Biochemical and Biophysical Research Communications 460 (2015) 308–313

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Hepatoprotective effect of phosphatidylcholine against carbon tetrachloride liver damage in mice

Ji-Young Na, Kibbeum Song, Sokho Kim*, Jungkee Kwon*

Department of Laboratory Animal Medicine, College of Veterinary Medicine, Chonbuk National University, Jeonju, Jeonbuk, 561-156, Republic of Korea

ARTICLE INFO

Article history:
Received 2 March 2015
Available online 14 March 2015

Keywords:
Phosphatidylcholine
Carbon tetrachloride
Hepatotoxicity
Oxidative stress
Fibrosis

ABSTRACT

It has been shown that phosphatidylcholine (PC) extracted from egg yolk possesses a variety of biological activities, such as anti-inflammatory and anti-oxidant effects, and prevents oxidative stress. The aim of this study was to evaluate the hepatoprotective effects of PC against carbon tetrachloride (CCl_4), which is a well-known hepatotoxicant that causes extensive oxidative liver damage, and to investigate the mechanisms involved in this protective effect. Mice were treated with PC (0.1 ml, 10 or 100 mg/kg, orally) once daily for 5 consecutive days prior to CCl_4 administration (0.1 ml, 20 mg/kg, intraperitoneally). The experimental data show that pretreatment with PC significantly prevented increases of serum aspartate transaminase, alanine transaminase, and alkaline phosphatase, and reduced reactive oxygen species levels. Histopathological evaluation of the liver also revealed that PC effectively ameliorated CCl_4-induced hepatic injury and fibrosis. In addition, PC significantly counteracted the increase in glutathione levels and glutathione-S-transferase activity induced by CCl_4. Concordantly, PC significantly decreased CCl_4-induced upregulation of apoptotic proteins in the liver. These results suggest that PC exerts its protective effects against CCl_4-induced hepatotoxicity via its activities as an anti-oxidant and free radical scavenger.

1. Introduction

Anti-oxidative action plays an important role in protection against carbon tetrachloride (CCl_4)-induced liver injury. Toxic chemicals, drugs, alcohol, and viral infections are known to cause liver injury. CCl_4 has been commonly used in animal models to study chemical toxin-induced liver injury [1]. Protective effects of various natural products against CCl_4 hepatotoxicity have been reported [2]. CCl_4-induced hepatotoxicity is characterized by varying degrees of hepatocyte degeneration and cell death [3], which are thought to result from the formation of reactive oxygen species (ROS), including superoxide and hydroxyl radicals, that are known to play an important role in liver disease pathology and the initiation of hepatic fibrogenesis [4,5]. ROS are associated with intoxication by CCl_4 [6]. A number of recent reports regarding CCl_4 and therapeutic agents targeting its effects suggest that exposure to this solvent causes acute liver injury [7–9]. One major defense mechanism for protection against and treatment of liver damage consists of reducing the production of reactive metabolites by raising the levels of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione (GSH), and decreasing lipid peroxidation [10,11].

Phosphatidylcholine (PC, 1,2-diacyl-sn-glycero-3-phosphocholine) is the main constituent of egg yolk and soybeans [12]. These molecules have additional bioactive properties, including anti-inflammatory [13], anti-oxidant, and anti-fibrotic activities [14]. Moreover, PC is known to inhibit fatty acid accumulation, and is used as a treatment for myocardial ischemia, cerebrovascular disease, dementia, and fatty liver-induced liver dysfunction [15]. Resent study shown that PC related to cytotoxicity and apoptosis induction in HepG2 cells [16].

Until now, PC studies have been limited to agricultural applications of the anti-inflammatory, anti-oxidant, anti-fibrotic, and antibiotic properties of this naturally produced compound. Although PC has various beneficial bioactive properties, its protective effect against CCl_4-induced hepatotoxicity has not previously been explored. Thus, the present study examines the effects

Abbreviations: PC, phosohocholine; CCl_4, carbon tetrachloride; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; GST, glutathione S-transferase; H&E, hematoxylin and eosin.

* Corresponding authors. Department of Laboratory Animal Medicine, College of Veterinary Medicine, Chonbuk National University, 664-14, Duckjin-Dong, Jeonju, Jeonbuk, 561-156, Republic of Korea. Fax: +82 63 270 3780.

E-mail addresses: raios@hanmail.net (S. Kim), jkwon@jbnu.ac.kr (J. Kwon).

http://dx.doi.org/10.1016/j.bbrc.2015.03.031
0006-291X/

of PC on mice treated with CCl_4 as a model for liver injury, evaluating the degree of hepatic lesions and the levels of anti-oxidant enzymes in the liver.

2. Materials and methods

2.1. Animals and experimental design

Male ICR mice (Seven weeks, n = 40) weighing 30–32 g were obtained from Dae Han Biolink (Daejeon, Korea). All animals were fed a standard laboratory diet and water *ad libitum*. They were maintained in micro-isolator cages under pathogen-free conditions on a 12 h light/12 h dark schedule for a week. The Animal Care and Use Committee of Chonbuk National University approved all experimental procedures involving animals. PC (Sigma, USA) in corn oil was orally administered at 10 mg or 100 mg/kg concentrations once daily for 5 consecutive days. Three hours after the final treatment, the mice were treated with CCl_4 (20 mg/kg, intraperitoneally (i.p.)) dissolved in corn oil (2.6%, v/v) as described previously (Lee et al., 2008). The control group animals were administered respective vehicles. Eighteen hours after CCl_4 administration, mice were anesthetized by isoflurane USP inhalation (Abbott Labs, Chicago, IL, USA), the abdominal cavity was opened, and approximately 1 ml of blood was removed by cardiac puncture to determine serum ALT, AST, and ALP activities. The blood was allowed to clot and the blood samples were centrifuged at 3000 rpm for 5 min at 4 °C. The samples were stored at −80 °C until they were analyzed for renal enzyme activity. The livers were excised from the mice, weighed, and frozen quickly on ice at −20 °C until GSH content and GST activity were assessed.

2.2. Serum biochemistry

Enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) as indicators of liver function were evaluated using a VETSTAT 8008 Chemistry Analyzer supplied by IDEXX (Seoul, Korea).

2.3. Hepatic GSH and GST activity

Livers were quickly removed, weighed, and perfused with ice-cold 0.15 M KCl, and then homogenized with 4 vol. (w/v) 10 mM Tris–HCl (pH 7.4) containing 0.15 M KCl, 0.1 mM EDTA, 1.0 mM dithiothreitol, and 0.01 mM phenylmethoxysulfonyl fluoride in a homogenizer. Hepatic microsomal fractions were obtained by differential centrifugation, and hepatic GSH levels were estimated colorimetrically using Ellman's reagent. Cytosolic GST activity was determined using 1-chloro-2,4-dinitrobenzene as the substrate. After incubation for a few seconds, the change in absorbance values was determined at an excitation wavelength of 340 nm.

2.4. Assessment of hepatic ROS generation

ROS were measured using the method of Ali [17]. Hepatic tissues were homogenized on ice with 1 mM ethylenediaminetetraacetic acid (EDTA)–50 mM sodium phosphate buffer (pH 7.4), and then 25 mM DCFH-DA (Invitrogen, Carlsbad, CA, USA) was added to the homogenates. After incubation for 30 min, the change in fluorescence values was determined at an excitation wavelength of 486 nm and emission wavelength of 530 nm.

2.5. Histopathological examination

For histopathological examination, liver samples fixed in 10% neutral buffered formaldehyde solution were embedded in paraffin and sectioned at 5 microns. The sections were deparaffinized with xylene, rehydrated with a graduated alcohol series to water, and stained with hematoxylin and eosin (H&E) and Masson's trichrome for collagen evaluation using conventional light microscopy.

2.6. Western blotting analysis

Total tissue extracts were prepared in RIPA buffer and then centrifuged at 15,000 RPM for 15 min at 4 °C. Quantification of the total protein was performed with BCA protein assay reagents (Bio-Rad Laboratories, Hercules, CA, USA). Proteins were resolved using sodium dodecyl sulfate polyacrylamide gel electrophoresis (12% acrylamide gels) and transferred to polyvinylidene difluoride membranes. After blocking in 5% skim milk in PBS with 0.1% Tween-20 (PBS-T), the membranes were incubated with specific primary antibodies for MDA (Abcam, Cambridge, MA, USA), Bcl-xL (Cell Signaling, Danvers, MA, USA), Bax (Santa Cruz Biotechnology, Santa Cruz, CA, USA), caspase-3 (Cell Signaling), and β-actin (Cell Signaling) diluted 1:1000 in 1% skim milk in PBS-T, overnight at 4 °C. After washing, the blots were incubated with peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Millipore, Temecula, CA, USA) diluted 1:10,000 in PBS-T at room temperature for 1 h. The signals were detected using the Supersignal West Dura extended duration substrate (Thermo, CA, USA) according to the manufacturer's instructions. Densitometric analysis was conducted directly from the blotted membrane using the Chemi Imager analyzer system (Alpha Innotech, San Leandro, CA, USA).

2.7. Statistical analysis

The control and CCl_4 groups were compared, and the CCl_4 and CCl_4 + PC groups were compared separately. Statistical analyses were performed using a Student's *t*-test and repeated-measures ANOVA followed by a Bonferroni test. The data are expressed as the mean ± SEM. Differences with p values <0.05 were considered statistically significant.

3. Results

3.1. Hepatic serum levels were decreased by PC

Biochemical measurement of liver function revealed that CCl_4 treatment induced a significant increase in plasma AST, ALT, and ALP levels, reaching 369.6, 259.3, and 133% of control values, respectively (Fig. 1). The treatment of mice with CCl_4 significantly increased the serum concentrations of AST, ALT, and ALP, while the serum levels of these same indicators were significantly attenuated in mice cotreated with PC.

3.2. ROS generation and MDA protein were suppressed by PC

As expected, CCl_4 induced a significant increase in ROS production compared to the control group (Fig. 2A). Concurrent treatment of mice with CCl_4 and PC significantly counteracted the oxidative stress effect of CCl_4. To evaluate the degree of oxidative injury, lipid peroxidation in liver tissue was determined by measuring the level of MDA, which is an end product of lipid peroxidation in liver damage [18]. As shown in Fig. 2B, a significant increase in MDA production was found in the livers of mice exposed to CCl_4, while pretreatment with PC significantly decreased MDA levels in liver tissue.

3.3. Hepatic GSH and GST activation were increased by PC

As oxidative stress in tissues generally involves the GSH system, we measured the level of GSH in livers from each test group. The

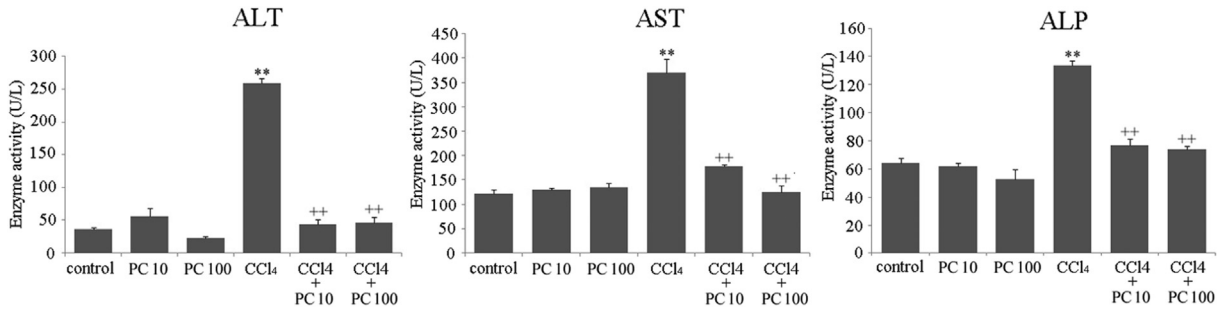

Fig. 1. Effects of PC on serum parameters associated with CCl_4-induced liver damage in mice. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) after co-treatment with CCl_4 (20 mg/kg) and PC (10 and 100 mg/kg). Data shown are the mean ± SEM (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. Control, $++p < 0.01$ vs. CCl_4.

treatment of mice with CCl_4 significantly diminished GSH, while co-treatment with PC significantly and dose-dependently prevented the GSH depletion induced by CCl_4 (Fig. 2C). In addition, decreased GST activity was observed in CCl_4-treated mice compared to the control group. Concurrent treatment of mice with PC and CCl_4 significantly counteracted the oxidative stress effect of CCl_4 (Fig. 2D). These results show that the protection afforded by PC against CCl_4-induced hepatotoxicity may be related to increased cellular GSH content and GST activity.

3.4. Liver histopathology and fibrosis

Histological evaluation by H&E stain showed significant differences among experimental groups (Fig. 3A). CCl_4 caused significant hepatocellular degeneration, including the formation of fibrous tissue infiltrated with inflammatory cells, compared to the control group, whereas these changes were ameliorated in CCl_4-exposed mice cotreated with PC relative to the CCl_4-alone group. Liver fibrosis was evaluated histologically by visualizing the blue color of collagen fibers using Masson's trichrome stain as an index of collagen accumulation. As shown in Fig. 3B, the CCl_4-treated mice had markedly numerous hepatic lobules surrounded by thick fibrotic tissue, resulting in the formation of continuous fibrotic septa, unlike the control group, whereas the area of fibrosis was markedly decreased in the mice cotreated with CCl_4 and PC.

3.5. Apoptotic protein production is decreased by PC

Production of the apoptotic protein Bax was higher in CCl_4-treated mice than in the control group, whereas levels of the anti-

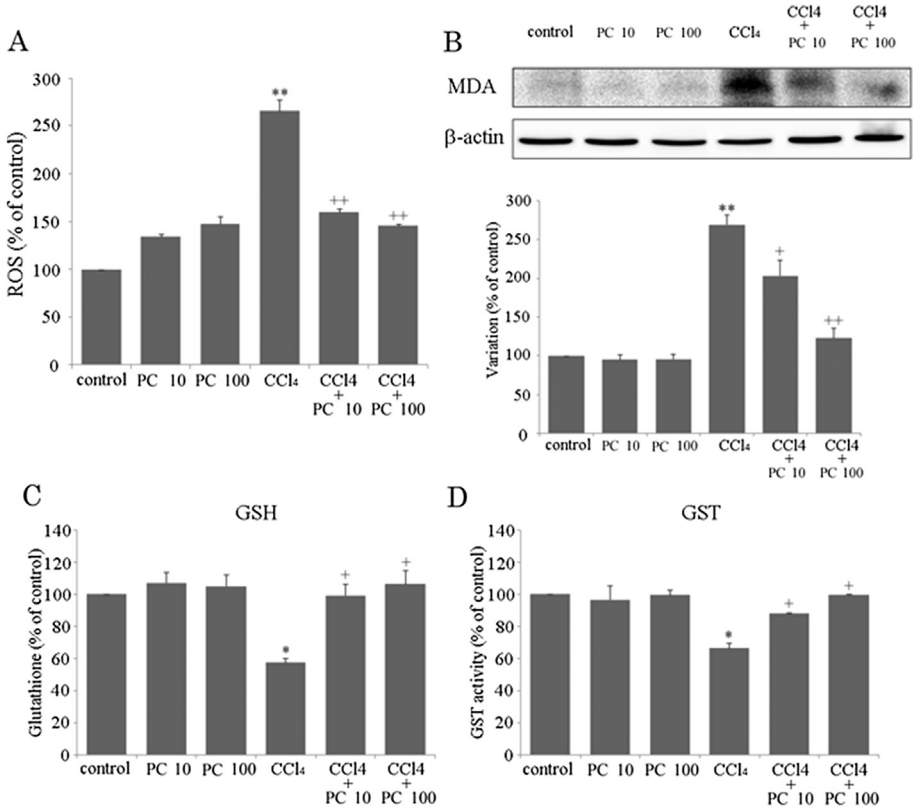

Fig. 2. The effect of PC on the generation of reactive oxygen species (ROS), malondialdehyde (MDA) protein, hepatic glutathione (GSH) and glutathione S-transferase (GST) activity associated with CCl_4-induced liver damage in mice. (A) The fluorescence intensity of renal ROS measurements in mice co-treated with CCl_4 (1.2 mg/kg) and PC (0.5 mg/kg). (B) Immunoblot analysis performed on liver tissue of mice co-treated with CCl_4 (20 mg/kg) and PC (10 and 100 mg/kg) confirmed the production of MDA. (C, D) The hepatic GSH content and GST activity after co-treatment with CCl_4 (20 mg/kg) and PC (10 and 100 mg/kg). Densitometric data are expressed as the mean ± SEM of triplicate analyses. The data shown are the means ± SEM (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. Control, $+p < 0.05$, $++p < 0.01$ vs. CCl_4.

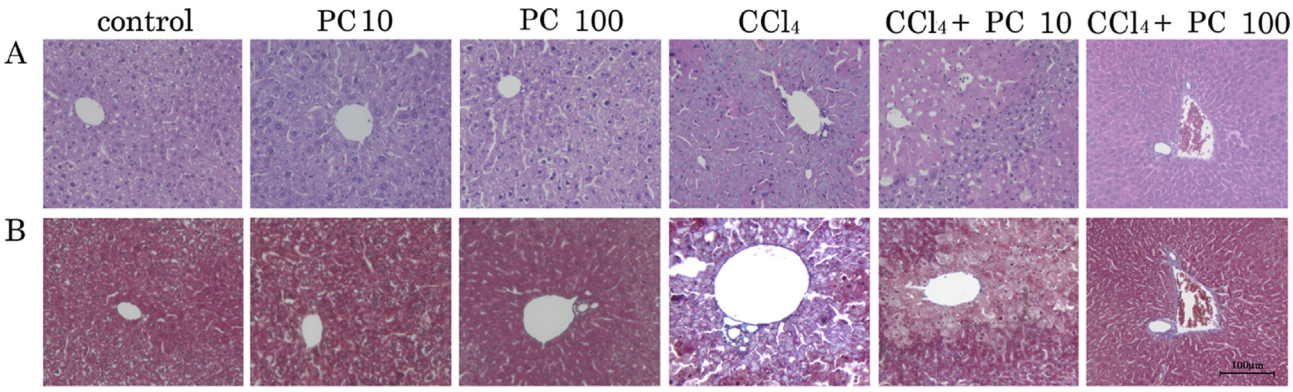

Fig. 3. The effect of PC on histopathological changes and collagen accumulation induced by CCl_4 in the livers of mice. (A) Histopathological changes demonstrated with hematoxylin and eosin (H&E) stain 5 days after exposure. (B) Collagen accumulation demonstrated with Masson's trichrome stain 5 days after exposure. Sections obtained from liver of CCl_4-intoxicated mice show excessive collagen fiber deposition and pseudolobule formation. Mice co-treated with CCl_4 (20 mg/kg) and PC (10 and 100 mg/kg). The number of mice per experimental point is 5.

apoptotic protein Bcl-xL were significantly lower in the CCl_4-treated mice. However, treatment of mice with PC significantly counteracted the respective effects of CCl_4 on the levels of both proteins. In addition, CCl_4-treated mice showed a significant increase in caspase-3 production compared to the control group, whereas caspase-3 production was decreased in the CCl_4- and PC-cotreated mice. These results suggest that PC protects liver tissue against CCl_4-induced oxidative stress by decreasing the production of apoptotic proteins and increasing anti-apoptotic protein production (Fig. 4).

4. Discussion

The anti-oxidant and free radical scavenging activities of many substances have been assessed, and have been found to exert anti-hepatotoxic effects associated with strong anti-oxidant activity [7,19,20]. Previous studies have demonstrated that PC has various beneficial effects, including anti-inflammatory, anti-oxidant, and anti-fibrotic activity [13,14]. PC was also found to exert a strong reactive oxygen species scavenging effect and prevented lipid peroxidation in an *in vivo* study of oxidative stress [21]. Moreover, in a severe sepsis rat model, MDA levels were decreased in the liver, heart and lung by PC treatment [22]. Based on these studies, we investigated the potential protective effects of PC in an experimental animal model of acute CCl_4-induced liver injury.

CCl_4-induced liver injury is the best-characterized system of xenobiotic-induced hepatotoxicity, and is the most commonly used model for assessing the hepatoprotective activities of drugs [23]. In the present study, the hepatotoxic effects of CCl_4 were assessed as a significant increase in liver enzymes, namely AST, ALT, and ALP. Increases in serum AST, ALT, and ALP levels in response to CCl_4 have been attributed to hepatic structural damage, because these

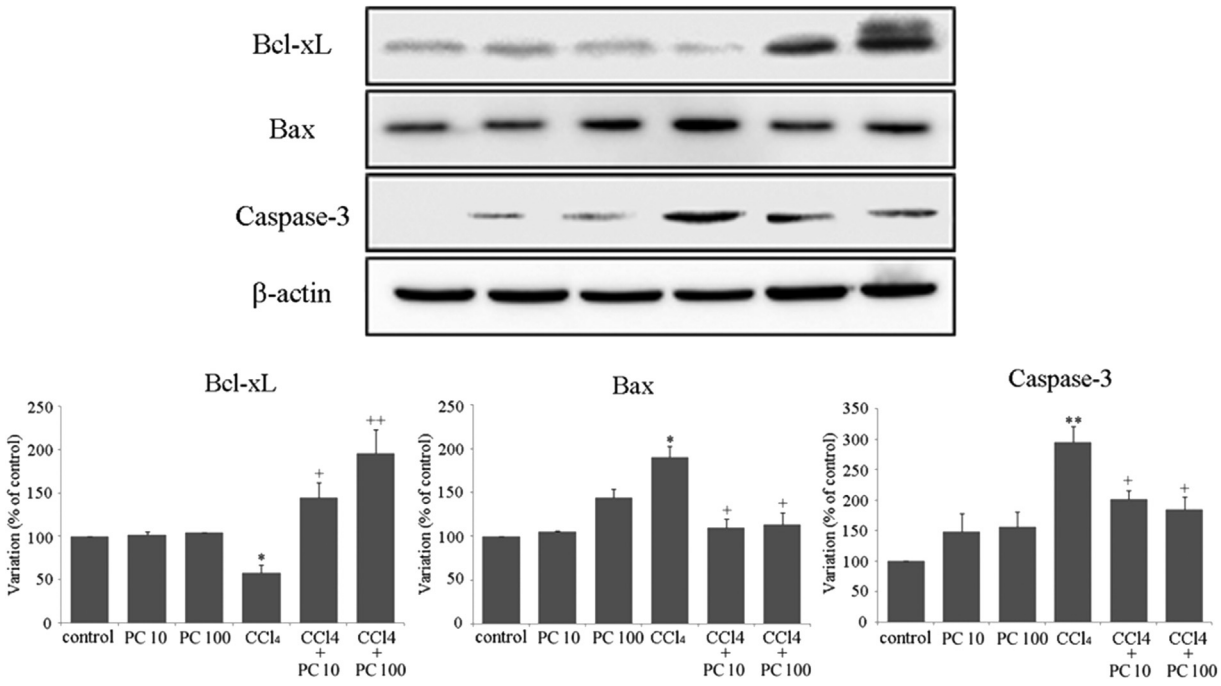

Fig. 4. Effect of PC on apoptotic protein production observed during CCl_4-induced liver damage in mice. The production of Bax and caspase-3 proteins was significantly increased in mice treated with CCl_4 compared to the control. These changes were prevented in mice co-treated with CCl_4 (20 mg/kg) and PC (10 and 100 mg/kg). In contrast, Bcl-xL production was significantly increased in mice co-treated with CCl_4 and PC. The data shown are the means ± SEM (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. Control, $+p < 0.05$, $++p < 0.01$ vs. CCl_4.

enzymes are normally localized to the cytoplasm and are released into circulation after cellular damage has occurred [24]. Pretreatment of mice with PC significantly counteracted the hepatotoxic effect of CCl_4 as indicated by significant decreases in AST, ALT, and ALP serum levels compared to those seen in treatment with CCl_4 alone. Previous studies have demonstrated the exhaustion of intracellular substances, such as GSH and free radicals, as a consequence of CCl_4-induced hepatotoxicity [24,25]. GSH is capable of preferentially conjugating with toxic metabolites and thus contributes to the detoxification of CCl_4, largely through the activity of GST. GST binds both GSH and the relevant endogenous or exogenous substance [26]. Therefore, the capacity for GSH conjugation is critical in alleviating CCl_4-induced hepatotoxicity, and is mediated by GST. In the present study, we observed that CCl_4 treatment resulted in a significant depletion of liver GSH, accompanied with a significant increase in ROS production and lipid peroxides, compared to the control group. Furthermore, GST activity was significantly decreased by CCl_4 treatment. However, pretreatment of mice with PC significantly counteracted GSH depletion and also significantly restored GST activity. These results indicate that PC's protective effect against CCl_4-induced hepatotoxicity may be related to increased cellular GSH content and increased GST activity.

The liver is the principal organ involved in the generation of ROS induced by drugs and toxic chemicals [27]. Free radicals and the lipid peroxidation triggered by them are involved in the chief mechanisms by which CCl_4 injures hepatocytes. The oxidative stress induced by CCl_4 is an established model for hepatotoxicity. CCl_4 induces ROS and depletes antioxidant defenses, including antioxidant enzymes and their substrates, thus causing oxidative stress in multiple tissues [28]. The toxic effect of CCl_4 is due to the peroxidation of membrane lipids by trichloromethyl or trichloromethyl peroxy radicals [29]. These radicals initiate lipid peroxidation chain reactions and cause severe cell damage, which induces the development of fibrosis in the liver. Liver fibrosis induced by CCl_4 is associated with the severity of lipid peroxidation and the depletion of antioxidants caused by damage in the cell membrane and organelles of hepatocytes. In agreement with biochemical studies, histopathological evaluation indicated hepatotoxic effects of CCl_4 and gave further evidence that pretreatment with PC attenuated the oxidative injuries and fibrosis induced by CCl_4. Based on these findings, we conclude that PC may have anti-fibrotic effects in addition to its anti-oxidant properties.

Excessive oxidative stress causes cellular damage to DNA, lipids, and proteins [19]. Here, we analyzed the effects of PC on apoptotic proteins such as Bcl-xL, Bax, and caspase-3. Production of the anti-apoptotic protein Bcl-xL was increased in response to CCl_4-induced hepatotoxicity following PC treatment. Bcl-xL is a potent cell death inhibitor, and its production prevents cell death. Conversely, the reduced level of the apoptotic protein Bax induced by PC treatment is consistent with the expression of caspase-3. The ratio between the two subsets helps to determine, in part, the susceptibility of cells to a death signal. They regulate the apoptotic process principally via the mitochondrial pathway, which is enacted via activation of caspase, the major executor of apoptosis, resulting from the release of cytochrome c from mitochondria [30]. Caspase-3 is a critical mediator of apoptosis in many cell types, including hepatocytes. Caspase-3 protein levels have been shown to increase after CCl_4 exposure. Our results show that pretreatment with PC reduced the induction of apoptotic proteins by increasing the activation of Bcl-xL, indicating that PC decreases apoptosis in hepatocytes.

The protective effects of PC were confirmed in a mouse model of CCl_4-induced acute liver injury. We found that PC effectively ameliorated CCl_4-induced oxidative stress damage as evidenced by reduced oxidative stress, decreased levels of apoptotic proteins, and improvement of hepatic lesions. The protective potential of PC correlates directly with its anti-oxidant properties, but its detailed mechanism and signaling pathway remain to be elucidated by further investigation.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This paper was supported by research funds of Chonbuk National University in 2014.

References

[1] L.W. Weber, M. Boll, A. Stampfl, Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, Crit. Rev. Toxicol. 33 (2003) 105–136.
[2] T.C. Jeong, H.J. Kim, J.I. Park, C.S. Ha, J.D. Park, S.I. Kim, J.K. Roh, Protective effects of red ginseng saponins against carbon tetrachloride-induced hepatotoxicity in Sprague Dawley rats, Planta Med. 63 (1997) 136–140.
[3] J. Wu, A. Danielsson, M.A. Zern, Toxicity of hepatotoxins: new insights into mechanisms and therapy, Expert Opin. Investig. Drugs 8 (1999) 585–607.
[4] R.S. Britton, Free Radical Damage and Lipid Peroxidation, Hepatotoxicology, 1991, p. 401.
[5] P. Vitaglione, F. Morisco, N. Caporaso, V. Fogliano, Dietary antioxidant compounds and liver health, Crit. Rev. Food Sci. Nutr. 44 (2004) 575–586.
[6] T.F. Slater, B.C. Sawyer, The stimulatory effects of carbon tetrachloride on peroxidative reactions in rat liver fractions in vitro. Inhibitory effects of free-radical scavengers and other agents, Biochem. J. 123 (1971) 823–828.
[7] X. Deng, K. Wu, J. Wan, L. Li, R. Jiang, M. Jia, Y. Jing, L. Zhang, Aminotriazole attenuated carbon tetrachloride-induced oxidative liver injury in mice, Food Chem. Toxicol. 50 (2012) 3073–3078.
[8] Y.W. Hsu, C.F. Tsai, W.K. Chen, F.J. Lu, Protective effects of seabuckthorn (Hippophae rhamnoides L.) seed oil against carbon tetrachloride-induced hepatotoxicity in mice, Food Chem. Toxicol. 47 (2009) 2281–2288.
[9] S. Sreelatha, P.R. Padma, M. Umadevi, Protective effects of Coriandrum sativum extracts on carbon tetrachloride-induced hepatotoxicity in rats, Food Chem. Toxicol. 47 (2009) 702–708.
[10] O.I. Aruoma, Nutrition and health aspects of free radicals and antioxidants, Food Chem. Toxicol. 32 (1994) 671–683.
[11] A.K. Bansal, M. Bansal, G. Soni, D. Bhatnagar, Protective role of vitamin E pretreatment on N-nitrosodiethylamine induced oxidative stress in rat liver, Chem. Biol. Interact. 156 (2005) 101–111.
[12] Y.B. Gandola, S.E. Perez, P.E. Irene, A.I. Sotelo, J.G. Miquet, G.R. Corradi, A.M. Carlucci, L. Gonzalez, Mitogenic effects of phosphatidylcholine nanoparticles on MCF-7 breast cancer cells, Biomed. Res. Int. 2014 (2014) 687037.
[13] S.J. Chung, C.H. Lee, H.S. Lee, S.T. Kim, U.D. Sohn, E.S. Park, J.S. Bang, J.H. Lee, Y.H. Chung, J.H. Jeong, The role of phosphatidylcholine and deoxycholic acid in inflammation, Life Sci. 108 (2014) 88–93.
[14] M. Akin, S. Demirbilek, S. Ay, K. Gurunluoglu, E. Turkmen, E. Tas, R.T. Aksoy, A. Baykarabulut, M.N. Edali, Attenuation of ureteral obstruction-induced renal injury by polyenylphosphatidylcholine, Int. J. Urol. 14 (2007) 350–356.
[15] Y. Noh, C.Y. Heo, The effect of phosphatidylcholine and deoxycholate compound injections to the localized adipose tissue: an experimental study with a murine model, Arch. Plast. Surg. 39 (2012) 452–456.
[16] Y. Suzuki, K. Nakagawa, S. Kato, N. Tatewaki, S. Mizuochi, J. Ito, T. Eitsuka, H. Nishida, T. Miyazawa, Metabolism and cytotoxic effects of phosphatidylcholine hydroperoxide in human hepatoma HepG2 cells, Biochem. Biophys. Res. Commun. 458 (2015) 920–927.
[17] S.F. Ali, C.P. LeBel, S.C. Bondy, Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity, Neurotoxicology 13 (1992) 637–648.
[18] L. Cesaratto, C. Vascotto, S. Calligaris, G. Tell, The importance of redox state in liver damage, Ann. Hepatol. 3 (2004) 86–92.
[19] N. Hamdy, E. El-Demerdash, New therapeutic aspect for carvedilol: anti-fibrotic effects of carvedilol in chronic carbon tetrachloride-induced liver damage, Toxicol. Appl. Pharmacol. 261 (2012) 292–299.
[20] H. Upur, N. Amat, B. Blazekovic, A. Talip, Protective effect of Cichorium glandulosum root extract on carbon tetrachloride-induced and galactosamine-induced hepatotoxicity in mice, Food Chem. Toxicol. 47 (2009) 2022–2030.
[21] K.P. Navder, E. Baraona, C.S. Lieber, Dilinoleoylphosphatidylcholine protects human low density lipoproteins against oxidation, Atherosclerosis 152 (2000) 89–95.
[22] S. Demirbilek, I. Gurses, N. Sezgin, A. Karaman, N. Gurbuz, Protective effect of polyunsaturated phosphatidylcholine pretreatment on stress ulcer formation in rats, J. Pediatr. Surg. 39 (2004) 57–62.

[23] Y.P. Hwang, J.H. Choi, H.G. Jeong, Protective effect of the Aralia continentalis root extract against carbon tetrachloride-induced hepatotoxicity in mice, Food Chem. Toxicol. 47 (2009) 75–81.
[24] E.A. Glende Jr., R.O. Recknagel, An indirect method demonstrating that CCl4-dependent hepatocyte injury is linked to a rise in intracellular calcium ion concentration, Res. Commun. Chem. Pathol. Pharmacol. 73 (1991) 41–52.
[25] A.T. Williams, R.F. Burk, Carbon tetrachloride hepatotoxicity: an example of free radical-mediated injury, Semin. Liver Dis. 10 (1990) 279–284.
[26] T.D. Boyer, D.A. Vessey, C. Holcomb, N. Saley, Studies of the relationship between the catalytic activity and binding of non-substrate ligands by the glutathione S-transferases, Biochem. J. 217 (1984) 179–185.
[27] L. Alric, C. Orfila, N. Carrere, M. Beraud, G. Carrera, J.C. Lepert, M. Duffaut, B. Pipy, J.P. Vinel, Reactive oxygen intermediates and eicosanoid production by kupffer cells and infiltrated macrophages in acute and chronic liver injury induced in rats by CCl4, Inflamm. Res. 49 (2000) 700–707.
[28] R.A. Khan, M.R. Khan, S. Sahreen, J. Bokhari, Prevention of CCl4-induced nephrotoxicity with Sonchus asper in rat, Food Chem. Toxicol. 48 (2010) 2469–2476.
[29] P. Abraham, G. Wilfred, Cathrine, Oxidative damage to the lipids and proteins pf the lungs, testis and kidney of rats during carbon tetrachloride intoxication, Clin. Chim. Acta 289 (1999) 177–179.
[30] B. Lu, Y. Xu, L. Xu, X. Cong, L. Yin, H. Li, J. Peng, Mechanism investigation of dioscin against CCl4-induced acute liver damage in mice, Environ. Toxicol. Pharmacol. 34 (2012) 127–135.