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Suppression of cytochrome *P*450 gene expression in the livers of mice with concanavalin A-induced hepatitis

Toshihiro Okamoto *

Research Laboratories, Nippon Chemiphar Co., Ltd., 1-22 Hikokawato, Misato, Saitama 341-0005, Japan

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Abstract

Cytochrome *P*450 (CYP) gene expression in the livers of mice with concanavalin A-induced hepatitis was examined. Treatment of mice with concanavalin A (10 mg/kg, i.v.) elevated plasma alanine aminotransaminase activity. In normal liver, CYP1A2, 3A and 2E1 mRNAs were expressed, and concanavalin A treatment differentially suppressed the expression of these CYP genes. Gadolinium chloride (40 mg/kg, i.p.) treatment, which inhibited the concanavalin A-induced elevation of plasma alanine aminotransferase activity without affecting concanavalin A-induced cytokine expression, counteracted the concanavalin A-induced suppression of CYP gene expression in the liver. Kupffer cell function or hepatic injury might contribute to the concanavalin A-induced suppression of CYP gene expression.

Keywords: Concanavalin A; Cytochrome *P*450; Hepatitis

1. Introduction

In human liver disease, hepatic cytochromes *P*450 (CYP) 1A2, 3A and 2E1 have been shown to be significantly decreased and this suppression is regulated at the level of mRNA expression (Guengerich and Turvy, 1991; George et al., 1995). In order to study the mechanism of this suppression of CYP mRNA expression, a suitable animal hepatitis model is required. Although the mechanism of the suppression of CYPs in the livers of humans with hepatitis remains unknown, hepatic CYPs are known to be suppressed by immunological reactions (Chang et al., 1978; Renton, 1986). The liver is a rich source of cytokines, and cytokines mediate the immune response in the liver. Proinflammatory cytokines such as interferon-γ and tumor necrosis factor-α (TNF-α) are thought to be responsible for the suppression of hepatic CYP enzymes through immunological reactions (Ghezzi et al., 1986; Abdel-Razzak et al., 1993). Furthermore, suppression of CYPs through immunological reactions is predominantly regulated at the gene expression level (Stanley et al., 1988; Armstrong and Renton, 1993). A mouse concanavalin A-induced hepatitis model is dependent on immunological reactions (Gantner et al., 1995). Intravenous injection of concanavalin A into mice activates T-cells, and proinflammatory cytokines, TNF-α and interferon-γ, are released by the activated T-cells (Gantner et al., 1995). However, it was not known whether CYPs in the liver are suppressed or not in this immune-mediated hepatitis model.

In the present study, the expression of CYP 1A2, 3A and 2E1 in the livers of mice with concanavalin A-induced hepatitis was studied by means of reverse-transcription polymerase chain reaction (RT-PCR).

2. Materials and methods

Female BALB/c mice obtained from Charles River Japan, (Atsugi, Japan) were used at 7–10 weeks of age. The animals were kept in an air-conditioned room and given water ad libitum. Experiments were conducted under the approval of the experimental protocols by the Institutional Ethics Committee. They were killed with ether. Concanavalin A and Gadolinium chloride ($GdCl_3$) were purchased from Sigma (St. Louis, MO, USA) and Wako (Osaka, Japan), respectively. Concanavalin A dissolved in pyrogen-free saline was administered to mice (10 mg/kg) via a tail vein (injection vol., 100 μl) and $GdCl_3$ was

* Tel.: +81-489-52-4311; fax: +81-489-52-0743.

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administered intraperitoneally. Plasma transaminase activity, i.e., that of alanine aminotransferase, and RT-PCR analysis were performed as previously described (Okamoto et al., 1996). RT-PCR with these gene-specific primers was performed within the range of the linear phase of amplification for each primer. RT-PCR with interleukin-2, interferon-γ, TNF-α and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene-specific primers was per-

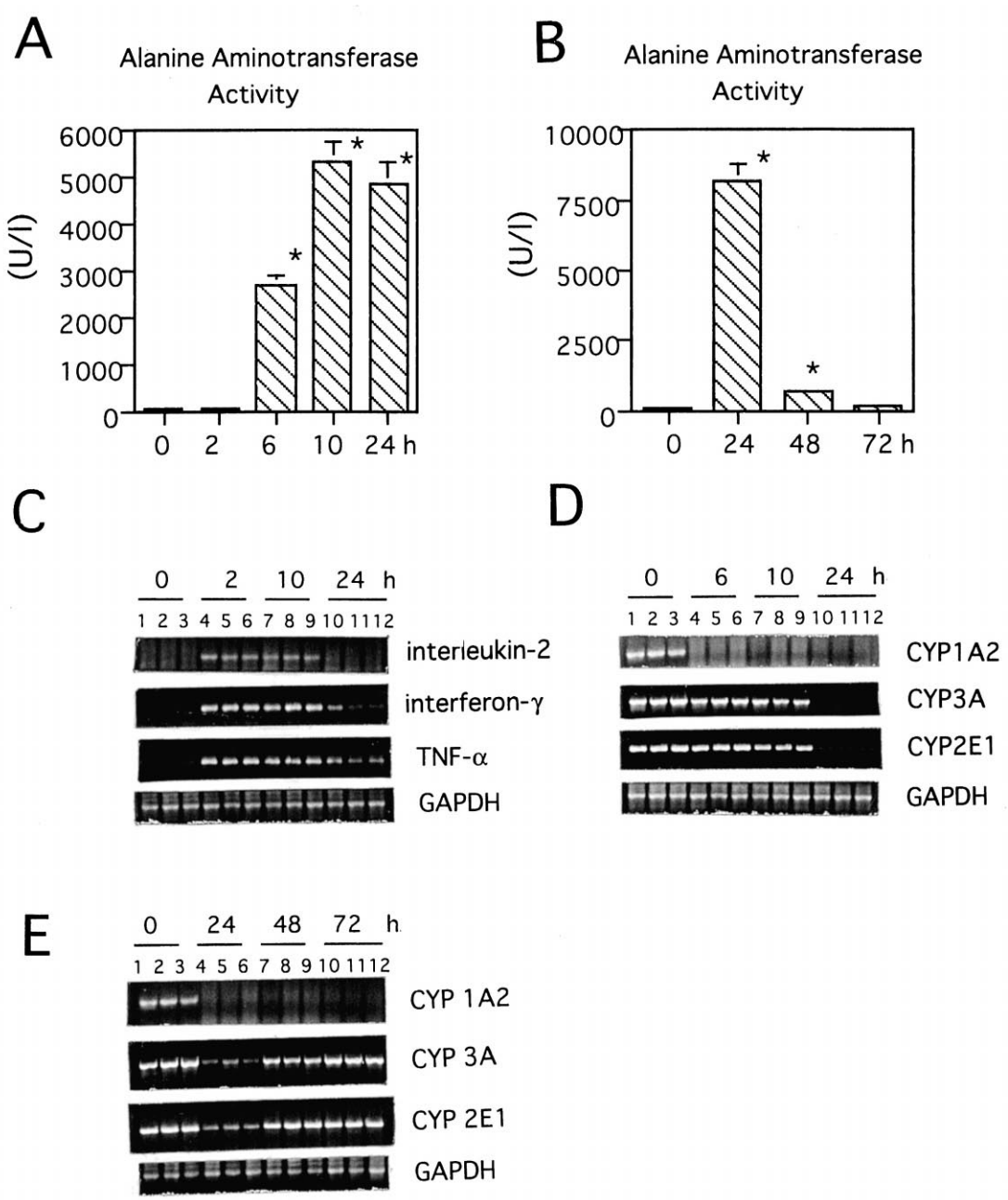

Fig. 1. (A) and (B) Increase in the plasma transaminase level after concanavalin A injection. For each time point, four mice were injected intravenously with concanavalin A (10 mg/kg). Data points represent the means ± S.E. of alanine aminotransferase activity (U/l) in plasma obtained at the indicated time points. * $P < 0.01$ vs. normal control. Livers were removed at the time indicated for RNA analysis. (A) Plasma was obtained at 2, 6, 10 and 24 h after the concanavalin A injection. (B) Plasma was obtained at 24, 48 and 72 h after the concanavalin A injection. (C) and (D) Effects of concanavalin A on cytokine and CYP gene expression in the liver. RNA was isolated from the liver samples in (A) and subjected to RT-PCR. (C) RT-PCR with interleukin-2, interferon-γ and TNF-α gene-specific primers. Lane 1, 2 and 3: normal liver samples; lane 4 to 12 were liver samples from mice treated with concanavalin A for the indicated period. (D) RT-PCR with CYP1A2, 3A and 2E1 gene-specific primers. Lane 1, 2 and 3: normal liver samples; lane 4 to 12 were liver samples from mice treated with concanavalin A for the indicated period. (E) Time course of the recovery of concanavalin A-induced suppression of CYP gene expression. The livers in (B) were removed at the indicated time points. RNA was isolated and subjected to RT-PCR with CYP1A2, 3A and 2E1 gene-specific primers. Lane 1, 2 and 3: normal liver samples; lane 4, 5 and 6: liver samples from mice treated with concanavalin A for 24 h; lane 7, 8 and 9: liver samples from mice treated with concanavalin A for 48 h; lane 10, 11 and 12: liver samples from mice treated with concanavalin A for 72 h.

formed for 30 cycles of amplification. RT-PCR amplification with CYP1A2, 3A and 2E1 gene-specific primers was performed for 35, 25 and 19 cycles, respectively. Gene-specific PCR primers were as follows:

Interleukin-2 (Genbank Acc#K02292)
(sense) 5′-ATGTACAGCATGCAGCTCGCATC-CTGTGTC-3
(antisense) 5-AGTCAAATCCAGAACATGC-CGCAGAGGTCC-3
Interferon-γ (Genbank Acc#K00083)
(sense) 5-ATCAGCAGCGACTCCTTTTCCGCTT-3
(antisense) 5-GAAAGCCTAGAAAGTCT-GAATAACT-3
TNF-α (Genbank Acc#M11731)
(sense) 5-AGCCCACGTCGTAGCAAACCACCAA-3
(antisense) 5-ACACCCATTCCCTTCACA-GAGCAAT-3
CYP1A2 (Genbank Acc#MUSCYP34A)
(sense) 5-AAGATCCATGAGGAGCTGGA-3
(antisense) 5-TCCCCAATGCACCGGCGCTTTCC-3
CYP3A (Genbank Acc#MMCP450M)
(sense) 5-GAAGCATTGAGGAGGATCAC-3
(antisense) 5-GGGTTGTTGAGGGAATCCAC-3
CYP2E1 (Genbank Acc#MUSP450E)
(sense) 5-GCGGTTCTTGGCATCACCGT-3
(antisense) 5ô-GCAGGGTGCACAGCCAATCA-3
GAPDH (Genbank Acc#X02231)
(sense) 5-ATGGTGAAGGTCGGTGTGAACG-3ô
(antisense) 5-GTTGTCATGGATGACCTTGGCC-3

The results were statistically analyzed by means of the Dunnett's multiple comparison test.

3. Results

3.1. Suppression of CYP gene expression in the liver

Treatment of mice with concanavalin A elevated plasma alanine aminotransferase activity at 6 h (Fig. 1A). At 24 and 48 h after concanavalin A treatment, plasma alanine aminotransferase activity was still significantly elevated (Fig. 1A and B).

Interleukin-2, interferon-γ and TNF-α gene expression in the liver was induced at 2 h after concanavalin A treatment (Fig. 1C). In normal liver, the CYP1A2, 3A and 2E1 genes were expressed (Fig. 1D). Treatment of mice with concanavalin A abolished the CYP1A2 gene expression at 6 h (Fig. 1D). CYP3A1 and 2E1 gene expression was decreased at 24 h after concanavalin A administration (Fig. 1D). The GAPDH gene was evenly expressed in the

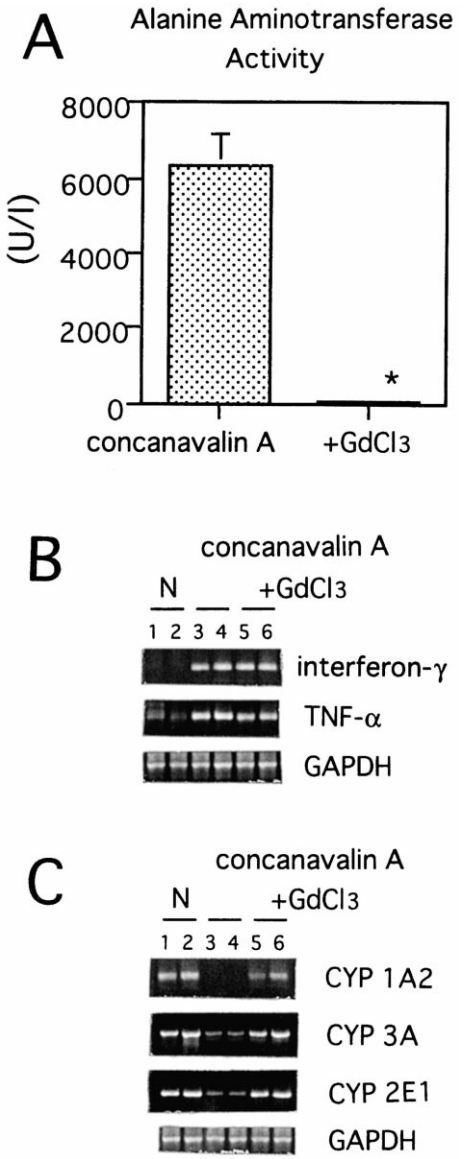

Fig. 2. Effect of $GdCl_3$ on concanavalin A-induced suppression of CYP gene expression in the liver. (A) Effect of $GdCl_3$ treatment on concanavalin A-induced elevation of plasma alanine aminotransferase activity. Each mouse was pretreated with $GdCl_3$ (40 mg/kg, i.p.), and 24 h later, concanavalin A was injected. Plasma was obtained at 24 h after concanavalin A treatment ($n = 5$). $^{*}P < 0.01$ vs. result with concanavalin A alone. (B) and (C) Typical agarose gel electrophoresis of RT-PCR products of the effects of $GdCl_3$ on concanavalin A-induced suppression of CYP gene expression. $GdCl_3$ (40 mg/kg, i.p.) was administered to each mouse, and 24 h later, concanavalin A was injected. Livers were removed and RNA was isolated for RT-PCR. (B) Effect of $GdCl_3$ treatment on concanavalin A-induced interferon-γ and TNF-α gene expression. Liver samples were obtained at 6 h after concanavalin A treatment. Lane 1 and 2: normal liver samples; lane 3 and 4: liver samples from mice treated with concanavalin A; lane 5 and 6: liver samples from mice treated with concanavalin A + $GdCl_3$. (C): Effect of $GdCl_3$ treatment on concanavalin A-induced suppression of CYP1A2, 3A and 2E1 gene expression. Liver samples were obtained at 24 h after concanavalin A treatment. Lane 1 and 2: normal liver samples; lane 3 and 4: liver samples from mice treated with concanavalin A; lane 5 and 6: liver samples from mice treated with concanavalin A + $GdCl_3$.

livers of normal and concanavalin A-treated mice. The stable expression of GAPDH indicated that there was no variation in overall expression.

The concanavalin A-induced suppression of CYP1A2 gene expression had not even recovered at 72 h (Fig. 1E). Although plasma alanine aminotransferase activity was still significantly elevated at 48 h after concanavalin A injection, the concanavalin A-induced suppression of CYP3A and 2E1 gene expression had recovered by this time (Fig. 1E).

3.2. Effects of GdCl3 treatment on concanavalin A-induced hepatitis and CYP gene expression

In the present study, $GdCl_3$ treatment inhibited the concanavalin A-induced elevation of plasma alanine aminotransferase activity (Fig. 2A). $GdCl_3$ treatment had not affected concanavalin A-induced interferon-γ or TNF-α gene expression in the liver at 6 h after concanavalin A treatment (Fig. 2B). However, this treatment did counteract the concanavalin A-induced suppression of CYP 1A2, 3A1 and 2E1 gene expression in the liver at 24 h (Fig. 2C).

4. Discussion

Suppression of hepatic CYPs by immunological reactions is predominantly regulated at the gene expression level (Stanley et al., 1988; Armstrong and Renton, 1993). In the present study, the induction of interleukin-2 mRNA expression by concanavalin A treatment suggested T-cell activation, and interferon-γ and TNF-α gene expression was induced in the liver. Thus, it is possible that transcripts of these concanavalin A-induced proinflammatory cytokine genes suppress CYPs in the liver. In the present study, suppression of CYP 1A2, 3A and 2E1 gene expression was clearly shown in the livers of the concanavalin A-treated mice. At 6 h after concanavalin A treatment, plasma alanine aminotransferase activity was elevated and CYP1A2 mRNA expression was suppressed. In contrast, CYP3A and 2E1 mRNA expression was inhibited at 24 h. Thus, the gene expression of CYP1A2, and CYP3A and 2E1 in the liver might be differently regulated. The time course of the reversal of the concanavalin A-induced suppression of this CYP gene expression was examined. At 72 h after concanavalin A treatment, CYP1A2 mRNA expression had not yet recovered from the concanavalin A-induced suppression. In contrast, although plasma alanine aminotransferase activity was significantly elevated at 48 h, expression of the CYP3A and 2E1 genes had already recovered from the concanavalin A-induced suppression. Thus, CYP1A2 gene expression seems to be sensitively suppressed in concanavalin A-induced hepatitis. In contrast, the concanavalin A-induced suppression of CYP3A and 2E1 gene expression might recover independently of liver injury.

$GdCl_3$ is a commonly used Kupffer cell inhibitor (Hardonk et al., 1992), and previously it was reported that $GdCl_3$ treatment protected mice from concanavalin A-induced hepatitis without affecting concanavalin A-induced cytokine gene expression in the liver (Okamoto et al., 1998). In the present study, $GdCl_3$ treatment inhibited concanavalin A-induced hepatitis, but affected neither concanavalin A-induced interferon-γ nor TNF-α gene expression in the liver. However, this treatment counteracted the concanavalin A-induced suppression of CYP1A2, 3A and 2E1 gene expression in the liver. Thus, proinflammatory cytokines do not seem to be the only factors involved in the suppression of hepatic CYP gene expression. Based on this, at least two possible mechanisms of concanavalin A-induced suppression of hepatic CYP gene expression can be considered: (1) since $GdCl_3$ treatment inhibits Kupffer cell function, it is possible that Kupffer cell function or factors released from Kupffer cells are involved in the concanavalin A-induced suppression of CYP gene expression. (2) Since $GdCl_3$ treatment inhibited the concanavalin A-induced elevation of plasma alanine aminotransferase activity, it is also possible that a factor, which is released during hepatitis participates in the inhibition of hepatic CYP gene expression.

The present results clearly showed the suppression of CYP 1A2, 3A and 2E1 gene expression in the livers of mice with concanavalin A-induced hepatitis. In order to examine the physiological effect of concanavalin A on CYP, further studies on CYP protein expression are required. In human chronic hepatitis, hepatic CYP 1A2, 3A and 2E1 are suppressed at the level of mRNA expression (Guengerich and Turvy, 1991; George et al., 1995). The mechanism of this suppression of CYP mRNA expression remains unknown due to the lack of a suitable animal model. From the results of the present study, the mouse concanavalin A-induced hepatitis model seems to be suitable for studying the mechanism of the suppression of hepatic CYP gene expression in human chronic hepatitis.

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