Role of nuclear receptor CAR in carbon tetrachloride-induced hepatotoxicity

Yuichi Yamazaki, Satoru Kakizaki, Norio Horiguchi, Hitoshi Takagi, Masatomo Mori, Masahiko Negishi

Abstract

AIM: To investigate the precise roles of CAR in CCl₄-induced acute hepatotoxicity.

METHODS: To prepare an acute liver injury model, CCl₄ was intraperitoneally injected in CAR⁺/+ and CAR⁻/- mice.

RESULTS: Elevation of serum alanine aminotransferase and extension of centrilobular necrosis were slightly inhibited in CAR⁻/- mice compared to CAR⁺/+ mice without PB. Administration of a CAR inducer, PB, revealed that CCl₄-induced liver toxicity was partially inhibited in CAR⁻/- mice compared with CAR⁺/+ mice. On the other hand, androstanol, an inverse agonist ligand, inhibited hepatotoxicity in CAR⁺/+ but not in CAR⁻/- mice. Thus, CAR activation caused CCl₄ hepatotoxicity while CAR inhibition resulted in partial protection against CCl₄-induced hepatotoxicity. There were no differences in the expression of CYP2E1, the main metabolizing enzyme for CCl₄, between CAR⁺/+ and CAR⁻/- mice. However, the expression of other CCl₄-metabolizing enzymes, such as CYP2B10 and 3A11, was induced by PB in CAR⁺/+ but not in CAR⁻/- mice. Although the main pathway of CCl₄-induced acute liver injury is mediated by CYP2E1, CAR modulates its pathway via induction of CYP2B10 and 3A11 in the presence of activator or inhibitor.

CONCLUSION: The nuclear receptor CAR modulates CCl₄-induced liver injury via induction of CCl₄-metabolizing enzymes in the presence of an activator. Our results suggest that drugs interacting with nuclear receptors such as PB might play critical roles in drug-induced liver injury or drug-drug interaction even though such drugs themselves are not hepatotoxic.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

Key words: CAR; Phenobarbital; Cytochrome P450; CCl₄; Drug-induced liver injury

Yamazaki Y, Kakizaki S, Horiguchi N, Takagi H, Mori M, Negishi M. Role of nuclear receptor CAR in carbon tetrachloride-induced hepatotoxicity. World J Gastroenterol 2005; 11 (38): 5966-5972

http://www.wjgnet.com/1007-9327/11/5966.asp

INTRODUCTION

Drug-induced liver injury is a clinical-relevant problem[1-3], and investigation of mechanisms of drug-induced liver injury is therefore of pharmacological and clinical importance. Animals induce drug-metabolizing enzymes to detoxify xenoc hemicals, including therapeutic drugs[4-6]. However, paradoxically, this can often result in bioactivation of toxic drugs and cause liver injury[4-6]. Thus, drug-metabolizing enzymes are a two-edge blade and have very crucial roles in drug-induced liver injury.

Recent studies indicate that the nuclear receptor constitutive androstane receptor (CAR) is a key regulator of drug-metabolizing enzymes such as cytochrome P450 (CYP)[7-9], UDP-glucuronosyltransferase (UGT)[10,11] and multidrug resistance-associated protein (MRP)[12,13]. CAR has also been reported as a key regulator for bile acid[14] and bilirubin liver injury[15,16]. As a cellular defense mechanism against toxicity and carcinogenicity, induction of metabolism and metabolism by CYPs usually leads to increased detoxification and elimination of xenobiotics[4-6]. The nuclear receptor CAR is implicated as an essential factor that mediates this inducible activation of drug-metabolizing enzymes[7-9]. CAR, acting as a heterodimer with the retinoid X receptor, binds to a nuclear receptor-binding site NR1 within the 51-bp phenobarbital-responsive enhancer module and activates genes such as CYP2B in response to phenobarbital (PB)-type inducers[7,9]. Expression analysis of hepatic genes in CAR⁻/- mice has also revealed that CAR is a key regulator of a group of xenoc hemical-metabolizing enzymes that include the CYP2B and CYP3A subfamilies[7]. Thus, CAR is closely associated with the regulation of drug-metabolizing enzymes. Because of its regulating roles for drug-metabolizing enzymes, CAR is thought to play an important role in the development of drug-induced liver injury.

Carbon tetrachloride (CCl₄) was once used widely as a solvent cleaner, anesthetic agent, and degreaser both for industrial and home use[16-20]. However, the high incidence...
of liver and renal problems following administration led to discontinuation of its use. CCl4 is used as a model drug for the study of hepatotoxicity in acute and chronic liver failure\(^{15, 18-20}\). CCl4 is metabolized by CYP2E1, CYP2B, and possibly CYP3A, to form the trichloromethyl radical, CCl3\(^{•}\), a highly reactive species. Thus, the metabolites of CCl4 cause the hepatic injury in the CCl4 acute liver injury model. Inducers of drug-metabolizing enzymes, such as PB or dichlorodiphenyltrichloroethane, are also known to cause severe liver toxicity with CCI4\[^{18-20}\].

The nuclear receptor CAR has recently been shown to play a role in CCl4 hepatotoxicity\[^{20-21}\]. The precise relationship between CAR and CCl4-induced liver injury is not fully understood. We investigated the transmission of the disrupted allele was detected by PCR\[^{17}\].

Materials and methods

Materials

PB was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). 5α-Androstan-3-ol (androstanol) was purchased from Steraloids (Newport, RI, USA). CCl4 was purchased from Kanto Chemistry (Tokyo, Japan). The total glutathione quantification kit was purchased from Dojindo Molecular Technologies Inc. (Gaithersburg, MD, USA).

Animals and treatment

CAR\(^+/+\) and CAR\(^{-/-}\) mice used in this study were generated as described previously\[^{24}\]. The background strain contained 98\% of the C3H/HeNCrBR markers by microsatellite analysis\[^{24}\]. All mouse work was performed in accordance with the guidelines for animal care and use established by Gunma University Graduate School of Medicine. Germline transmission of the disrupted allele was detected by PCR\[^{17}\]. Mice were injected intraperitoneally with the indicated concentration of CCl4 dissolved in olive oil at 8–10 wk of age and were killed, and their sera and livers were collected at specified time points. PB was administered in H2O at a dose of 100 mg/kg body weight for 12 and 36 h before CCl4 administration. Androstanol was administered in olive oil at a dose of 100 mg/kg body weight for 1 h before CCl4 administration. For control mice, 100 μL of H2O or olive oil per 25 g of body weight was injected. Serum alanine aminotransferase (ALT), total bilirubin, and blood urea nitrogen (BUN) levels were measured with an auto-analyzer at each point. Liver tissues were fixed in 4% formaldehyde, embedded in paraffin and stained with hematoxylin-eosin. At a magnification of ×40, 10 areas of centrilobular necrosis were measured in a blinded fashion for each group using NIH Image 1.62 software (National Institute of Health, MD, USA). The index of centrilobular necrosis was scored as follows: area of centrilobular necrosis divided by whole area.

Reverse transcription-polymerase chain reaction

Total RNA extraction from liver and subsequent synthesis of first-strand cDNA were performed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and SuperScript\(^{TM}\) preamplification system (Invitrogen, Carlsbad, CA, USA), respectively. cDNAs were amplified using the following sets of primers: CYP2B10 mRNA, 5′-AAGTTCCCGTGGCAACTTC-3′ and 5′-CATCCCAAAGTCTCTCATGG-3′; CYP3A11 mRNA, 5′-CTCAAGGCCACTGTC-3′ and 5′-CCGATGTTCTTAGACACTGCC-3′; GSTpi, 5′-CTTGCTCAGGCCCACCATTGC-3′ and 5′-ATGGACGCGTTCCATGTTTC-3′; CYP2E1, 5′-GGATGAATTGGCTACCTTCTCAGGTACC-3′ and 5′-TGATGGGCAGCAGGCTTCAAATGC-3′. β-actin mRNA level was also measured as an internal control. One-twentieth of each cDNA synthesized from 5 μg of RNA was subjected to PCR. PCR was performed using Taq DNA polymerase (Promega, Madison, WI, USA) with TaqStart antibody (Clontech, Palo Alto, CA, USA) at 95 °C for 60 s, 55 °C for 60 s, and 72 °C for 60 s. The amplified DNA was separated on a 1.5% agarose gel and visualized with staining by ethidium bromide. The expected sizes of the amplified cDNA were 340, 423, 468, and 486 bp for CYP2B10, CYP3A11, CYP2E1, and GSTPi, respectively.

Hepatic glutathione concentration

Total hepatic reduced glutathione concentration was determined by the total glutathione quantification kit (Dojindo Molecular Technologies Inc.). Each liver was excised and homogenized with 5% trichloroacetic acid, and centrifuged at 4 °C, 2,000 r/min for 15 min. The supernatant was used for total glutathione assay using the total glutathione quantification kit according to the manual provided by the manufacturer.

Statistical analysis

All experimental data are shown as mean±SD. Differences in serum ALT levels and the index of centrilobular necrosis were determined by one-way factorial analysis of variance for each group. The level of significance for all statistical analyses was set at P<0.05.

Results

Phenobarbital pretreatment induces CCl4 toxicity in CAR\(^{+/+}\) mice

CAR\(^{+/+}\) and CAR\(^{-/-}\) mice were administered a 25-mg/kg dose of CCl4 by intraperitoneal injection (n = 6 per treatment group) and the ALT level was measured at 24 h (Figure 1A). The serum levels of ALT increased following CCl4 administration in both groups (P<0.001). The level of ALT was slightly higher in CAR\(^{+/+}\) mice than in CAR\(^{-/-}\) mice (P<0.001). There were no significant changes in the serum levels of total bilirubin and BUN in both groups (data not shown). CAR\(^{+/+}\) and CAR\(^{-/-}\) animals pretreated with PB (12 and 36 h before CCl4 administration) were then administered the same dose of CCl4. PB pretreatment caused marked elevation of ALT in CAR\(^{+/+}\) mice. The CAR\(^{-/-}\) mice showed some elevation of ALT with PB pretreatment, but the level was less than that in wild-type mice. The difference of ALT levels became clear between CAR\(^{+/+}\) and CAR\(^{-/-}\) mice in vivo.
Histological changes associated with CCl₄ toxicity

CAR⁺/⁺ or CAR⁻/⁻ mice were administered a 25-mg/kg dose of CCl₄ by intraperitoneal injection with or without PB (n = 6 per treatment group). Liver sections from each treatment were examined by hematoxylin and eosin staining. Liver samples from all treated animals were analyzed but representative histology is presented in Figure 2A showing marked centrilobular necrosis. The extent of the necrotic area with CCl₄ in the CAR⁺/⁺ liver was slightly larger than that in CAR⁻/⁻ mice. PB pretreatment caused extensive centrilobular necrosis in CAR⁺/⁺ mice. The indices of centrilobular necrosis (Figure 2B) in CAR⁺/⁺ mice and CAR⁻/⁻ mice treated with CCl₄ alone were 17.44±1.02 and 13.11±1.41, respectively (P<0.001). The indices of centrilobular necrosis in CAR⁺/⁺ mice treated with PB plus CCl₄ were 39.08±2.10 and 26.33±2.33, respectively (P<0.001).

Dose dependency and time course of CCl₄ toxicity

CAR⁺/⁺ or CAR⁻/⁻ mice were given 25-, 50-, or 100-mg/kg doses of CCl₄. Blood samples were collected 24 h later, and serum ALT levels were measured (n = 4). CAR⁻/⁻ animals were significantly less sensitive than CAR⁺/⁺ mice to CCl₄ toxicity (P<0.001). Data are mean±SD.

Indices of centrilobular necrosis

| CCl₄ (mg/kg) | CAR⁺/⁺ | CAR⁻/⁻ |
|-------------|--------|--------|
| 25          | 17.44±1.02 | 13.11±1.41 |
| 50          | 39.08±2.10 | 26.33±2.33 |
| 100         | 39.08±2.10 | 26.33±2.33 |

PB (P<0.001). B: Dose dependency of CCl₄ toxicity. CAR⁺/⁺ and CAR⁻/⁻ mice were given 25-, 50-, or 100-mg/kg doses of CCl₄. Blood samples were collected 24 h later, and serum ALT levels were measured (n = 4). CAR⁻/⁻ animals were significantly less sensitive than CAR⁺/⁺ mice to CCl₄ toxicity (P<0.001). Data are mean±SD.
were killed and blood samples were collected (Figure 1B). No elevation of ALT was observed at 5 h (data not shown). At 24 h, ALT was significantly elevated and dose dependency was observed in CCl₄ toxicity in both groups of mice. The serum ALT concentrations of CAR−/− mice were lower than those of the CAR+/+ mice (P<0.001). Thus, CAR+/+ mice were apparently more sensitive to CCl₄ liver toxicity compared with CAR−/− mice.

**Hepatic mRNA levels of CCl₄-metabolizing enzymes**

CCl₄ is reported to be metabolized and activated by CYP2E1, CYP2B, and possibly CYP3A, to form the trichloromethyl radical, CCl₃•. To evaluate the relationship between CAR and CCl₄-metabolizing enzymes, CAR+/+ or CAR−/− animals were treated with a 100-mg/kg PB intraperitoneally (n = 6 per treatment group, Figure 3). There were no differences in the basal expression of CYP2E1, the main metabolizing enzyme of CCl₄ between CAR+/+ and CAR−/− mice. Among the genes associated with CCl₄ metabolism, PB treatment did not influence CYP2E1 mRNA levels but induced CYP2B10, CYP3A11 mRNAs in CAR+/+ mice. On the other hand, no induction of the enzymes CYP2E1, CYP2B10, and CYP3A11 was observed in CAR−/− mice. Thus, PB activated CCl₄-metabolizing enzymes, CYP2B10 and CYP3A11, via CAR. Because CAR is reported to regulate GSTP1, the enzyme that enhances glutathione depletion in acetaminophen toxicity[18-20], GSTP1 mRNA, was also measured. GSTP1 mRNA was induced by PB in CAR+/+ mice stronger than in CAR−/− mice.

**Figure 3**  Hepatic mRNA level of drug-metabolizing enzymes with PB treatment. Total liver RNA was prepared from CAR+/+ or CAR−/− animals treated with a 100-mg/kg dose of PB by intraperitoneal injection (n = 6 per treatment group). Total liver RNA was prepared 12 h after PB treatment and subjected to PCR analysis with the indicated primers in Materials and methods. β-Actin mRNA level was also measured as an internal control. The amplified DNA was separated on a 1.5% agarose gel and visualized with staining by ethidium bromide. The expected sizes of the amplified cDNA are described in Materials and methods.

**Hepatic glutathione level**

For detoxification, metabolites of CCl₄ such as CCl₃• decreased the amount of reduced glutathione. A decrease of glutathione in the liver reflects increased production of CCl₄ metabolites. Liver samples treated with CCl₄ and/or PB were collected 24 h after each treatment and glutathione levels were measured (n = 6 per treatment group, Figure 4). Hepatic glutathione levels in the PB plus CCl₄-treated CAR+/+ mice were significantly different from those of the CAR−/− mice (P<0.001). CAR+/+ mice treated with PB plus CCl₄ showed about a 50% decrease in hepatic glutathione level compared with CAR−/− mice.

**Figure 4** Hepatic glutathione (GSH) level. Liver samples treated with the indicated chemicals were collected 24 h after each treatment and glutathione levels were measured (n = 6 per treatment group) using the total glutathione quantification kit. Hepatic glutathione levels in the PB plus CCl₄ treated CAR+/+ mice significantly decreased, compared with those of the CAR−/− mice (P<0.001). Data are means±SD.

**Androstanol reduced CCl₄-induced hepatotoxicity in CAR+/+ mice to the level of CAR−/− mice**

CAR+/+ or CAR−/− mice were treated with 100-mg/kg CCl₄ intraperitoneally, with or without pretreatment with androstanol (100 mg/kg). Serum ALT levels were measured 24 h later (n = 6 per treatment group, Figure 5). Without androstanol, CAR+/+ mice treated with CCl₄ showed significantly higher ALT concentrations than did CAR−/− mice (P<0.001). Surprisingly, androstanol reduced the ALT concentration of CAR+/+ mice to the level of CAR−/− mice. Liver sections from the same animals, as indicated, were stained with hematoxylin and eosin (Figure 5B). Androstanol pretreatment reduced the hepatic centrilobular necrosis (Figure 5C) of CAR+/+ mice to the level of CAR−/− mice. The indices of centrilobular necrosis of CAR+/+ mice and CAR−/− mice treated with CCl₄ alone were 33.39±5.04 and 26.49±2.87, respectively (P<0.001). With androstanol treatment, the indices of centrilobular necrosis in CAR+/+ mice and CAR−/− mice decreased to 23.64±1.89 and 25.15±2.36, respectively (P = 0.15). Thus, androstanol, the inverse agonist of CAR, reduced the CCl₄-induced hepatotoxicity of CAR+/+ mice to the level of CAR−/− mice. To evaluate the effect of androstanol on CCl₄-metabolizing enzymes, hepatic mRNA levels were determined with RT-PCR. Androstanol treatment did not influence CYP2E1 mRNA levels but slightly repressed CYP3A11 mRNA in CAR−/− mice as previously reported[20]. Because of low basal expression of CYP2B10 mRNA, the repression of CYP2B10 mRNA by androstanol could not be demonstrated with RT-PCR in CAR−/− mice. However, androstanol was reported to repress the basal promoter activity of CYP2B10 by luciferase reporter assay[20]. On the other hand, no induction or repression of the enzymes CYP2E1, CYP2B10, and CYP3A11 was observed in CAR−/− mice. Thus, androstanol repressed basal expression of CCl₄-metabolizing enzymes,
CYP3A11 and may be CYP2B10, via CAR. Androstanol treatment did not influence GSTPi mRNA levels in CAR+/+ and CAR-/- mice by RT-PCR.

DISCUSSION
In this study, we showed that CAR partially modulates the CCl4 toxicity that is associated with induction of some CCl4-metabolizing enzymes in the presence of agonist. The loss of CAR function results in partial resistance to CCl4 toxicity. However, the CCl4 liver toxicity is not fully inhibited in CAR-/- mice and differences between CAR+/+ and CAR-/- mice were small in the absence of an inducer, PB. Metabolites of CCl4 and may be CYP2B10, via CAR. Androstanol treatment did not influence GSTPi mRNA levels in CAR+/+ and CAR-/- mice by RT-PCR.

Figure 5
Androstanol prevented CCl4-induced hepatotoxicity. A: CAR+/+ or CAR-/- mice were given a 100-mg/kg dose of CCl4 by intraperitoneal injection, with or without pretreatment with androstanol (100 mg/kg). Serum ALT levels were measured 24 h later (n = 6 per treatment group). Androstanol-pretreatment reduced the ALT level of CAR+/+ mice to the level of CAR-/- mice. An: androstanol. B: Liver sections from four animals 24 h after different treatments, as indicated, were stained with hematoxylin and eosin. (a) CAR+ mice, control; (b) CAR+/+ mice, control; (c) CAR-/- mice, androstanol; (d) CAR+/+ mice, androstanol; (e) CAR-/- mice, CCl4; (f) CAR+/+ mice, CCl4; (g) CAR-/- mice, androstanol plus CCl4; and (h) CAR+/+ mice, androstanol plus CCl4. Androstanol pretreatment reduced the hepatic centrilobular necrosis in CAR+/+ mice. Arrows indicate areas of hepatic necrosis. C: The indices of centrilobular necrosis in CAR+/+ mice and CAR-/- mice. Androstanol-pretreatment reduced hepatic centrilobular necrosis of CAR+ mice to the level of CAR-/- mice. Data in (A) and (C) are mean±SD. D: Hepatic mRNA level of drug-metabolizing enzymes with androstanol treatment. Total liver RNA was prepared from CAR+/+ or CAR-/- animals treated with a 100-mg/kg dose of androstanol by intraperitoneal injection (n = 6 per treatment group) and subjected to PCR analysis with the indicated primers in Materials and methods. β-actin mRNA level was also measured as an internal control. The amplified DNA was separated on a 1.5% agarose gel and visualized with staining by ethidium bromide. The expected sizes of the amplified cDNA are described in Materials and methods.

Figure 6
Schematic representation of CAR-mediated CCl4 hepatotoxicity.
of CCl₄ such as CCl₃⁺ and CCl₃OO⁺ are known to cause the hepatic injury associated with CCl₄[18-20]. CCl₄ is metabolized by CYP2E1, CYP2B, and possibly CYP3A, to form the trichloromethyl radical, CCl₃⁺[18-20]. The main CCl₄-metabolizing enzyme is CYP2E1 and it is expressed in CAR⁺/⁺ and CAR⁻/⁻ mice in the same way. Therefore, CCl₄ liver toxicity occurs despite an absence of CAR. Thus, CYP2B or CYP3A may only have supplementary roles in CCl₄ metabolism in the absence of PB. On the other hand, in the presence of CAR inducers, it results in the induction of CYP2B and CYP3A, and CCl₄ liver toxicity is severe, as seen in CAR⁻/⁻ mice. Inducers of drug-metabolizing enzymes including PB have long been known to cause severe liver toxicity with CCl₄[18-20]. In this study, we showed that PB activated CYP2B10 and 3A11 via receptor CAR and that it caused the severe CCl₄ liver toxicity (Figure 6). Thus, the xenobiotic receptor CAR will have a mean with the presence of its agonist/antagonist. PB treatment resulted in enhanced CCl₄ liver toxicity in CAR⁻/⁻ mice although to a lesser degree than that in CAR⁺/⁺ mice. PB may impact on pathways distinct from CAR to cause CCl₄ liver toxicity. Indeed, cDNA microarray analysis of CAR⁻/⁻ mice showed CAR-independent genes in response to PB treatment[18]. A total of 138 genes were detected to be either induced or repressed in response to PB treatment, of which about half were not under CAR regulation[18]. Enzymes such as amino levulinate synthase 1 and squalene epoxidase displayed CAR-independent induction by PB[18]. CYP4A10 and CYP4A14 represented the group of genes induced by PB only in CAR⁻/⁻ mice, indicating that CAR may be a transcription blocker that prevents these genes from being induced by PB[18]. The enzymes having CCl₄-metabolizing activity may be included in these CAR-independent PB response genes.

CAR was also identified as a key regulator of acetaminophen metabolism and hepatotoxicity[28]. CAR regulates GSTPi, the enzyme that enhances glutathione depletion and so promotes acetaminophen toxicity[27-29]. In this study, CAR also induced GSTPi with PB treatment. The metabolites of CCl₄ such as CCl₃⁺ and CCl₃OO⁺ induce hepatotoxicity and expend glutathione and cause a decrease of hepatic glutathione level. In addition, induction of GSTPi expression may cause further depletion of glutathione and severe toxicity in CCl₄-treated mice.

Although CAR acted as a hepatotoxic regulator in this study, CAR could act as a protective manner in other situations[30,31]. PB is a well-established therapy for hyperbilirubinemia in Crigler-Najjar type II patients[32]. CAR has the ability to activate expression of known components of the bilirubin pathway including UGT1A1, OATP2, MRPs, and GSTA1[33]. It was also reported that paralysis by the muscle relaxant zoxazolamine was avoided by pretreatment with CAR activators but was prolonged in CAR⁻/⁻ mice[34]. Thus, CAR coordinates the induction of metabolic activity and increases elimination of the drugs in a protective manner in some cases.

Transactivation of target genes by CAR can be blocked by the inverse agonist androstanol[35]. Androstanol is reported to not only prevent induction but also to decrease basal expression of CAR target genes including CYP3A11[35]. Androstanol reduced the liver toxicity of CAR⁺/⁺ mice to the level of CAR⁻/⁻ mice in this study. This effect of androstanol may result in decrease of the basal expression of CAR target genes including CYP3A11. Androstanol treatment was also reported to decrease the toxicity of acetaminophen[36].

The nuclear receptor CAR has only a small role in CCl₄ toxicity without its agonist/antagonist. However, because of its nature as a xenobiotic sensor, CAR exhibits its ability to the full in the presence of agonist/antagonist. Since some kinds of CAR inducers such as PB and phenytoin are used clinically, CAR may have an important clinical role in drug-induced liver injury or drug-drug interaction. Nuclear receptors regulating drug-metabolizing enzymes such as CAR are a two-edge blade in animals. In some cases, activation of CAR increases production of drug-metabolizing enzymes and increases toxicity. Thus, CAR inverse agonists such as androstanol may have potential for treatment of these kinds of drug-induced liver injury. On the other hand, activation of CAR by appropriate inducers acts in a hepato-protective manner in some cases such as bilirubin toxicity. Conversely, induction of CAR activity reduced the liver injury in these cases.

Thus, a nuclear receptor that regulates drug-metabolizing enzymes is a key factor in drug-induced liver injury. Regulation of CAR activity may be an important clinical strategy for treatment of drug-induced liver injury. Both CAR and nuclear receptor regulating drug-metabolizing enzymes such as pregnane X receptor and peroxisome proliferator activated receptors are thought to be associated with drug-induced liver injury. Activating or inverse agonists for nuclear receptors may provide a clinically useful means to treat drug-induced liver injury.

REFERENCES

1. Sgro C, Clinard F, Ouazir K, Chanay H, Allard C, Guillemint L, Lenoir C, Lemoine A, Hillon P. Incidence of drug-induced hepatic injuries: a French population-based study. Hepatology 2002; 36: 451-455
2. Chitturi S, Farrell GC. Drug-induced cholestasis. Semin Gastrointest Dis 2001; 12: 113-124
3. Hanzel I, Perez E, Vidal X, Lapeor J. Grup d’Estudi Multicentric d’Hepatotoxicitat Aguda de Barcelona (GEMHAB). Prospective surveillance of acute serious liver disease unrelated to infectious, obstructive, or metabolic diseases: epidemiological and clinical features, and exposure to drugs. J Hepatol 2002; 37: 592-600
4. Guengerich FP, Liebler DC. Enzymatic activation of chemicals to toxic metabolites. Crit Rev Toxicol 1985; 14: 259-307
5. Guengerich FP, Shimada T. Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol 1991; 4: 391-407
6. Zhou S, Gao Y, Jiang W, Huang M, Xu A, Paxton JW. Interactions of herbs with cytochrome P450. Drug Metab Rev 2003; 35: 35-98
7. Honjakoski P, Moore R, Washburn K, Negishi M. Activation by diverse xenoc hemicals of the 51-base pair phenobarbital-responsive enhancer module in the CYP2B10 gene. Mol Pharmacol 1998; 53: 597-601
8. Honjakoski P, Zelko I, Sueyoshi T, Negishi M. The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. Mol Cell Biol 1998; 18: 5652-5658
9. Sueyoshi T, Kawamoto T, Zelko I, Honjakoski P, Negishi M. The repressed nuclear receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. J Biol Chem 1999; 274: 6043-6046
10. Sugatani J, Kojima H, Ueda A, Kakizaki S, Yoshinari K, Gong QH, Owens IS, Negishi M, Sueyoshi T. The phenobar-
nuclear receptor CAR. *Hepatology* 2001; 33: 1232-1238

11 Xie W, Yeuh MF, Radominska-Pandya A, Saini SP, Negishi Y, Bottroff BS, Cabrera GY, Tukey RH, Evans RM. Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. *Proc Natl Acad Sci USA* 2003; 100: 4150-4155

12 Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, Edwards PA. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *J Biol Chem* 2002; 277: 2908-2915

13 Cherrington NJ, Hartley DP, Li N, Johnson DR, Klaassen CD. Organ distribution of multidrug resistance proteins 1, 2, and 3 (Mrp1, 2, and 3) mRNA and hepatic induction of Mrp3 by constitutive androstane receptor activators in rats. *J Pharmacol Exp Ther* 2002; 300: 97-104

14 Saini SP, Sonoda J, Xu L, Toma D, Uppal H, Mu Y, Ren S, Moore DD, Evans RM, Xie W. A novel constitutive androstane receptor-mediated and CYP3A-independent pathway of bile acid detoxification. *Mol Pharmacol* 2004; 65: 292-300

15 Huang W, Zhang J, Chua SS, Qatanani M, Han Y, Granata R, Moore DD. Induction of bilirubin clearance by the constitutive androstane receptor (CAR). *Proc Natl Acad Sci USA* 2003; 100: 4156-4161

16 Huang W, Zhang J, Moore DD. A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. *J Clin Invest* 2004; 113: 137-144

17 Ueda A, Hamadeh HK, Webb HK, Yamamoto Y, Sueyoshi T, Afshari CA, Lehmann JM, Negishi M. Diverse roles of the nuclear orphan receptor CAR in regulating hepatic genes in response to phenobarbital. *Mol Pharmacol* 2002; 61: 1-6

18 Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol* 2003; 33: 105-136

19 Jones IW. Chloroform anaesthesia in Liverpool. *Anaesthesia* 1983; 38: 578-580

20 Clawson GA. Mechanisms of carbon tetrachloride hepatotoxicity. *Pathol Immunopathol Res* 1989; 8: 104-112

21 Wei P, Zhang J, Egan-Hafley M, Liang S, Moore DD. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. *Nature* 2000; 407: 920-923

22 Wang H, LeChuyse EL. Role of orphan nuclear receptors in the regulation of drug-metabolising enzymes. *Clin Pharmacokinet* 2003; 42: 1331-1357

23 Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, Liddle C, Blanchard SG, Willson TM, Collins JL, Kliewer SA. Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J Biol Chem* 2000; 275: 15122-15127

24 Yamamoto Y, Moore R, Goldsworthy TL, Negishi M, Maronpot RR. The orphan nuclear receptor CAR is essential for liver tumor promotion by phenobarbital in mice. *Cancer Res* 2004; 64: 7197-7200

25 Wei P, Zhang J, Dowhan DH, Han Y, Moore DD. Specific and overlapping functions of the nuclear hormone receptors CAR and PXR in xenobiotic response. *Pharmacogenomics* 2002; 2: 117-126

26 Tzameli I, Chua SS, Cheskis B, Moore DD. Complex effects of rexinoids on ligand dependent activation or inhibition of the xenobiotic receptor, CAR. *Nucl Recept* 2003; 1: 2

27 Zhang J, Huang W, Chua SS, Wei P, Moore DD. Modulation of acetaminophen-induced hepatotoxicity by the xenobiotic receptor CAR. *Science* 2002; 298: 422-424

28 Morel F, Fardel O, Meyer DJ, Langouet S, Gilmore KS, Meunier B, Tu CP, Kessler TW, Ketterer B, Guillouzo A. Preferential increase of glutathione S-transferase class alpha transcripts in cultured human hepatocytes by phenobarbital, 3-methylcholanthrene, and dithiolethiones. *Cancer Res* 1993; 53: 231-234

29 Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995; 30: 445-600

30 Berk PD, Martin JF, Blaschke TF, Scharschmidt BF, Plotz PH. Unconjugated hyperbilirubinemia. Physiologic evaluation and experimental approaches to therapy. *Ann Intern Med* 1975; 82: 552-570

31 Guo GL, Lambert G, Negishi M, Ward JM, Brewer HB Jr, Kliewer SA, Gonzalez FJ, Sinal CJ. Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity. *J Biol Chem* 2003; 278: 45062-45071

32 Forman BM, Tzameli I, Choi HS, Chen J, Simha D, Seol W, Evans RM, Moore DD. Androstanone metabolites bind to and deactivate the nuclear receptor CAR-beta. *Nature* 1998; 395: 612-615

Science Editor Guo SY  Language Editor Elsevier HK