Toxicology 316 (2014) 55–60

Contents lists available at ScienceDirect

Toxicology

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

Chronological changes in circulating levels of soluble tumor necrosis factor receptors 1 and 2 in rats with carbon tetrachloride-induced liver injury

Yoshio Ijiri[a,*], Ryuji Kato[a], Maiko Sadamatsu[a], Mina Takano[b], Yoshikatsu Okada[c], Kazuhiko Tanaka[d], Tetsuya Hayashi[a]

[a] Cardiovascular Pharmacotherapy and Toxicology, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan
[b] Pharmacotherapy II, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan
[c] Department of Pathology, Osaka Medical College, 2-7 Daigaku-machi, Takatsuki, Osaka 569-8686, Japan
[d] Kidney Center, Shirasagi Hospital, 7-11-23 Kumata, Higashisumiyoshi-ku, Osaka 546-0002, Japan

ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form 6 December 2013
Accepted 22 December 2013
Available online 3 January 2014

Keywords:
Drug-induced liver injury (DILI)
Carbon tetrachloride (CCl_4)
Tumor necrosis factor-α (TNF-α)
Soluble TNF-receptor 1 (sTNF-R1)
Soluble TNF-receptor 2 (sTNF-R2)

ABSTRACT

Carbon tetrachloride (CCl_4) facilitates the generation of hepatotoxins that can result in morphologic abnormalities, and these abnormalities are reasonably characteristic and reproducible for each particular toxin. It is also known that tumor necrosis factor-alpha (TNF-α) may participate in CCl_4-induced liver injury (CILI). In this study, we observed the chronological changes in circulating soluble tumor necrosis factor receptors 1 and 2 (sTNF-R1 and -R2) in rats with CILI. Laboratory data; circulating levels of TNF-α, sTNF-R1, and sTNF-R2; and TNF-α levels in liver tissues were measured at various time-points.

In the CCl_4 group, the plasma aspartate aminotransferase (AST, 7694 ± 3041 IU/l)/alanine aminotransferase (ALT, 3241 ± 2159 IU/l) levels peaked at 48 h after CCl_4 administration, but the other laboratory data did not differ significantly from the corresponding data in the controls. Centrilobular hepatocyte necrosis and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells near the central vein area were observed via hematoxylin eosin (HE) and TUNEL staining, respectively, at 24 and 48 h after CCl_4 administration. Compared to the control group, the CCl_4 group did not show significantly the increased circulating TNF-α levels. But TNF-α levels in the liver tissues first peaked at 1 h (5261 ± 2253 pg/g liver), and a second peak was observed at 12 h (3806 ± 533 pg/g liver) after CCl_4 administration. Compared to the control group, the CCl_4 group showed significantly increased circulating levels of both sTNF-R1 (797 ± 121 pg/ml) and sTNF-R2 (5696 ± 626 pg/ml) 1 h after CCl_4 administration. Since the hepatocyte apoptosis may be resulted from binding of TNF-α with TNF-R1 at 24 h after administration, and consequently the circulating TNF-R2 level might be approximately 10-fold higher than the circulating TNF-R1 level.

In conclusion, increased circulating levels of sTNF-R1 and -R2 potentially contribute to drug-induced liver injury, together with AST/ALT.

1. Introduction

Carbon tetrachloride (CCl_4) has historically been used as a coolant, extinguishing agent, anesthetic agent, and agricultural chemical from the mid-19th century to the mid-20th century, but its use has been restricted since it became clear that it causes hepatotoxicity (Masuda, 2006). It is metabolized in the liver by cytochrome P450 (CYP) 2E, yielding trichloromethyl radical (CCl_3-radical) (Raucy et al., 1993; Shimizu et al., 2001). It has also been noted that the inflammatory cytokine tumor necrosis factor-alpha (TNF-α) induces apoptosis of hepatocytes, and may be involved in the pathogenesis of CCl_4 (Nagaki et al., 2000; Wen and Xiao, 2004). Accordingly, it has been utilized in studies of acute liver injury and liver cirrhosis in animals, as well as for investigating hepatic regeneration (Hayashi et al., 2005; Robert and David, 2006).

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AMY, amylase; AST, aminotransferase; BUN, blood urea nitrogen; CCl_4, carbon tetrachloride; CCl_3-radical, trichloromethyl radical; CK, creatine kinase; CRP, C-reactive protein; CYP, cytochrome P450; DAB, diaminobenzidine; D-Bil, direct bilirubin; DILI, drug-induced liver injury; γ-GT, gamma-glutamyl transpeptidase; HCV, hepatitis C virus; HE, hematoxylin and eosin; Id-Bil, indirect bilirubin; K, potassium; LPS, lipopolysaccharide; PBS, phosphate buffered saline; POD, peroxidase; S-Cr, serum creatinine; SD, standard deviation; sTNF-R, soluble TNF receptor; TANEL, TdT-mediated dUTP-biotin nick end labeling (TUNEL) stain; T-Bil, total bilirubin; TDT, terminal deoxynucleotidyl transferase; TNF-α, tumor necrosis factor-alpha; TPCs, TUNEL positive cells.

* Corresponding author. Tel.: +81 72 690 1044; fax: +81 72 690 1044.
E-mail address: ijiri@gly.oups.ac.jp (Y. Ijiri).

0300-483X/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tox.2013.12.004

There are two major signaling receptors for TNF-α, TNF-R1 and -R2, and their molecular weights are 55 kDa and 75 kDa, respectively (Idriss and Naismith, 2000; Modzelewski, 2003). The former is expressed in the epithelium, blood vessel endothelium, normal fibroblasts, and tumor cells (Stewart et al., 2005). The latter is mostly expressed in immune cells (the antigen-presenting cells) such as peripheral blood lymphocytes or macrophages, and mediates the biological responses (Lien et al., 1995). In inflammation, TNF-α is primarily expressed as a 26 kDa type II transmembrane protein (membrane-bound TNF-α, mTNF) and is subsequently cleaved by the metalloproteinase-disintegrin TNF-α converting enzyme (TACE) into the secreted 17 kDa monopeptide TNF-α (soluble TNF-α, sTNF) (Stewart et al., 2005; Yoshida et al., 2006). At the same time, zinc metalloproteinases in modulating the generation of sTNF-R1 and -R2 proteolytically cleaved from TNF-R1 and -R2 ectodomain, respectively (Reddy et al., 2000). TNF-Rs are reportedly released into circulation after lipopolysaccharide (LPS) stimulation, resulting in increased circulating levels of soluble TNF-Rs (sTNF-Rs) (Stewart et al., 2005). By various stimuli including TNF-α itself, both of sTNF-R1 and -R2 can be detected at high concentrations for a prolonged period of time in the circulation of patients with various inflammatory diseases such as septicemia, leukemia, hepatitis C virus infection, and rheumatoid arthritis (Kaplanski et al., 2002; McKenna et al., 2007; Navarro et al., 2012; Trentin et al., 1995).

In this study, we observed chronological changes in circulating TNF-α, sTNF-R1, and sTNF-R2 levels, and TNF-α in liver tissues of rats with CILI at various time points.

2. Materials and methods

2.1. Animals

Five- to six-week old male Wistar/ST rats (Japan SLC Inc., Shizuoka, Japan) were used in the study. They were acclimated for at least one week under controlled environmental conditions, where 12 h dark–light cycles were maintained with free access to food and water. All experimental procedures were conducted according to the guidelines for the use of experimental animals and animal facilities established by Osaka University of Pharmaceutical Sciences.

2.2. Reagents

The following regents were purchased and used: CCl_4 (Wako Pure Chemical Industries Ltd., Osaka, Japan); olive oil (Sigma–Aldrich Co., MO, USA); HEPES (Wako Pure Chemical Industries Ltd., Osaka, Japan); 10% Neutral buffered formalin (Wako Pure Chemical Industries Ltd., Osaka, Japan); Mayer's hematoxylin solution (Sakura Finetek Japan Co. Ltd., Tokyo, Japan); Eosin (Sakura Finetek Japan Co. Ltd., Tokyo, Japan); Marinol (Muto Pure Chemical Co. Ltd., Tokyo, Japan). Other reagents and solvents were commercially available, extra-pure grade chemicals.

2.3. Preparation and administration of chemicals

Subjects were divided into the following groups: the CCl_4-administered group where a CCl_4 solution was intraperitoneally administered (CCl_4: olive oil = 1:1; 2 ml/kg), the vehicle group where an olive oil solution was intraperitoneally administered (olive oil = 2 ml/kg), and the control group where no reagent was administered.

2.4. Collecting blood samples

Blood samples were taken from the abdominal aorta at 1, 3, 6, 9, 12, 24, 48, 72, 120, and 168 h after CCl_4 administration. The portal vein and the hepatic artery were ligated under anesthesia. After the hepatic vein was cut, physiological saline was perfused through the portal vein to remove blood in the liver. They were put in 200 IU heparin-treated microtubes and centrifuged for 10 min at 1500 × *g*, 4 °C, the supernatant of which was taken as a plasma sample. Thus the obtained plasma was immediately stored at −20 °C until measurement.

2.5. Laboratory data

The liver enzymes in the plasma samples, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were measured with the JSCC reference method. In addition, the following were measured: total bilirubin (T-Bil), direct bilirubin (D-Bil), indirect bilirubin (Id-Bil), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GT), amylase (AMY), creatine kinase (CK), potassium (K), blood urea nitrogen (BUN), serum creatinine (S-Cr), and C-reactive protein (CRP). Meanwhile, direct bilirubin and indirect bilirubin were measured to distinguish the type of drug-induced liver injury (e.g., a liver injury of cholestasis type increases direct bilirubin, and that of necrosis type increases id-bilirubin). The measured value of the control group is defined as the control value.

2.6. Measurement of TNF-α level in liver tissues and plasma, and sTNF-R1 and -R2 plasma levels

Measurement was performed using Quantikine® Immunoassay Rat TNF-α, Quantikine® Immunoassay Mouse sTNF RI, and Quantikine® Immunoassay Mouse sTNF RII (R&D Systems Inc., MN, USA). These assay kits were recognized for the quantitative determination of mouse sTNF-R1 and -R2 concentrations in cell cultures, mouse serum/plasma, and rat serum/plasma (Defer et al., 2007). The measured value of the control group was defined as the control value.

The liver was drained of blood at 1, 3, 6, 9, 12, 24, 48, 72, 120, and 168 h after CCl_4 administration, after which an approximately 2 g section was cut out from the quadrate lobe. It was then snap frozen in liquid nitrogen and stored at −80 °C. TNF-α level in the liver cytosol fraction sample was measured with the Quantikine® Immunoassay Rat TNF-α in a similar fashion to TNF-α level measurement in plasma. TNF-α level measurement in liver tissues represented in terms of TNF-α amount per 1 g liver. The measured value of the control group is defined as the control value.

2.7. HE and TUNEL staining

The animals were sacrificed under anesthesia for the following procedures at 1, 3, 6, 9, 12, 24, 48, 72, 120, and 168 h after CCl_4 administration. After the portal vein and the hepatic artery were ligated, and the hepatic vein was cut, physiological saline was perfused through the portal vein to remove blood in the liver. The liver was drained of blood, after which a 5 mm square section was cut out from the quadrate lobe which was then fixed in 10% neutral buffered formalin. The liver sections were washed with tap water for 1.5 h, dehydrated by routine method. They were embedded by paraffin and cut into 5 μm sections on a microtome in this study (Cioban et al., 2013). They were then stained in Mayer's hematoxylin and eosin. TUNEL staining was performed using apoptosis in situ detection kit (Wako Pure Chemical Industries Ltd., Osaka, Japan). The stained tissues were observed through an optical microscope (BX51; Olympus Co. Ltd., Tokyo, Japan) and were photographed.

2.8. Statistical analysis

All data are presented as mean ± standard deviation. The Student's *t*-test and one-way ANOVA test were used for the statistical analysis. Values below 5% ($p < 0.05$) on both sides were considered to be statistically significant.

3. Results

3.1. Laboratory data

In control value, the plasma AST level was 85.9 ± 10.3 IU/l, while the ALT level was 45.8 ± 3.4 IU/l (Fig. 1). The CCl_4 administered group showed an AST level of 573 ± 443 IU/l an ALT level of

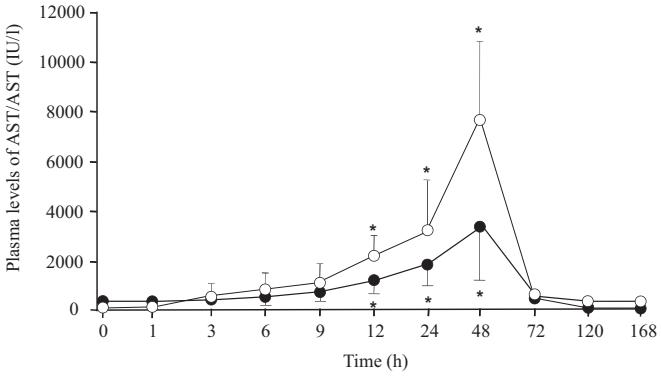

Fig. 1. Plasma concentration–time curves of AST (○) and ALT (●) in rats treated with CCl_4. CCl_4 were administered intraperitoneally 2 mg/kg in rats. 0 h represents the control group. CCl_4, carbon tetrachloride; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Each bar indicates mean ± SD ($n = 4–8$). $^{**}p < 0.01$ vs 0 h.