 240 min after treatment was analyzed by the trapezoidal method for each mouse strain.

NF-κB transcription factor assays in nuclear protein extracts

For the cytokine assay, sections of the liver were homogenized with tissue protein extraction reagent (Thermo Scientific, Rockford, Illinois, USA) containing protease inhibitors. The nuclear protein extract from sections of frozen livers was prepared using a CelLytic NuCLEAR extraction kit (Sigma Aldrich, St Louis, Missouri, USA) for the transcription factor assays. The protein concentration of the liver homogenates and nuclear protein extracts was measured with the Pierce BCA protein assay kit (Thermo Scientific). Activation of nuclear factor (NF-κB) was measured in the nuclear protein extracts of the liver using TransAM NF κB p52, p50, and p65 kits according to the manufacturer's instructions (Active Motif, Inc., Carlsbad, California, USA).

Statistical analysis

Two-way analysis of variance (ANOVA) was used to evaluate the effects of genotypes and 1,2-DCP doses. Comparisons among multiple groups within the same genotype were analyzed by one-way ANOVA. If the ANOVA was statistically significant, a Dunnett's *post hoc* test was performed to determine which exposure group was different from the

corresponding control group. Statistical significance was set at $p < 0.05$. A logarithmic transformation was applied to plasma ALT and AST activities before statistical analysis.

Results

Liver and BW

Table 1 shows the changes in liver weight and liver/BW ratios following 1,2-DCP treatment. After injec-tion of 1,2-DCP, one mouse of *cyp2e1*$^{+/+}$ died in the 300 mg/kg BW group. The liver weight of *cyp2e1*$^{+/+}$ mice was significantly higher in the 300 mg/kg BW group than in the control group, while the liver/BW ratios of *cyp2e1*$^{+/+}$ mice at 200 and 300 mg/kg BW groups were also significantly greater than the control group. In contrast, *cyp2e1*$^{-/-}$ mice did not exhibit changes in the liver weight at any 1,2-DCP dose, although the liver/BW ratio in the 200 mg/kg BW group was significantly higher than in the controls.

Biochemical and hematological changes

Plasma AST and ALT were used to assess 1,2-DCP-induced liver damage. In *cyp2e1*$^{+/+}$ mice, plasma AST activities were significantly increased at the high dose of 1,2-DCP (genotypes: $F = 20.597$, $p < 0.001$; dose: $F = 20.218$, $p < 0.001$; genotype + dose: $F = 9.528$, $p < 0.001$) and ALT activities at both the medium and the high-dose groups (genotypes: $F = 44.981$, $p < 0.001$; dose: $F = 30.939$, $p < 0.001$; genotype + dose: $F = 23.459$, $p < 0.001$; Table 1). However, in *cyp2e1*$^{-/-}$ mice, no change was observed in the activities of the two enzymes after 1,2-DCP treatment, even at the highest dose. The hematological examinations were not found to be influenced by 1,2-DCP administration (data not shown).

DNA damage in liver tissue

Comet assays were used to detect early DNA damage in the liver. However, it was found that TI value, the index of DNA damage extent, did not significantly increase at any dose of 1,2-DCP in either genotype mice (data not shown). Considering the sampling time as 16 h after the treatment (though sufficient for the pathological observations as shown below), DNA damage, if any, might have been repaired at this time point.

Histopathology

Remarkable pathological changes were observed in *cyp2e1*$^{+/+}$ mice in a dose-dependent manner 16 h after 1,2-DCP intraperitoneal administration. These changes included necrosis of hepatocytes especially around the central veins of the hepatic lobule, ballooned hepatocytes around the portal veins, and nucleolar hypertrophy between the two zones (Figure 1(c)). To a lesser extent, these changes were also found in the 200 mg/kg BW group, but there was no such obvious change in the 100 mg/kg BW group (Figure 1(f) and (g)). However, in *cyp2e1*$^{-/-}$ mice, only minor changes such as hypertrophy of nucleoli were observed in the high-dose 1,2-DCP treatment group (Figure 1(d)), and there was even no change in the medium-and low-dose groups (data not shown). In addition, moderate-to-severe macrovesicular steatosis and congestion of blood were also observed in *cyp2e1*$^{+/+}$, but not *cyp2e1*$^{-/-}$, mice dosed with 1,2-DCP at 200 and 300 mg/kg BW (Figure 1(g) and (h)).

In vitro *and* in vivo *metabolism of 1,2-DCP*

To investigate differences in metabolism of 1,2-DCP in the liver of *cyp2e1*$^{+/+}$ versus *cyp2e1*$^{-/-}$ mice, we conducted *in vitro* assays with the liver homogenate as the enzyme source. The metabolic rate in *cyp2e1*$^{+/+}$ mice was 21.86 nmol/min/mg protein, while it was only marginally detectable (0.22 nmol/min/mg protein) in *cyp2e1*$^{-/-}$ mice (Figure 2), suggesting that the first step in the metabolism of 1,2-DCP is exclusively catalyzed by CYP2E1 in mouse liver.

The decay curves of 1,2-DCP in blood after intraperitoneal administration of 1,2-DCP at 300 mg/kg BW are shown in Figure 3. In *cyp2e1*$^{+/+}$ mice, blood concentration of 1,2-DCP peaked at 10 min, the first sampling time point, and decayed quickly from the blood, with marginal value at 240 min, suggesting that the clearance rate exceeded absorption rate around 10 min in mice of this type. On the other hand, in *cyp2e1*$^{-/-}$ mice, the blood concentration of 1,2-DCP increased gradually, reached its peak at 60 min, and then declined. The mean maximum concentrations of blood 1,2-DCP were 16.18 and 33.94 nmol/ml for *cyp2e1*$^{+/+}$ and *cyp2e1*$^{-/-}$ mice, respectively. The $t_{1/2}$ of blood 1,2-DCP after administration was 47.51 and 50.97 min, respectively. The AUC calculated from 10 min to 240 min was 1398 for *cyp2e1*$^{+/+}$ mice but increased to 3878 in *cyp2e1*$^{-/-}$ mice. These results clearly indicate that 1,2-DCP was cleared from the body of *cyp2e1*-null mice much slower than in wild-type mice.

Hepatic NF-κB p52 in mice after 1,2-DCP treatment

NF-κB p52 nuclear-binding activities after intraperitoneal administration of 1,2-DCP are shown in Figure 4. In *cyp2e1*$^{+/+}$ mice, NF-κB p52 binding activities were significantly increased at the high dose of 1,2-DCP. However, in *cyp2e1*$^{-/-}$ mice, no change was observed in binding activities after 1,2-DCP treatment even at the high dose. On the other hand, no change was observed in NF-κB p50 and p65 binding activities after 1,2-DCP treatment for either genotype (data not shown).

Discussion

In this study, we found that CYP2E1 is responsible for the occurrence of 1,2-DCP-induced hepatic lesions in mice expressing this enzyme, as shown both biochemically and pathologically. In *cyp2e1*$^{-/-}$ mice that lack this enzyme, no obvious hepatic lesions were observed even after treatment with a high dose of 1,2-DCP. The observation of necrosis of hepatocytes was localized within the perivenular region of liver lobule, consistent with the perivenous expression of CYP2E1 (Lieber, 1997). These results suggest that CYP2E1 mediates a metabolic step of 1,2-DCP, leading to the occurrence of liver damage.

We also found that CYP2E1 is the only enzyme involved in the oxidative reaction of the solvent in mouse liver. Our results are in agreement with the findings from the reconstituted systems containing purified human P450 2E1 by Guengerich and his colleagues (1991a, 1991b, 1980). Their *in vitro* experiments demonstrated that 1,2-DCP was only metabolized in liver tissue from mice expressing CYP2E1, but not *cyp2e1*-null mice. Other forms of cytochrome P450, including CYP1A2, CYP2A1, CYP2C6, and CYP3A1, are known to be

expressed in the liver of *cyp2e1*$^{-/-}$ mice (Lee et al., 1996), though their metabolizing capacity is less than half that of CYP2E1 (Lee et al., 1996; Ramdhan et al., 2008). However, it seems that these forms of P450 are not involved in the metabolism of 1,2-DCP, whereas CYP2E1 shows high substrate specificity for the solvent.

NF-κB represents a family of dimeric transcription factors, which in mammals comprises p65, RelB, c-Rel, p50, and p52. Two major types of signaling pathways have been implicated in NF-κB activation. A variety of stimuli induce the classical pathway with rapid phosphorylation and subsequent degradation of IκB and p105, IκBα is the primary regulator of p65- and p50 and p50 complexes (Liu and Chen, 2011; Scheidereit, 2006). A subset of inducers stimulates the alternative pathway, where IkappaB kinase (IKK) α-mediated phosphorylation and processing of p100, generating p52/RelB heterodimers' (Scheidereit, 2006; Sun and Ley, 2008). NF-κB p52-messenger RNA (mRNA) expression and its significantly positive correlations with plasma ALT activity levels have been observed in a study of trichloroethylene exposure, suggesting the involvement of p52 in mediating liver damage (Ramdhan et al., 2008). In this study, NF-κB p52-binding activities were significantly increased for the high-dose 1,2-DCP treatment group of *cyp2e1*$^{+/+}$ mice, suggesting that the alternative NF-κB p52 pathway could lead to 1,2-DCP-induced liver damage. However, we did not observe a correlation between NF-κB p52 mRNA expression and plasma ALT activity levels. In this study, it was showed that levels of the NF-κB p50 and p65 binding activities were not changed, after 1,2-DCP treatment for either genotype (data not shown). In the study of the hepatotoxicity of trichloroethylene, NF-κB p50 and p65 signaling were inhibited by trichloroacetic acid, one of the trichloroethylene metabolites, via peroxisome proliferator-activated receptor-α PPARα agonist way (Maloney and Waxman, 1999; Ramdhan et al., 2008;). However, we did not investigate the activation of PPARα, and it is not known whether 1,2-DCP shares the same way as trichloroethylene in the induction of hepatic injury. In the future, it might be necessary to investigate the relation of 1,2-DCP metabolites and PPARα/NF-κB signaling.

Ethanol-inducible CYP2E1 is known to be involved in the oxidation of many low-molecular-weight cancer suspects (Guengerich et al., 1991a) and also plays a key role in the metabolism and activation of toxic substrates such as toluene, chloroform (Lipscomb et al., 2004; Wang and Nakajima, 1991), and carbon tetrachloride (Lin et al., 2012) and drugs like acetaminophen (Bajt et al., 2011; Comporti et al., 2010; Liang et al., 2012). It is generally believed that the generation of reactive oxygen species from CYP2E1-mediated oxidation can lead to membrane lipid peroxidation and cell toxicity, meaning that excessive exposure to various chemicals can result in hepatic injury (Lieber, 1997). Imberti et al. (1990) reported that the administration of 1,2-DCP caused GSH depletion in rat liver and blood, which was associated with marked increase in serum ALT and AST levels, and that pretreatment with the GSH-depleting agent buthionine sulfoximine enhanced the hepatotoxicity of 1,2-DCP. A GSH conjugation reaction follows the CYP2E1-mediated oxidation reaction, though the specific molecular species of GST involved has yet to be identified. An intermediate existing between the oxidation and conjugation reactions may be a candidate for the hepatotoxicity of 1,2-DCP. The metabolism of other halogenated organic solvents, such as 1,2-dichloroethane and 1,2-dibromoethane, generally encompasses two routes, namely, the CYP pathway and GSH pathway (Jones and Gibson, 1980; Ross and

Pegram, 2003; Thomas et al., 2001; Watanabe et al., 2007). The former oxidative pathway is usually high affinity with limited capacity, which is saturated at relatively low solvent concentrations in rats. In contrast, the latter GSH-dependent pathway has low affinity for solvents but does not appear to become saturated even at high concentrations (Tornero-Velez et al., 2004). A similar metabolic pathway for 1,2-DCP has been proposed as well (Bartels and Timchalk, 1990). However, our finding of undiminished substrate concentration in the liver homogenate reaction system from *cyp2e1*$^{-/-}$ mice implies that a direct GSH conjugation pathway for 1,2-DCP may not exist. However, this needs to be investigated in detail. Oxidation of 1,2-DCP to 1-chloro-2-hydroxypropane and ultimately the mercapturic acid, N-acetyl-S-(2-hydroxypropyl)cysteine, is an established metabolic pathway of 1,2-DCP (Jones and Gibson, 1980). However, details remain unknown as to the relation of specific metabolite production and the occurrence of liver damage. In this study, we selected intraperitoneal administration as the exposure way by which the chemical is rapidly absorbed and then metabolized in the liver. The exposure pathways that are inferred from the workplace are primarily inhalation routes by which the chemical enters the blood through the lungs and then metabolized in the liver. With the intraperitoneal administration as an alternative way to inhalation exposure, we tried to focus on the action of CYP2E1 after systemic circulation in the metabolism of the liver. Furthermore, we set the highest dose at approximately one-third of the median lethal dose obtained in mice from oral administration of 1,2-DCP (Ministry of Health, Labour and Welfare, Japan, 2013a).

Overall, our study demonstrated that the oxidative metabolism of 1,2-DCP is exclusively catalyzed by CYP2E1 in mice. While further studies on the subtypes of other enzymes like GSTs and detailed genotoxicity of 1,2-DCP are necessary, our results reveal clues to the possibility and mechanism of 1,2-DCP exposure leading to human cholangiocarcinoma in the offset printing industry.

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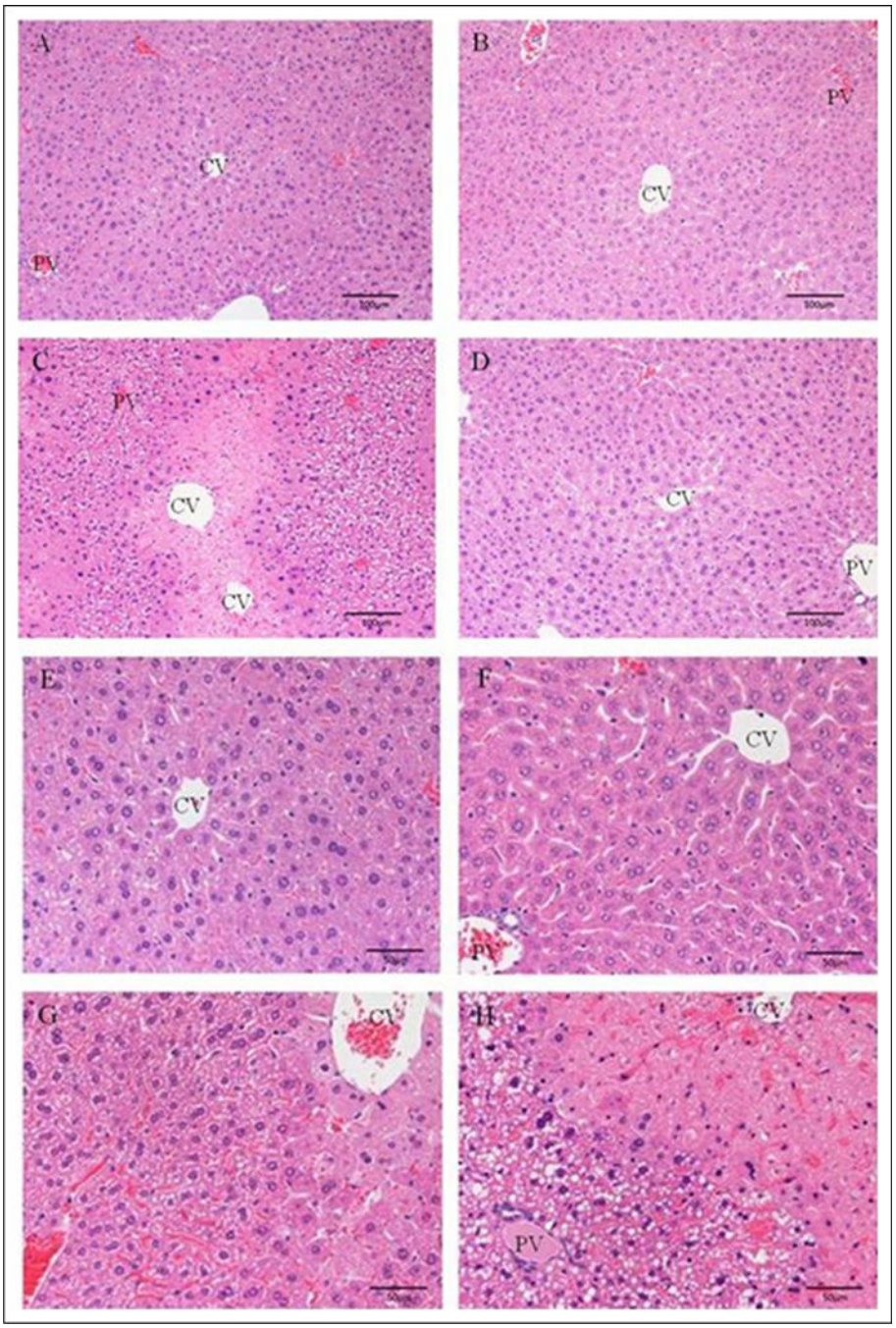

Figure 1.

Liver histopathological findings after 1,2-DCP treatment. All sections were stained with H&E. (a and b) Liver tissue of untreated *cyp2e1*$^{+/+}$ and *cyp2e1*$^{-/-}$ mice, respectively. (c) Liver tissue of representative *cyp2e1*$^{+/+}$ mouse treated with 1,2-DCP at 300 mg/kg BW. Moderate necrosis around the centrilobular area and microvesicular steatosis around the portal vein were observed. (d) Liver tissue of representative *cyp2e1*$^{-/-}$ mouse treated with 1,2-DCP at 300 mg/kg BW. No obvious histological changes were found. Portal vein (PV); central vein (CV); original magnification ×200. Sections (e–h) are images at original

magnification 400. (e) Liver tissue of representative untreated *cyp2e1*$^{+/+}$ mouse. (f–h) Liver tissue of representative *cyp2e1*$^{+/+}$ mice treated with 1,2-DCP at 100, 200, and 300 mg/kg BW, respectively. In addition to necrosis and microvesicular steatosis, congestion of blood was also observed. 1,2-DCP: 1,2-dichloropropane; H&E: hematoxylin–eosin; BW: body weight.

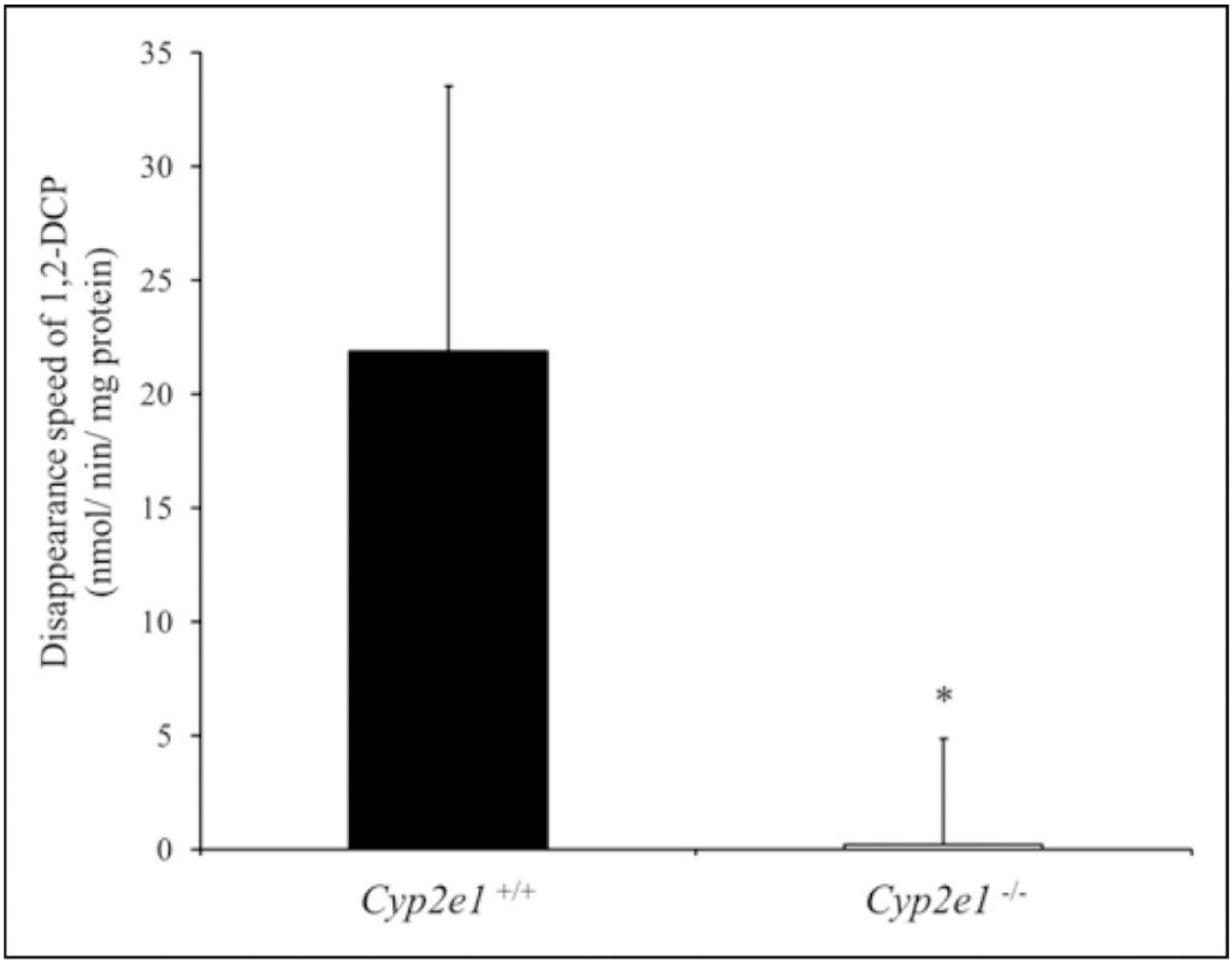

Figure 2.

In vitro metabolism of 1,2-DCP. Each bar represents the mean ± SD of five mice. *$p < 0.01$ (t-test): significantly different from the mean value of the wild-type mice. 1,2-DCP: 1,2-dichloropropane.

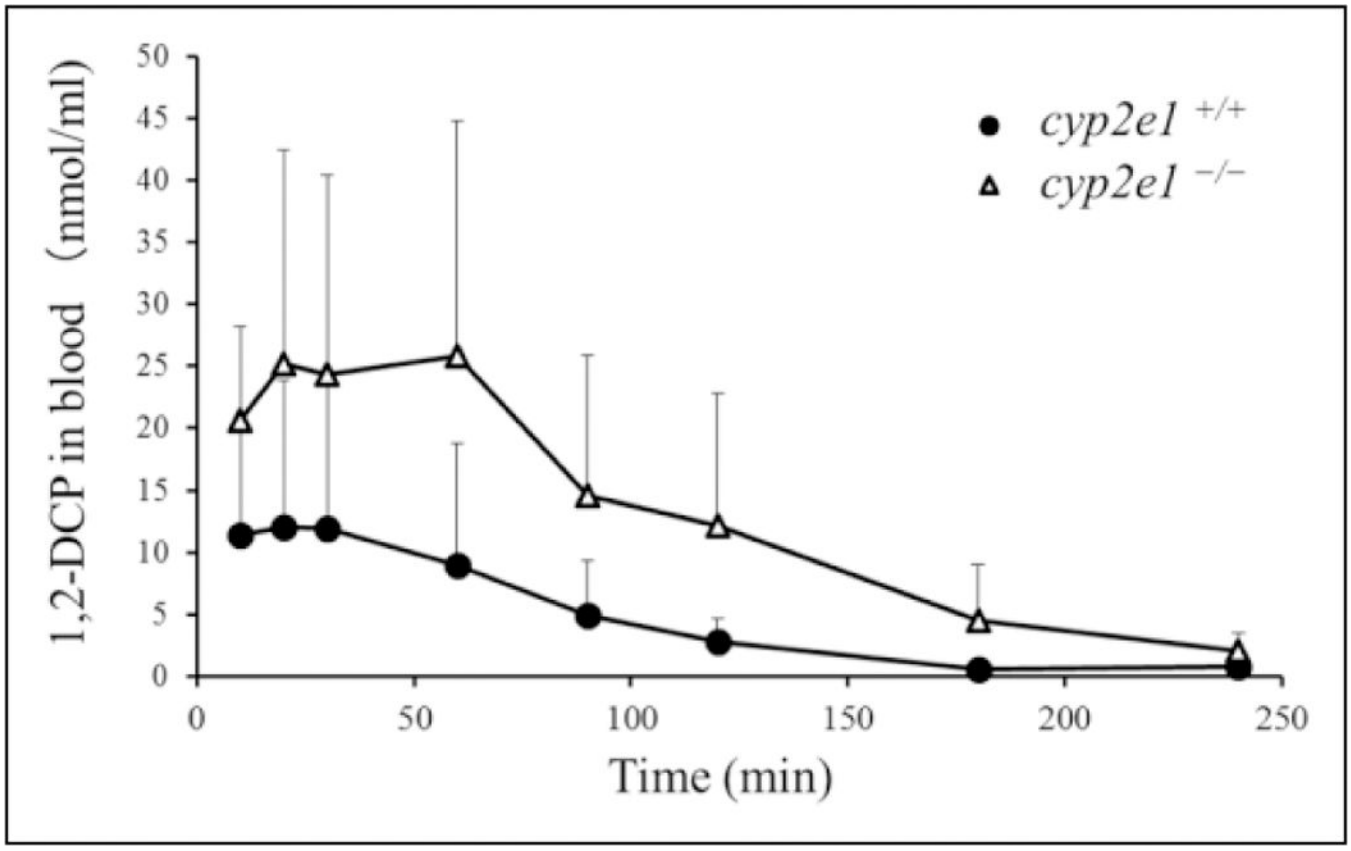

Figure 3.

Decay curve of 1,2-DCP in blood after intraper-itoneal administration at a dose of 300 mg/kg to *cyp2e1*$^{+/+}$ (circle) and *cyp2e1*$^{-/-}$ mice (triangle). Each point represents the mean for five mice. 1,2-DCP: 1,2-dichloropropane.

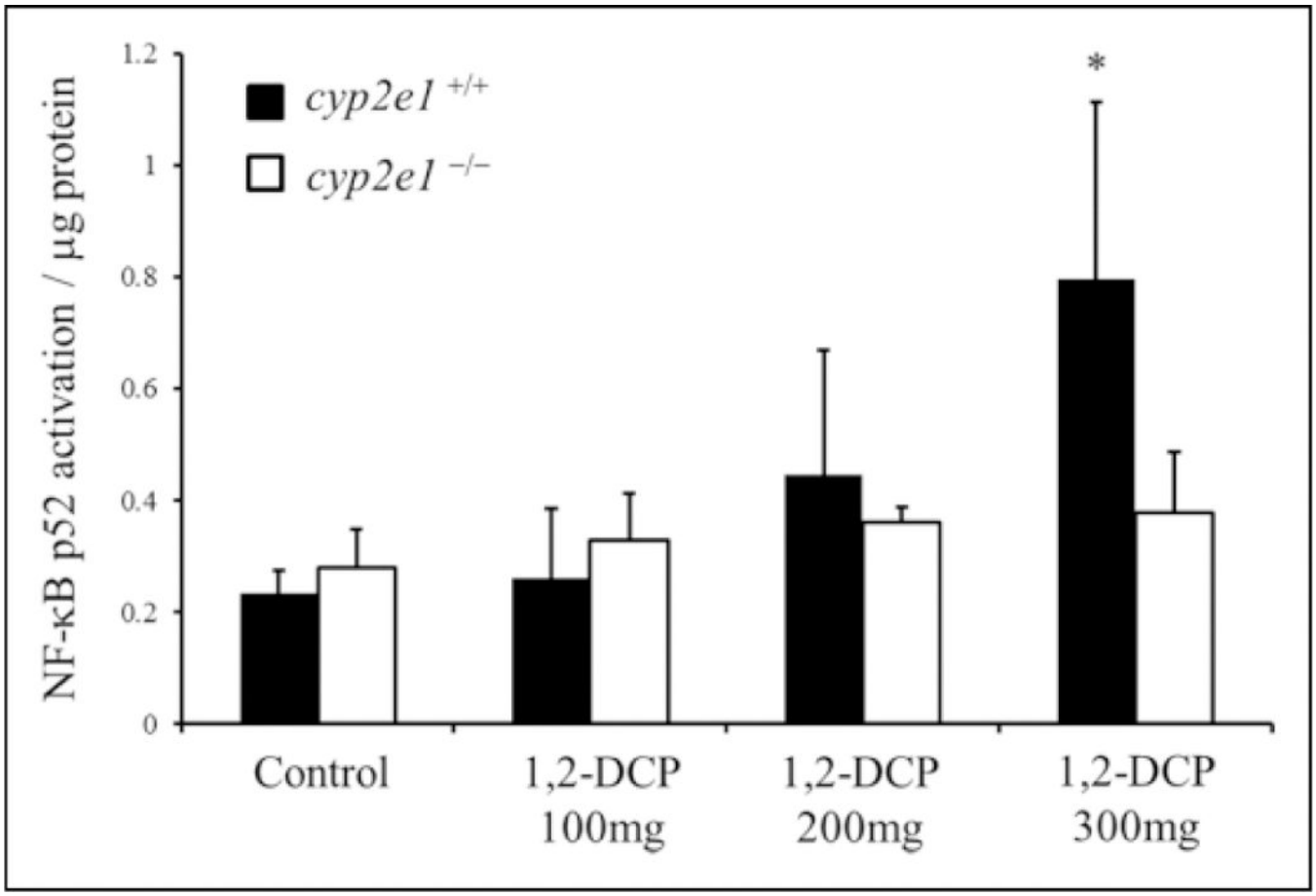

Figure 4.

Activation of NF-κActivation of NF-loropropane. epresents the mean for d you ± SD of five to six mice. *$p < 0.05$: versus control (Dunnett's *post hoc* test); statistically significant. Comparisons among multiple groups with the same genotypes were made using the one-way ANOVA test. If the ANOVA was statistically significant, Dunnett's *post hoc* test was performed to determine which exposure group was different from the corresponding control group. NF: nuclear factor; ANOVA: analysis of variance.

Table 1.

Liver weight and blood biochemical parameters after treatment with 1,2-DCP.[a]

Genotype	1,2-DCP (mg/kg i.p.)	Liver weight (g)	Liver weight/BW (%)	AST (IU/l)	ALT (IU/l)
cyp2e1$^{+/+}$	0	0.91 ± 0.06	4.38 ± 0.35	62.5 ± 9.57	18.5 ± 3.5
	100	0.90 ± 0.08	4.19 ± 0.14	68.7 ± 6.07	17 ± 2.89
	200	1.03 ± 0.11	4.68 ± 0.53[b]	207.2 ± 287.39	214.3 ± 357.55[b]
	300	1.09 ± 0.14[b]	4.88 ± 0.31[b]	46540.4 ± 85374.43[b]	4010 ± 4240.77[b]
Cyp2e1$^{-/-}$	0	0.96 ± 0.17	4.25 ± 0.69	55.0 ± 7.98	22.0 ± 6.0
	100	1.24 ± 0.23	5.05 ± 0.55	52.5 ± 13.07	21.3 ± 6.6
	200	1.30 ± 0.23	5.17 ± 0.84[b]	55.3 ± 18.51	17.2 ± 3.0
	300	1.20 ± 0.15	4.97 ± 0.43	62.5 ± 9.57	30.3 ± 14.23

1,2-DCP: 1,2-dichloropropane; i.p.: intraperitoneally; AST: aspartate aminotransferase; ALT: alanine aminotransferase. Cyp2e1: cytochrome P450 2E1; BW: body weight.

[a]Data for each parameter represent the mean ± SD of five mice in *cyp2e1*$^{+/+}$ 300 mg/kg group and six mice in other groups.

[b]$p < 0.05$ (Dunnett's *post hoc* test): significantly different from the mean value of the control group