ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 550–559

THE JOURNAL OF Nutritional Biochemistry

L-tryptophan administration promotes the reversion of pre-established chronic liver injury in rats treated with carbon tetrachloride

Yoshiji Ohta[a,*], Daizo Sahashi[b]

[a]*Department of Chemistry, School of Medicine, Fujita Health University, Toyoake, Japan*
[b]*Sahashi Internal Medicine Clinic, Toyota, Japan*

Received 9 November 2001; received in revised form 25 March 2002; accepted 15 April 2002

Abstract

We examined the effect of L-tryptophan (Trp) administration on the reversion of CCl_4-induced chronic liver injury after hepatotoxicant withdrawal in rats. When rats treated with CCl_4 twice a week for 6 weeks were released from CCl_4 treatment for 2 weeks, there was an incomplete reversion of liver injury. The reversion was enhanced by 2 weeks of daily intraperitoneal administration of Trp (50 mg/kg body weight), starting just after CCl_4 withdrawal. There were increases in the levels of thiobarbituric acid reactive substances, an index of lipid peroxidation, Ca^{2+}, triglycerides, and Trp, and decreases in tryptophan 2,3-dioxygenase activity and serum triglyceride concentrations in the liver of rats treated with CCl_4 for 6 weeks. Serum albumin concentrations and in vitro hepatic protein synthesis activity did not change in the CCl_4-treated rats. The changes in the CCl_4-treated rats were partially attenuated 2 weeks after CCl_4 withdrawal. The attenuation was enhanced by 2 weeks of daily Trp administration. The increases in hepatic thiobarbituric acid reactive substances and triglycerides and the decreases in hepatic tryptophan 2,3-dioxygenase activity and serum triglyceride concentrations observed 2 weeks after CCl_4 withdrawal were almost completely attenuated by Trp administration. In vitro hepatic protein synthesis in CCl_4-treated and untreated rats was increased by 2 weeks of daily Trp administration. These results indicate that Trp administration promotes the reversion of pre-established chronic liver injury in rats treated with CCl_4, and suggest that Trp exerts this effect by enhancing the improvement of several parameters of liver dysfunction associated with chronic liver injury and by stimulating hepatic protein synthesis.

Keywords: L-tryptophan; Carbon tetrachloride; Chronic liver injury (rat); Liver dysfunction; Tryptophan 2'3-dioxygenase

1. Introduction

L-Tryptophan (Trp), one of the essential amino acids, is used for protein synthesis in various tissues including the liver. More than 90% of plasma Trp is metabolized in the liver, where it is converted to kynurenine by tryptophan 2,3-dioxygenase (TDO) and then to nicotinamide adenine dinucleotide (NAD) and acetyl coenzyme A via the kynurenine pathway [1]. Exogenous Trp stimulates the synthesis of proteins, including albumin, by enhancing both mRNA synthesis and its translocation from the nucleus to the cytoplasm in the liver of rats and mice [2]. Trp and its metabolites (produced by the kynurenine pathway), such as 3-hydroxykynurenine and 3-hydroxylanthranilic acid, have antioxidant actions in vitro [3,4].

Carbon tetrachloride (CCl_4) is widely used to experimentally produce chronic liver injury or cirrhosis as well as acute liver injury. There are similarities between CCl_4-induced liver cirrhosis and human liver cirrhosis [5]. Serum albumin concentrations decrease in rats with chronic CCl_4 intoxication at an advanced stage of liver cirrhosis [6–8]. Oxidative damage has been implicated in the pathogenesis of CCl_4-induced chronic liver injury and cirrhosis [8–15]. Fat, i.e., triglycerides, accumulates in the liver of rats with CCl_4–induced chronic liver injury and cirrhosis [6,8,9,12]. An increase in Ca^{2+} levels followed by an increase in phospholipase A_2 activity occurs in the liver of rats with CCl_4-induced chronic liver injury [11]. In addition, liver TDO activity is reduced in rats with CCl_4-induced chronic liver injury and cirrhosis [7,8].

Sidransky et al. [16] reported that a single oral dose of Trp stimulated liver protein synthesis in rats with CCl_4-induced liver cirrhosis. We recently reported that daily parenteral administration of Trp alleviated CCl_4-induced

* Corresponding author. Tel.: +81-562-93-1193; fax: +81-562-93-1193/4593.
E-mail address: yohta@fujita-hu.ac.jp (Y. Ohta).

0955-2863/02/$ – see front matter
PII: S0955-2863(02)00203-6

chronic liver injury in rats, possibly by maintaining liver protein synthesis [8]. Liver cirrhosis or fibrosis in rats chronically treated with CCl_4 is reversed by withdrawal of the hepatotoxicant at an early stage of liver cirrhosis or fibrosis [17–19]. It is not known, however, whether Trp administration promotes the reversion of pre-established chronic liver injury in rats treated with CCl_4.

The present study examined whether Trp administration promotes the reversion of chronic liver injury in rats treated with CCl_4 after hepatotoxicant withdrawal. The effect of daily administration of Trp for 2 weeks after withdrawal of 6 weeks of intermittent CCl_4 treatment, on several parameters of liver function was investigated. In addition, hepatic Trp levels and TDO activity were examined in rats 2 weeks after withdrawal of CCl_4 treatment.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin and hematin were obtained from Sigma Chemical Co. (St. Louis, MO. USA). L-[U-^{14}C]Leucine (Leu) was purchased from Du Pont/NEN Research Products (Wilmington, DE, USA); CCl_4, Ca^{2+} standard solution, ethylenediaminetetraacetic acid (EDTA), lanthanum chloride ($LaCl_3$), olive oil, tetramethoxypropane, 2-thiobarbituric acid, Trp, and other chemicals from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). All chemicals were of reagent grade and were used without further purification.

2.2. Animals and treatments

Male Wistar rats (n = 50, 200g, 5 weeks old) were purchased from Japan SLC Co. (Hamamatsu, Japan). The animals were housed in temperature- (25°C) and humidity-(50%) controlled room with a 12-h light cycle and standard rat chow (Oriental MF rat chow; Oriental Yeast Co., Tokyo, Japan) and tap water were available ad libitum throughout the study. The animals, 6 weeks of age, were classified into the following six groups: control pre-withdrawal group (CON-pre), 5 rats received subcutaneous (s.c.) injection of olive oil (1.0 ml/kg body weight) twice a week for a 6-week period; CCl_4 pre-withdrawal group (CCl_4-pre), 10 rats received s.c. injection of a 50% (v/v) CCl_4 solution in olive oil (2.0 ml/kg body weight) twice a week for a 6-week period; control post-withdrawal group (CON-post), 5 rats received daily intraperitoneal (i.p.) injection of 1.0 ml 0.85% NaCl/kg body weight, starting after 6 weeks of olive oil treatment, for 2 weeks; Trp post-withdrawal group (Trp-post), 5 rats received daily i.p. injection of a 50 mg/ml Trp solution in 0.85% NaCl (1.0 ml/kg body weight), starting after 6 weeks of olive oil treatment, for 2 weeks; CCl_4 post-withdrawal group (CCl_4-post), 10 rats received daily i.p. injection of 1.0 ml 0.85% NaCl/kg body weight for 2 weeks, starting after 6 weeks of CCl_4 treatment; CCl_4-Trp post-withdrawal group (CCl_4-Trp-post), 10 rats received daily i.p. injection of a 50 mg/ml Trp solution in 0.85% NaCl (1.0 ml/kg body weight) for 2 weeks, starting after 6 weeks of CCl_4 treatment. CCl_4 and Trp treatments were conducted between 9:00 and 10:00 a.m. and between 5:00 and 6:00 p.m., respectively. The dose of Trp (50 mg/kg body weight per day) used in the present study was chosen because daily i.p. administration of Trp at a dose of 50 mg/kg body weight alleviates CCl_4-induced chronic liver injury in rats [8]. Rats in each group were weighed just before sacrifice. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of Fujita Health University.

2.3. Determination of serum and liver enzymes and components

All rats were killed under ether anesthesia at which time blood was collected from the inferior vena cava, and subsequently separated to obtain serum. Immediately after sacrifice, the livers were perfused through the portal vein with ice-cold 0.15 M KCl for 5 min to remove as much blood as possible, isolated, washed with ice-cold 0.15 M KCl, blotted on a filter paper, and then weighed. The isolated liver and serum were stored at −80°C until use.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using a commercial kit, Iatrozyme TA-LQ (Dai-Iatron Co., Tokyo, Japan). Albumin and triglyceride levels were determined using commercial kits, Albumin Test-Wako (based on the bromcresol green method [20]) and Triglyceride G Test-Wako (based on the enzymatic method [21]) (Wako Pure Chemical Ind., Ltd., Osaka, Japan), respectively.

For determination of total liver hydroxyproline (Hyp), protein, triglycerides, and thiobarbituric acid reactive substances (TBARS; used as an index of lipid peroxidation), the liver was homogenized with nine volumes of ice-cold 0.15 M KCl-1.0 mM EDTA using a glass homogenizer with a Teflon pestle. The prepared homogenate was hydrolyzed in 6 M HCl at 110°C for 20 h, and the resultant hydrolysate was used for determination of total Hyp by the method of Bondjers and Björkerud [22]. Protein was measured by the method of Lowry et al. [23] using bovine serum albumin as a standard. Triglyceride levels were measured using a commercial kit, Triglyceride Test-Wako (based on the acetylacetone method [24]) (Wako Pure Chemical Ind., Ltd., Osaka, Japan). TBARS was measured using the thiobarbituric acid method of Ohkawa et al. [25] except that 1.0 mM EDTA was added to the reaction medium. Tetramethoxypropane was used as an external standard. The amount of TBARS is expressed in malondialdehyde (MDA) equivalents. For determination of TDO, Trp, and Ca^{2+}, the liver was homogenized with nine volumes of ice-cold 0.25 M sucrose. TDO in the homogenate was assayed in the presence of added hematin (2 μM) at 37°C under aerobic conditions with agitation according to the method of Metzler et

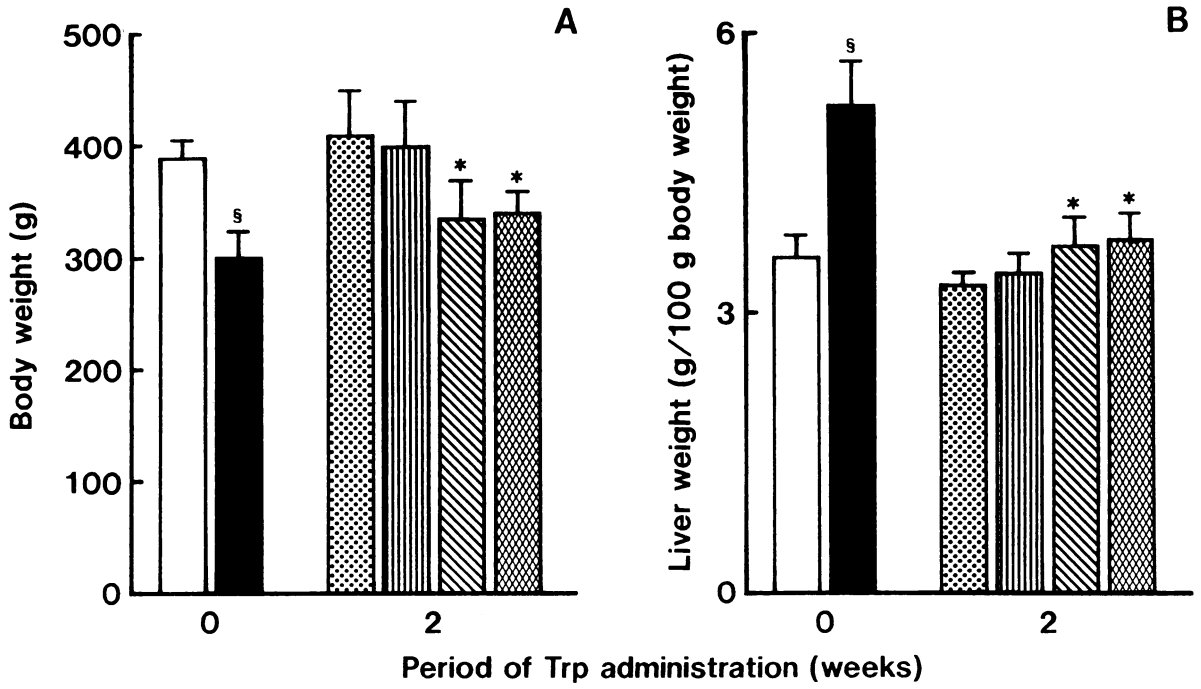

Fig. 1. Body weight (A) and relative liver weight (B) in rats with chronic CCl_4 treatment, chronic CCl_4 treatment and subsequent withdrawal of the toxicant treatment, or daily Trp injection during the withdrawal period. Open bar, CON-pre group; closed bar, CCl_4-pre group; dotted bar, CON-post group; stripped bar, Trp-post group; hatched bar, CCl_4-post group; crosshatched bar, CCl_4-Trp-post group. Results are expressed as means ± SD (n = 5–10). $^{\S}P < 0.05$ when the CCl_4-pre group was compared with the CON-pre group; $^{*}P < 0.05$ when the CCl_4-post group or the CCl_4-Trp-post group was compared with the CON-post group.

al. [26]. One unit of TDO activity is expressed as the amount of enzyme producing 1 μmol kynurenine per hour. After deproteinization of the homogenate with perchloric acid in a final concentration of 0.2 M, Trp was determined using high-performance liquid chromatography with electrochemical detection as described previously [27]. Determination of Ca^{2+} in the homogenate was conducted as follows: 1.0 ml of the homogenate was mixed with 1.0 ml of 10% trichloroacetic acid containing 0.3 M HCl and 5% $LaCl_3$, and the mixture was centrifuged at 3,000 rpm for 5 min after storing at 4°C for 15 h, as described by Carafoli and Tiozzo [28]. Ca^{2+} in the resultant supernatant was measured in a Hitachi polarized Zeeman atomic absorption spectrophotometer model Z-1800 (Hitachi Co., Tokyo, Japan). The amount of Ca^{2+} in the homogenate was calculated from the standard curve of Ca^{2+}, which was made using a standard solution of Ca^{2+}. In vitro protein synthesis activity was determined by the method of Ovravec and Sourks [29], using post-mitochondrial fractions isolated from 45 mg (original wet weight) of fresh rat liver and 18.5 kBq [^{14}C]Leu. This synthesis activity is expressed as the amount of radioactivity incorporated into the acid-insoluble fraction in 30 min at 37°C.

2.4. Statistical analysis

All data obtained are expressed as the mean ± SD. Results were analyzed by a computerized statistical program (StatView, Abacus Concepts Inc., Berkeley, CA, USA). Mean values were compared by one-way analysis of variance and Fisher's protected least significance difference for multiple comparisons as the post hoc test. A *P*-value of less than 0.05 was considered to be statistically significant.

3. Results

The body weight of rats treated with CCl_4 for 6 weeks (CCl_4-pre) was significantly lower than that of rats treated with olive oil for the same period (CON-pre) (Fig. 1A). The body weight of rats treated with saline for 2 weeks after 6 weeks of CCl_4 treatment (CCl_4-post) was significantly lower than that of rats treated with saline for 2 weeks after 6 weeks of olive oil treatment (CON-post), but was not different from that of rats treated with Trp for 2 weeks after 6 weeks of CCl_4 treatment (CCl_4-Trp-post) (Fig. 1A). The body weight in the CON-post group was not different from rats treated with Trp for 2 weeks after 6 weeks of olive oil treatment (Trp-post) (Fig. 1A). There was no difference in body weight between the CON-pre and CON-post groups (Fig. 1A). The CCl_4-pre group had significantly higher liver weight (1.4-fold higher) relative to body weight than the CON-pre group (Fig. 1B). The relative liver weight of the CCl_4-post group was significantly higher than that of the CON-post group, but the CCl_4-post group had a 1.1-fold higher relative liver weight than the CON-post group (Fig.

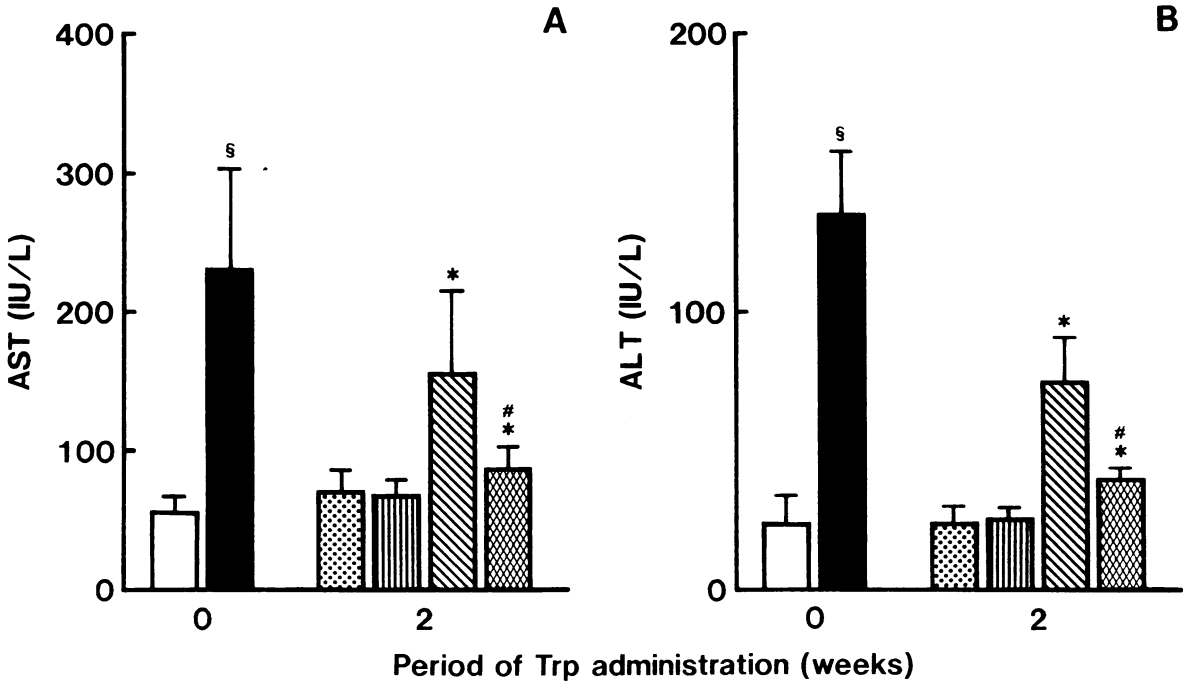

Fig. 2. Serum AST (A) and ALT (B) activities in rats with chronic CCl_4 treatment, chronic CCl_4 treatment and subsequent withdrawal of the toxicant treatment, or daily Trp injection during the withdrawal period. Open bar, CON-pre group; closed bar, CCl_4-pre group; dotted bar, CON-post group; stripped bar, Trp-post group; hatched bar, CCl_4-post group; crosshatched bar, CCl_4-Trp-post group. Results are expressed as means ± SD (n = 5–10). $^{\S}P < 0.05$ when the CCl_4-pre group was compared with the CON-pre group; $^{*}P < 0.05$ when the CCl_4-post group or the CCl_4-Trp-post group was compared with the CON-post group; $^{\#}P < 0.05$ when the CCl_4-Trp-post group was compared with the CCl_4-post group.

1B). There was no difference in relative liver weight between the CCl_4-post and CCl_4-Trp-post groups or between the CON-post and Trp-post groups (Fig. 1B).

Serum AST and ALT activities in the CCl_4-pre group were 4.4- and 6.9-fold, respectively, higher than those in the CON-pre group (Fig. 2). The CCl_4-post group had significantly higher serum AST (2.2-fold higher) and ALT (3.2-fold higher) activities than the CON-post group (Fig. 2). Serum AST and ALT activities in the CCl_4-Trp-post group were significantly lower than those in the CCl_4-post group and 1.2- and 1.7-fold, respectively, higher than those in the CON-post group (Fig. 2). There were no differences in serum AST and ALT activities between the CON-pre and CON-post groups or between the CON-post and Trp-post groups (Fig. 2).

The CCl_4-pre group had a significantly higher hepatic total Hyp content (2.1-fold higher) than the CON-pre group (Fig. 3). The liver of the CCl_4-post group had a significantly higher total Hyp content (1.5-fold higher) than the liver of the CON-post group (Fig. 3). The CCl_4-Trp-post group had a significantly lower hepatic total Hyp content than the CCl_4-post group and a 1.3-fold higher hepatic total Hyp content than the CON-post group (Fig. 3). There was no difference in hepatic total Hyp content between the CON-pre and CON-pos groups or between the CON-post and Trp-post groups (Fig. 3).

The CCl_4-pre group had significantly higher hepatic TBARS levels (1.6-fold higher) than that in the CON-post group (Fig. 4A). The CCl_4-post group had significantly higher hepatic TBARS levels (1.3-fold higher) than the CON-post group (Fig. 4A). Hepatic TBARS levels in the CCl_4-Trp-post group were significantly lower than in the CCl_4-post group and did not differ form those in the CON-post group (Fig. 4A). The hepatic TBARS levels did not differ between the CON-pre and CON-post groups or between the CON-post and Trp-post group (Fig. 4A). The Ca^{2+} content in the liver of the CCl_4-pre group was significantly higher (3.1-fold higher) than that of the CON-pre group (Fig. 4B). The CCl_4-post group had a significantly higher hepatic Ca^{2+} content (2.6-fold higher) than the CON-post group (Fig. 4B). The hepatic Ca^{2+} content in the CCl_4-Trp-post group was significantly lower than that in the CCl_4-post group and was 1.5-fold higher than that in the CON-post group (Fig. 4B). There was no difference in the amount of hepatic Ca^{2+} between the CON-pre and CON-post groups or between the CON-post and Trp-post groups (Fig. 4B).

The CCl_4-pre group had a significantly lower serum triglyceride concentration (25%) than the CON-pre group (Fig. 5A). The CCl_4-post group had a significantly lower serum triglyceride concentration (62%) than the CON group (Fig. 5A). The concentration of serum triglyceride in the CCl_4-Trp-post group was significantly higher than that in the CCl_4-post group and was almost equal to that in the CON-post group (Fig. 5A). There was no difference in serum triglyceride concentration between the CON-pre and CON-post groups or between the CON-post and Trp-post groups (Fig. 5A). Hepatic triglyceride levels in the CCl_4-pre

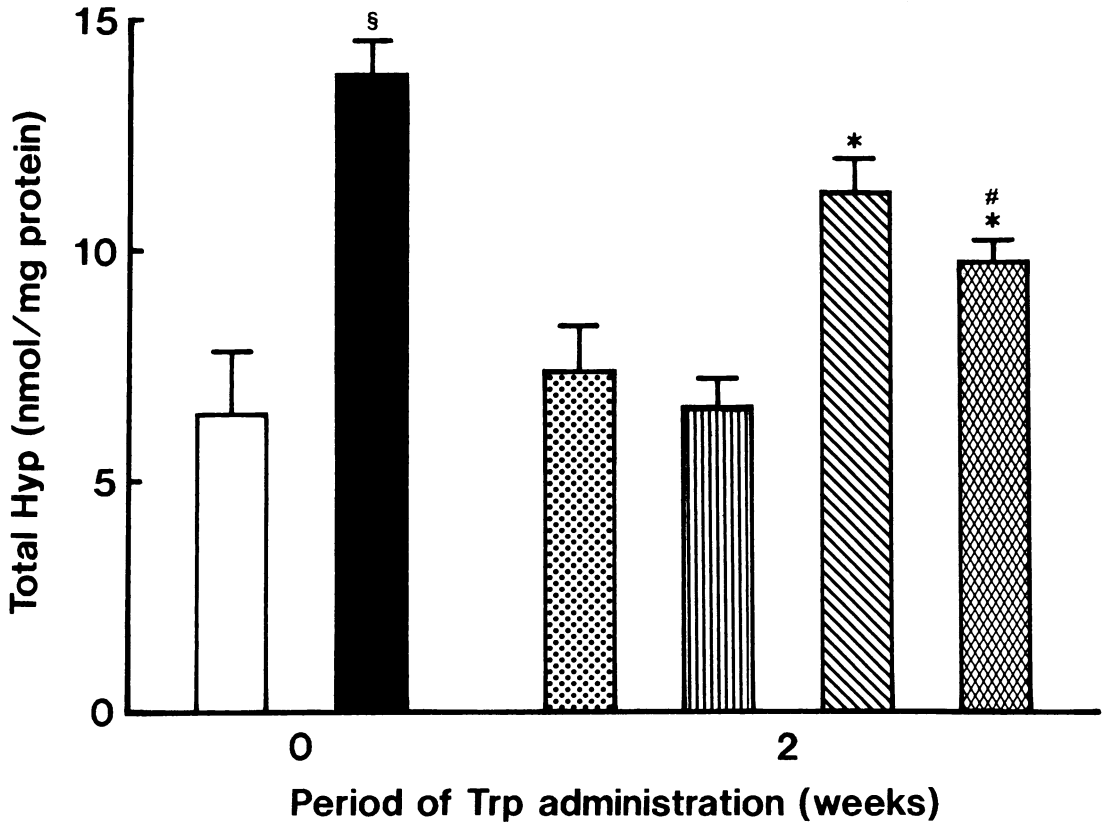

Fig. 3. Total Hyp content in the liver of rats with chronic CCl_4 treatment, chronic CCl_4 treatment and subsequent withdrawal of the toxicant treatment, or daily Trp injection during the withdrawal period. Open bar, CON-pre group; closed bar, CCl_4-pre group; dotted bar, CON-post group; stripped bar, Trp-post group; hatched bar, CCl_4-post group; crosshatched bar, CCl_4-Trp-post group. Results are expressed as means ± SD (n = 5–10). $^{\S}P < 0.05$ when the CCl_4-pre group was compared with the CON-pre group; $^{*}P < 0.05$ when the CCl_4-post group or the CCl_4-Trp-post group was compared with the CON-post group; $^{\#}P < 0.05$ when the CCl_4-Trp-post group was compared with the CCl_4-post group.

group were significantly higher (5.4-fold higher) than those in the CON-pre group (Fig. 5B). The CCl_4-post group had significantly higher hepatic triglyceride levels (1.6-fold higher) than the CON-post group (Fig. 5B). Hepatic triglyceride levels in the CCl_4-Trp-post group were significantly lower than those in the CCl_4-post group and were almost equal to those in the CON-post group (Fig. 5B). There was no difference in hepatic triglyceride levels between the CON-pre and CON-post group or between the CON-post and Trp-post groups (Fig. 5B).

There was no significant difference in serum albumin concentration between the CCl_4-pre and CON-pre groups, between the CCl_4-post and either the CON-post or CCl_4-Trp-post groups, between the Trp-post and CON-post groups or between the CON-pre and CON-post groups, although the albumin concentration in the Trp-post group tended to be higher than that in the CON-post group (Fig. 6A). There was no significant difference in the in vitro hepatic protein synthesis activity between the CCl_4-pre and CON-pre groups or between the CCl_4-post and CON-post groups or between the CON-pre and CON-post groups (Fig. 6B). The CCl_4-Trp-post group had a significantly higher in vitro hepatic protein synthesis activity (1,2-fold) than the CON-post group (Fig. 6B). The in vitro hepatic protein synthesis activity in the Trp-post group was significantly higher (1.3-fold higher) than that in the CON-post group (Fig. 6B).

The hepatic TDO activity in the CCl_4-pre group was significantly lower (53%) than that in the CON-pre group (Fig. 7A). The CCl_4-post group had a significantly lower hepatic TDO activity (75%) than the CON-post group (Fig. 7A). The hepatic TDO activity in the Trp-post group was significantly higher than that in the CCl_4-post group and almost equal to that in the CON-post group (Fig. 7A). There was no difference in hepatic TDO activity between the CON-post and Trp-post groups or between the CON-pre and CON-post groups, although the activity in the Trp-post group tended to be higher than that in the CON-post group (Fig. 7A). The CCl_4-pre group had a significantly higher hepatic Trp content (2.7-fold higher) than the CON-pre group (Fig. 7B). The CCl_4-post group had a significantly higher hepatic Trp content (2.4-fold higher) than the CON-post group (Fig. 7B). The hepatic Trp content in the CCl_4-Trp-post group was significantly lower than that in the CCl_4-post group and was 1.8-fold higher than in the CON-post group (Fig. 7B). The hepatic Trp content in the Trp-post group was significantly higher than that in the CON-post group but tended to be lower than that in the CCl_4-Trp-post group (Fig. 7B). There was no significant

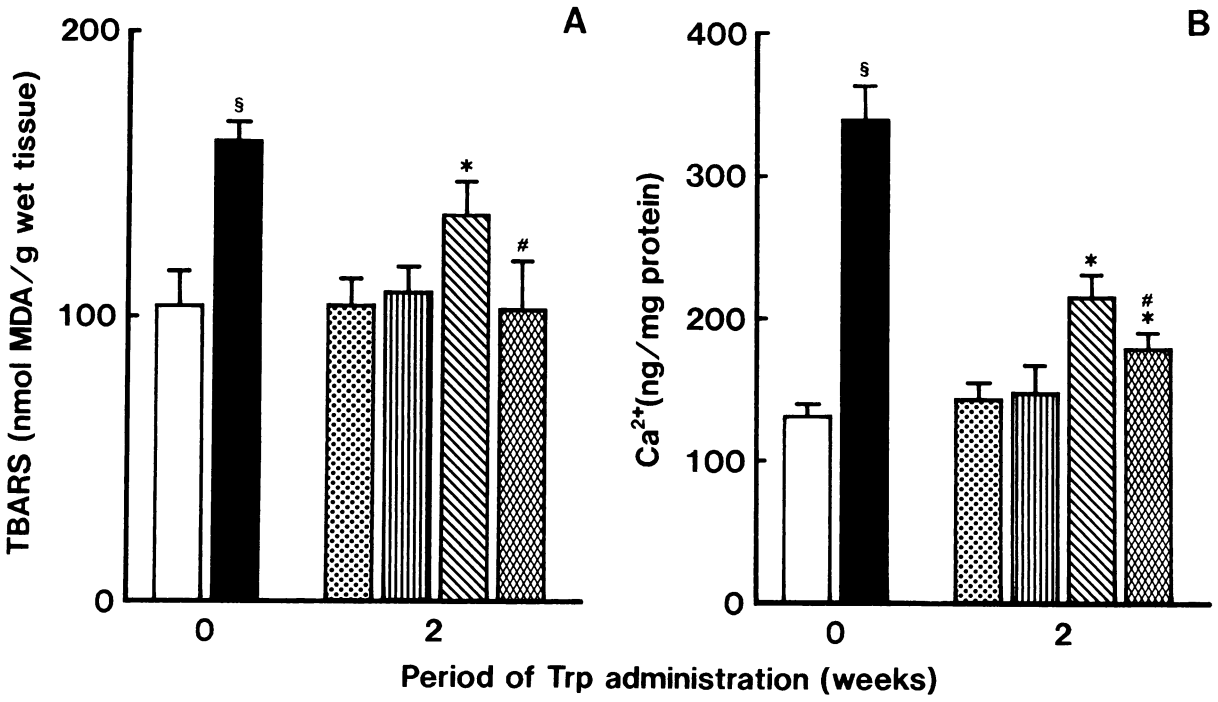

Fig. 4. TBARS (A) and Ca^{2+} (B) contents in the liver of rats with chronic CCl_4 treatment, chronic CCl_4 treatment and subsequent withdrawal of the toxicant treatment, or daily Trp injection during the withdrawal period. Open bar, CON-pre group; closed bar, CCl_4-pre group; dotted bar, CON-post group; stripped bar, Trp-post group; hatched bar, CCl_4-post group; crosshatched bar, CCl_4-Trp-post group. Results are expressed as means ± SD (n = 5–10). $^{\S}P < 0.05$ when the CCl_4-pre group was compared with CON-pre group; $^{*}P < 0.05$ when the CCl_4-post group or the CCl_4-Trp-post group was compared with the CON-post group; $^{\#}P < 0.05$ when the CCl_4-Trp-post group was compared with the CCl_4-post group.

difference in the hepatic Trp content between the CON-pre and CON-post groups (Fig. 7B).

4. Discussion

The present study demonstrates that Trp administration enhances reversion of CCl_4-induced chronic liver injury after withdrawal of hepatotoxicant in rats. Increases in serum AST and ALT activities, indices of liver cell damage, and hepatic total Hyp content, an index of fibrosis, of rats intermittently treated with CCl_4 for 6 weeks were partially attenuated by 2 weeks of hepatotoxicant withdrawal, as reported previously [17–19]. Daily administration of Trp (50 mg/kg body weight, i.p.) for 2 week after withdrawal of chronic CCl_4 treatment enhanced this reversion. The decreased body weight and the increased relative liver weight in rats treated with CCl_4 for 6 weeks were partially reversed by 2 weeks of hepatotoxicant withdrawal; these partial recoveries, however, were not enhanced by 2 weeks of daily Trp administration.

In the present study, TBARS content, an index of lipid peroxidation, increased in the liver of rats treated with CCl_4 for 6 weeks, as reported previously [8–15]. This increase in hepatic TBARS content was partially attenuated by a 2-week withdrawal of CCl_4 treatment and almost completely reversed by daily Trp administration during the withdrawal period. Trp administration during the same period in CCl_4-untreated (Trp-post) rats had no effect on hepatic TBARS levels (see Fig. 4A). A large increase in hepatic Ca^{2+} occurred in rats treated with CCl_4 for 6 weeks, as reported previously [11]. This increase in hepatic Ca^{2+} was partially attenuated by 2-week CCl_4 withdrawal, and further attenuated by daily Trp administration. Trp administration during the same period in CCl_4-untreated (Trp-post) rats had no effect on hepatic Ca^{2+} levels (see Fig. 4B). Both an increase in hepatic triglycerides and a decrease in serum triglycerides occurred in rats treated with CCl_4 for 6 weeks, as reported previously [6,8,9,12]. These changes in hepatic and serum triglyceride levels were partially reversed by 2-week CCl_4 withdrawal and were almost completely reversed by daily Trp administration, although Trp administration during the same period in CCl_4-untreated (Trp-post) rats did not affect hepatic or serum triglyceride levels (see Fig. 5). There were no significant changes in the serum albumin concentration or the in vitro hepatic protein synthesis activity in rats treated with CCl_4 for 6 weeks or those with 2-week CCl_4 withdrawal after CCl_4 treatment. Daily Trp administration had no effect on serum albumin concentration but caused a significant increase in the in vitro hepatic protein synthesis activity in rats with CCl_4 withdrawal. Trp administration tended to increase serum albumin concentration and caused a significant increase in the in vitro hepatic protein synthesis activity in rats without CCl_4

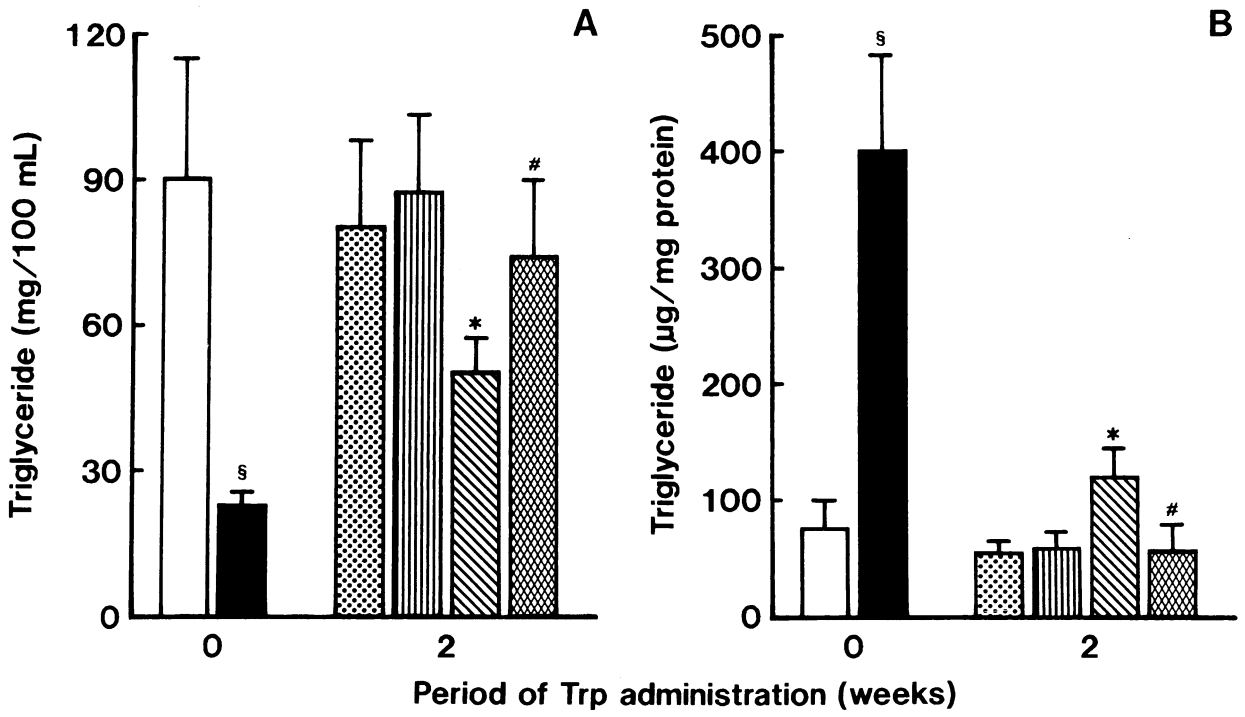

Fig. 5. Serum triglyceride concentration (A) and liver triglyceride content (B) in rats with chronic CCl_4 treatment, chronic CCl_4 treatment and subsequent withdrawal of the toxicant treatment, or daily Trp injection during the withdrawal period. Open bar, CON-pre group; closed bar, CCl_4-pre group; dotted bar, CON-post group; stripped bar, Trp-post group; hatched bar, CCl_4-post group; crosshatched bar, CCl_4-Trp-post group. Results are expressed as means ± SD (n = 5–10). $^{\S}P < 0.05$ when the CCl_4-pre group was compared with the CON-pre group; $^{*}P < 0.05$ when the CCl_4-post group was compared with the CON-post group; $^{\#}P < 0.05$ when the CCl_4-Trp-post group was compared with the CCl_4-post group.

treatment (see Fig. 6). Thus, daily Trp administration stimulated hepatic protein synthesis in rats with withdrawal of chronic CCl_4 treatment as observed in rats without CCl_4 treatment. Six weeks of CCl_4 treatment decreased TDO activity in the liver of rats by approximately half, as reported previously [7,8]. This marked decrease in hepatic TDO activity following chronic CCl_4 treatment was partially attenuated by withdrawing the toxicant for 2 weeks. The attenuation of the decreased hepatic TDO activity by CCl_4 withdrawal was enhanced by daily Trp administration and the decreased activity was almost completely returned to the level of CCl_4-untreated rats. In addition, Trp administration during the same period in CCl_4-untreated (Trp-post) rats tended to increase hepatic TDO activity more than in CCl_4-untreated rats without Trp administration (CON-post) (see Fig. 7A). There was a 2-fold increase in the Trp content in the liver of rats by 6 weeks of CCl_4 treatment. This increase in hepatic Trp content following chronic CCl_4 treatment was partially attenuated by 2-week CCl_4 withdrawal. Daily Trp administration enhanced the attenuation of the increased hepatic Trp content by CCl_4 withdrawal, although the attenuation was not complete. Trp administration during the same period in CCl_4-untreated (Trp-post) rats significantly increased the hepatic Trp content in CCl_4-untreated rats without Trp administration (CON-post) (see Fig. 7B). Accordingly, Trp accumulated in the liver of rats with 2 weeks of daily Trp administration after withdrawal of chronic CCl_4 treatment similarly to CCl_4-untreated (Trp-post) rats during the same period of Trp administration. In addition, the change in Trp content in the liver of rats after chronic treatment and subsequent withdrawal of CCl_4 in the presence or absence of daily Trp administration was inversely related to the change in TDO activity in the liver.

There is a significant direct correlation between lipid peroxidation and proline hydroxylase (prolyl hydroxylase) activity in the liver of rats with CCl_4-induced liver fibrosis [10]. Hepatic TDO participates not only as the first metabolizing enzyme but also as the rate-limiting enzyme in the kynurenine pathway [1]. Trp itself and some metabolites of the kynurenine pathway, such as 3-hydroxykynurenine and 3-hydroxyanthranic acid, function as antioxidants in vitro [3,4]. As described above, both the recovery of TDO activity and the reduction in the Trp accumulation in the liver of rats with chronic treatment and subsequent withdrawal of CCl_4 were enhanced by Trp administration during the withdrawal period. These results indicate that Trp is actively metabolized via the recovered TDO in the liver of rats with chronic treatment and subsequent withdrawal of CCl_4. Therefore, the enhancing effect of Trp on the attenuation of enhanced lipid peroxidation in the liver of rats with chronic treatment and subsequent withdrawal of CCl_4 can might be due to the antioxidant action of Trp and some kynurenine pathway metabolites.

Melatonin, which is produced from Trp via the serotonin

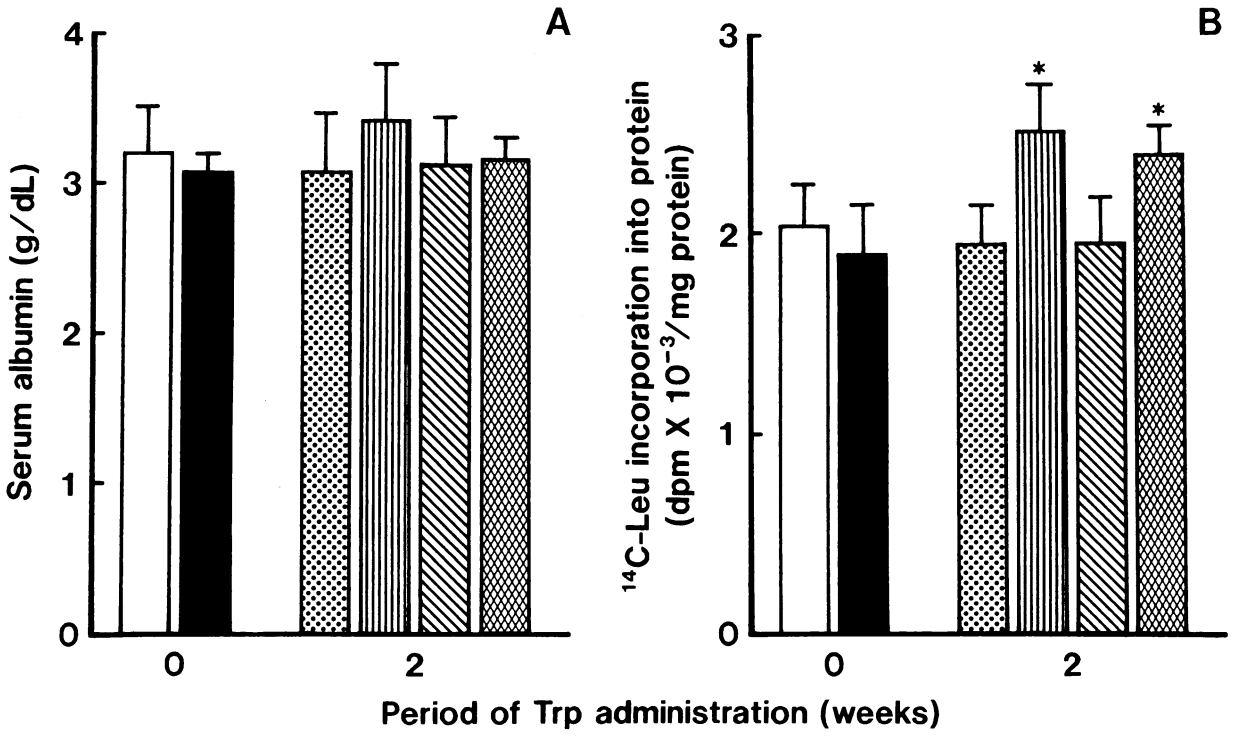

Fig. 6. Serum albumin concentration (A) and in vitro liver protein synthetic activity (B) in rats with chronic CCl_4 treatment, chronic CCl_4 treatment and subsequent withdrawal of the toxicant treatment, or daily Trp injection during the withdrawal period. Open bar, CON-pre group; closed bar, CCl_4-pre group; dotted bar, CON-post group; stripped bar, Trp-post group; hatched bar, CCl_4-post group; crosshatched bar, CCl_4-Trp-post group. Results are expressed as means ± SD (n = 5–10). *$P < 0.05$ when the Trp-post group or the CCl_4-Trp-post group was compared with the CON-post group.

pathway in extrahepatic tissues, such as the pineal gland and gut, possesses antioxidant activity in vitro and in vivo [30]. Exogenous melatonin exerts a protective or therapeutic effect on CCl_4-induced acute liver injury by preventing enhanced lipid peroxidation, possibly through its antioxidant action, in rats [31,32]. Thus, it is possible that the antioxidant action of melatonin produced from Trp via the serotonin pathway in extrahepatic tissues is associated with the enhancing effect of Trp administration on the attenuation of enhanced lipid peroxidation in the liver of rats with chronic treatment and subsequent withdrawal of CCl_4. These findings suggest that the enhancing effect of Trp administration on the attenuation of enhanced lipid peroxidation in the liver of rats with chronic treatment and subsequent withdrawal of CCl_4 contributes to reversion of pre-established chronic liver injury in CCl_4-treated rats.

Accumulation of Ca^{2+} due to impaired calcium homeostasis followed by activation of phospholipase A_2 might disrupt cell membrane function, leading to cell death, in the liver of CCl_4-treated rats [33,34]. An increase in Ca^{2+} levels leads to increased phospholipase A_2 activity and lipid peroxidation in the liver of rats chronically treated with CCl_4 [11]. These findings suggest that the enhancing effect of Trp on the attenuation of increased hepatic Ca^{2+} content in rats with chronic treatment and subsequent withdrawal of CCl_4 contributes to reversion of pre-established chronic liver injury in CCl_4-treated rats.

Exogenous Trp stimulates hepatic protein synthesis in normal rats [2]. Trp alleviates CCl_4-induced chronic liver injury in rats by maintaining the protein synthesis activity in the liver [8]. These findings suggest that the stimulatory effect of Trp administration on the hepatic protein synthesis activity in rats with chronic treatment and subsequent withdrawal of CCl_4 contributes to reversion of pre-established chronic liver injury in CCl_4-treated rats.

The major mechanism underlying the accumulation of triglycerides in the liver of rats after CCl_4 treatment is an impairment of triglyceride secretion into the circulation due to the inhibited secretion of lipoproteins such as very low density lipoprotein and high density lipoprotein and the changed apolipoprotein composition, which is associated with reduced de novo synthesis, in both lipoproteins in the liver [35–37]. Vitamin E administration has no effect on hepatic triglyceride accumulation in rats with chronic CCl_4 treatment [9,12]. As described above, Trp administration stimulates hepatic protein synthesis in rats with chronic treatment and subsequent withdrawal of CCl_4. Therefore, the mechanism underlying the enhancing effect of exogenous Trp on the attenuation of increased hepatic triglyceride content and decreased serum triglyceride concentrations in rats with chronic treatment and subsequent withdrawal of CCl_4 might be as follows: administered Trp enhances the recovery of impaired triglyceride secretion into the circulation following withdrawal of chronic CCl_4 treatment by

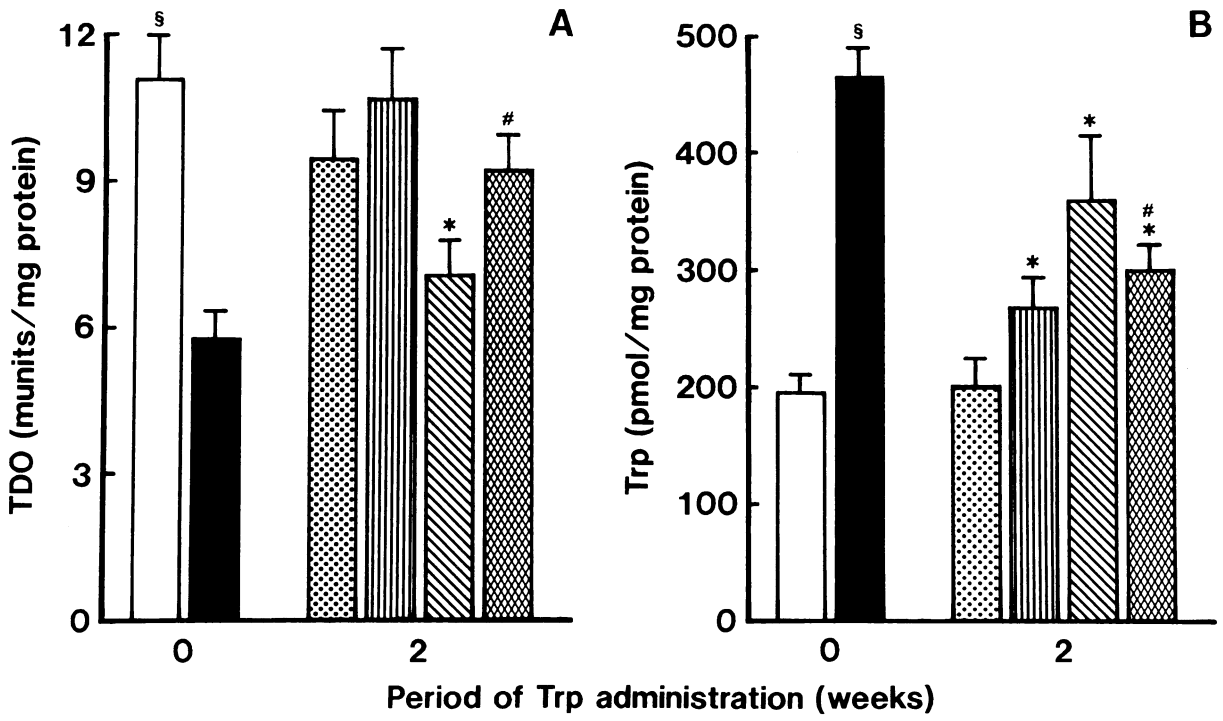

Fig. 7. TDO activity (A) and Trp content (B) in the liver of rats with chronic CCl_4 treatment, chronic CCl_4 treatment and subsequent withdrawal of the toxicant treatment, or daily Trp injection during the withdrawal period. Open bar, CON-pre group; closed bar, CCl_4-pre group; dotted bar, CON-post group; stripped bar, Trp-post group; hatched bar, CCl_4-post group; crosshatched bar, CCl_4-Trp-post group. Results are expressed as means ± SD (n = 5–10). $^{\S}P < 0.05$ when the CCl_4-pre group was compared with the CON-pre group; $^{*}P < 0.05$ when the Trp-post group, the CCl_4-post group or the CCl_4-Trp-post group was compared with the CON-post group; $^{\#}P < 0.05$ when the CCl_4-Trp-post group was compared with the CCl_4-post group.

stimulating the recovery of the reduced de novo synthesis of apolipoproteins in very low density lipoproteins and/or high density lipoproteins in the liver, which might be associated with reversion of pre-established chronic liver injury in CCl_4-treated rats.

TDO exists in two forms, i.e., the inactive form of the apo-enzyme that lacks heme, a prosthetic group, and the active form of the holo-enzyme possessing heme, in the liver of rats [1]. Trp administration induces hepatic TDO by stabilizing the enzyme through the conversion of the apo-enzyme to the holo-enzyme rather than by stimulating the synthesis of the enzyme in normal rats [38]. We reported that the reduction in hepatic TDO activity is mainly dependent on the decrease of apo-TDO rather than holo-TDO in rats with chronic CCl_4 treatment [7]. Hepatic TDO synthesis in rats with CCl_4-induced acute liver injury is impaired at the translational, rather than the transcriptional level [39]. As described above, Trp administration stimulates hepatic protein synthesis in rats with chronic treatment and subsequent withdrawal of CCl_4. Therefore, it is likely that the enhancing effect of Trp administration on the attenuation of the decreased TDO activity in the liver of rats with chronic treatment and subsequent withdrawal of CCl_4 is due to enhanced recovery of the impaired synthesis of the apo-enzyme rather than stabilization of the enzyme in the liver. The change in Trp content in the liver of rats with chronic treatment and subsequent withdrawal of CCl_4 in the presence or absence of daily Trp administration is inversely related to the change in TDO activity in the liver. Accordingly, Trp administered to rats with chronic treatment and subsequent withdrawal of CCl_4 might stimulate the recovery of impaired hepatic Trp metabolism by enhancing the attenuation of the reduced hepatic TDO activity, which might contribute to reversion of pre-established chronic liver injury in CCl_4-treated rats in terms of the above-described lipid peroxidation.

In conclusion, the results of the present study indicate that Trp administration promotes the reversion of pre-established chronic liver injury in rats treated with CCl_4, and suggest that Trp improves liver dysfunction parameters associated with chronic liver injury, such as enhanced lipid peroxidation, triglyceride accumulation, and impaired calcium homeostasis and Trp metabolism, as well as by stimulating hepatic protein synthesis. The detailed mechanism by which Trp administration promotes the reversion of pre-established chronic liver injury in rats treated with CCl_4, however, remains unclear. Further investigation is required.

References

[1] D.A. Bender, Biochemistry of tryptophan in health and disease, Mol Aspect Med 6 (1982) 101–109.

[2] H. Sidransky, Tryptophan, Unique action by an essential amino acid, in: Sidransky (Ed.), Nutritional Pathology. Pathobiochemistry of Dietary Imbalance, Dekker, New York, NY, 1985, pp. 1–62.
[3] E. Meucci, M.C. Mele, Amino acid and plasma antioxidant capacity, Amino Acids 12 (1997) 373–377.
[4] S. Christen, E. Peterhans, R. Stocker, Antioxidant activities of some tryptophan metabolites: Possible implication for inflammatory diseases, Proc Natl Acad Sci USA 87 (1990) 2506–2510.
[5] R.P. Tamayo, Is cirrhosis of the liver experimentally produced by CCl_4 an adequate model of human cirrhosis? Hepatology 3 (1983) 112–120.
[6] M. Hayashi, K. Uchiyama, T. Icho, Y. Kajiwara, T. Uchiyama, The correlation between morphometric analysis by a computer processing system and serum biochemical parameters in carbon tetrachloride-induced chronic hepatic injury in rats, J Med Soc Toho Japan (Toho Igakukai Zasshi) 40 (1993) 134–143.
[7] Y. Ohta, M. Uemura, K. Saito, E. Sasaki, I. Ishiguro, Relationship between the level of serum L-tryptophan and its hepatic uptake and metabolism in rats with carbon tetrachloride-induced liver cirrhosis, Amino Acids 10 (1996) 369–378.
[8] Y. Ohta, D. Sahashi, E. Sasaki, I. Ishiguro, Alleviation of carbon tetrachloride-induced chronic liver injury and related dysfunction by L-tryptophan in rats, Ann Clin Biochem 36 (1999) 504–510.
[9] M. Parola, G. Leonarduzzi, F. Biasi, E. Albano, M.E. Biocca, G. Poli, M.U. Dianzani, Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis, Hepatology 16 (1992) 1014–1021.
[10] J. Camps, T. Bargallo, A. Gimenez, S. Alie, J. Caballeria, A. Pares, J. Joven, L. Masana, J. Rodes, (1992) Relationship between hepatic lipid peroxidation and fibrosis in carbon tetrachloride-treated rats: effect of zinc administration, Clin Sci 83 (1992) 695–700.
[11] S.V.S. Rana, S. Rastogi, Antioxidant enzymes in the liver of rats treated with carbon-tetrachloride after parathyroidectomy, Physiol Chem Phys Med NMR 25 (1993) 41–47.
[12] A.-B. Halim, O. El-Ahmady, S. Hassab-Allah,F. Abdel-Galil, Y. Hafez, A. Darwish, Bicohemical effect of antioxidants on lipids and liver function in experimentally-induced liver damage, Ann Clin Biochem 34 (1997) 656–663.
[13] I. Castilla-Cortazar, M. Garcia, B. Muguerza, J. Quiroga, R. Perez, S. Santidrian, J. Prieto, Hepatoprotective effects of insulin-like growth factor I in rats with carbon tetrachloride-induced cirrhosis, Gastroenterology 113 (1997) 1682–1691.
[14] M. Cabré, J. Camps, J.L. Paternáin, N. Ferré, J. Joven, Time-course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride-induced cirrhosis, Clin Exp Pharmacol Physiol 27 (1999) 694–699.
[15] R. Deulofeu, A. Pares, M. Rubio, M. Gassó, J. Román, A. Giménez, G. Varela-Moreiras, J. Caballeria, A.M. Ballesta, J. Mto, J. Rodés, S-Adenosylmethionine prevents hepatic tocopherol depletion in carbon tetrachloride-injured rats, Clin Sci 99 (2000) 315–320.
[16] H. Sidransky, E. Verney, R.N. Kurl, T. Razavi, Effect of tryptophan on toxic cirrhosis induced by intermittent carbon tetrachloride intoxication in the rat, Exp Mol Pathol 49 (1988) 102–110.
[17] P.S. Quinn, J. Higginson, Reversible and irreversible changes in experimental cirrhosis, Am J Pathol 47 (1965) 353–369.
[18] F. Hutterer, M. Eisenstadt, E. Rubin, Turnover of hepatic collagen in reversible and irreversible fibrosis, Experientia 26 (1970) 244–245.
[19] T. Watanabe, M. Niioka, S. Hozawa, K. Kameyama, T. Hayashi, M., Arai, A. Ishikawa, K. Maruyama, I. Okazaki, Gene expression of intestinal collagenase in both progression and recovery phase of rat liver fibrosis induced by carbon tetrachloride, J Hepatol 33 (2000) 224–235.
[20] B.T. Doumas, A. Watson, H.G. Biggs, Albumin standards and the measurement of serum albumin with bromcresol green, Clin Chim Acta 31 (1971) 87–96.
[21] M.J. Fletcher, A colorimetric method for estimating serum triglyceride, Clin Chim Acta 22 (1968) 393–397.
[22] G. Bondjers, S. Björkerud, Spectrophotometric determination of hydroxyproline in connective tissue on the nanogram level, Anal Biochem 52 (1973) 496–504.
[23] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J Biol Chem 183 (1951) 265–275.
[24] M.S. Vishwanath, A.M. Joan, The determination of triglyceride in plasma and tissues, Clin Chem 14 (1968) 156–161.
[25] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal Biochem 95 (1979) 351–358.
[26] H. Metzler, R. Gebhart, Y. Mecke, A convenient and highly sensitive spectrophotometric assay for tryptophan 2,3-dioxygenase, Anal Biochem 121 (1982) 10–16.
[27] K. Saito, E. Sasaki, Y. Ohta, Y. Nagamura, I. Ishiguro, (1986) Mode of L-tryptophan uptake into rat hepatocytes via trypsin-sensitive high-affinity transport system, Biochem Int 13 (1986) 873–883.
[28] E. Carafoli, R. Tiozzo, A study of energy-linked calcium transport in liver mitochondria during CCl_4 intoxication, Exp Mol Pathol 19 (1968) 131–140.
[29] M. Oravec, T.L, Sourks, Inhibition of hepatic protein synthesis by α-methyl-DL-tryptophan in vivo. Further studies on the glyconeogenetic action of α-methyltryptophan, Biochemistry 22 (1970) 4458–4464.
[30] R.J. Reiter, The oxidant/antioxidant network: role of melatonin, Biol Signals Recept 8 (1999) 56–63.
[31] W.M.U. Daniels, R.J. Reiter, E. Sewerynek, M.I., Pablos, G.G. Oritz, Melatonin counteracts lipid peroxidation induced by carbon tetrachloride but does not restore glucose-6-phoshatase activity, J Pineal Res 19 (1995) 1–6.
[32] Y. Ohta, M. Kongo, E. Sasaki, K. Nishida, I. Ishiguro, Therapeutic effect of melatonin on carbon tetrachloride-induced acute liver injury in rats, J Pineal Res 28 (2000) 119–126.
[33] R.O. Recknagel, E.A. Glende, Jr., J.A. Dolak, Mechanism of carbon tetrachloride toxicity, Pharmacol Ther 43 (1989) 139–154.
[34] A. Ryu, H. Itabe, M. Mutoh, I. Kudo, H. Arai, K. Inoue, Enhanced degradation of phospholipids by phospholipase A_2 in liver of carbon tetrachloride-treated rat, J Health Sci 46 (2000) 275–281.
[35] B. Lombardi, G. Ugazio, Serum lipoproteins in rats with carbon tetrachloride-induced fatty liver, J Lipid Res 6 (1963) 498–505.
[36] G. Poli, E. Gravela, E. Albano, M.D. Diazani, Studies on fatty liver with isolated hepatocytes. II. The action of carbon tetrachloride on lipid peroxidation, protein, and triglyceride synthesis and secretion, Exp Mol Pathol 30 (1979) 116–127.
[37] M. Boll, L.W.D. Weber, E. Becker, A. Stampfl, Hepatocyte damage induced by carbon tetrachloride: Inhibited lipoprotein secretion and changed lipoprotein composition, Z Naturforsch 56c (2001) 283–290.
[38] W.E. Knox, The regulation of tryptophan pyrrolase activity by tryptophan, in: G. Weber (Ed.), Advances in Enzyme Regulation: Pergamon Press, Oxford, vol. 4, 1966, pp. 287–297.
[39] Y. Sato, M. Maruyama, The inhibition of tryptophan pyrrolase synthesis in rat liver by carbon tetrachloride, J Biochem 72 (1972) 1129–1137.