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SHORT COMMUNICATION

Methylenedioxy Group as Determinant of Schisandrin in Enhancing Hepatic Mitochondrial Glutathione in Carbon Tetrachloride-Intoxicated Mice

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ABSTRACT. As a preliminary approach to exploring whether the methylenedioxy group of the dibenzocyclooctadiene skeleton of schisandrins plays an important role in hepatic mitochondrial-reduced glutathione (GSH) stimulatory activity, we examined the effects of three schisandrins, namely schisandrin A (Sch A), schisandrin B (Sch B), and schisandrin C (Sch C), on carbon tetrachloride (CCl_4) hepatotoxicity and hepatic mitochondrial GSH status in mice. Pretreating mice with Sch A at a daily oral dose of 1 mmol/kg for 3 days did not protect against CCl_4 hepatotoxicity, whereas pretreatment with Sch B or Sch C at the same dosage regimen produced almost complete protection. The hepatoprotection afforded by Sch B or Sch C pretreatment was associated with significant increases in the hepatic mitochondrial GSH level and glutathione reductase (EC 1.6.4.2) activity. Our results indicate that the methylenedioxy group of the dibenzocyclooctadiene skeleton of schisandrin is an important structural determinant in the stimulation of hepatic mitochondrial GSH, particularly under conditions of CCl_4 intoxication. BIOCHEM PHARMACOL **54**;2:317–319, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. schisandrin; mitochondrial glutathione; glutathione reductase; carbon tetrachloride; liver

Fructus schisandrae, the fruit of *Schisandra chinensis*, is a widely used herbal material in traditional Chinese medicine. Previous studies in our laboratory have demonstrated the hepatoprotective effect of schisandrin B (Sch B)§ (see Fig. 1b), a dibenzocyclooctadiene derivative isolated from *Fructus schisandrae*, on carbon tetrachloride (CCl_4) toxicity in mice [1]. The hepatoprotection was associated with an enhancement in the functioning of the hepatic glutathione antioxidant system [1]. A recent study also indicated that the hepatoprotection afforded by Sch B pretreatment against CCl_4 toxicity was paralleled by the enhancement of hepatic mitochondrial reduced glutathione (GSH) status and glutathione reductase (EC 1.6.4.2) (GRD) activity [2]. Results obtained from a study examining the hepatoprotective action of dibenzocyclooctadiene derivatives against CCl_4- and galactosamine-induced cellular damage in primary hepatocyte cultures suggested that the methylenedioxy group of the dibenzocyclooctadiene skeleton may play an important role in antihepatotoxic activity [3]. However, it is unclear whether this structure–activity relationship is still valid with regard to the hepatic mitochondrial GSH stimulating activity of schisandrins. As a preliminary approach to exploring this relationship, we examined the effects of three schisandrins, namely schisandrin A (Sch A), Sch B, and schisandrin C (Sch C), which differ structurally by the presence or absence of the methylenedioxy group (see Fig. 1), on CCl_4 hepatotoxicity and hepatic mitochondrial GSH status in mice.

MATERIALS AND METHODS

Chemicals

EDTA, GSH, and Tris[hydroxymethyl] aminomethane (Tris) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade. Solvents used for high-performance liquid chromatography were of HPLC grade; they were filtered and degassed prior to use. Dried fruits of *Schisandra chinensis* were imported from China. Schisandrins (dibenzocyclooctadiene derivatives), including Sch A, Sch B, and Sch C, were purified from the petroleum ether extract of *Fructus schisandrae* by silica gel column chromatography as previously described [1].

The chemical structures of Sch A, Sch B, and Sch C were confirmed by comparing the silica gel TLC and spectral characteristics (^1H- and ^{12}C-NMR and mass spectra) with authentic standards obtained from the Institute of Materia Medica, Chinese Academy of Sciences, Beijing. The purity of the compounds, as assessed by HPLC, was found to be higher than 95% (w/w).

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§ *Abbreviations*: ALT, alanine aminotransferase; CCl_4, carbon tetrachloride; GRD, glutathione reductase; GSH, reduced glutathione; Sch A, schisandrin A; Sch B, schisandrin B; Sch C, schisandrin C; Tris, Tris[hydroxymethyl] aminomethane.

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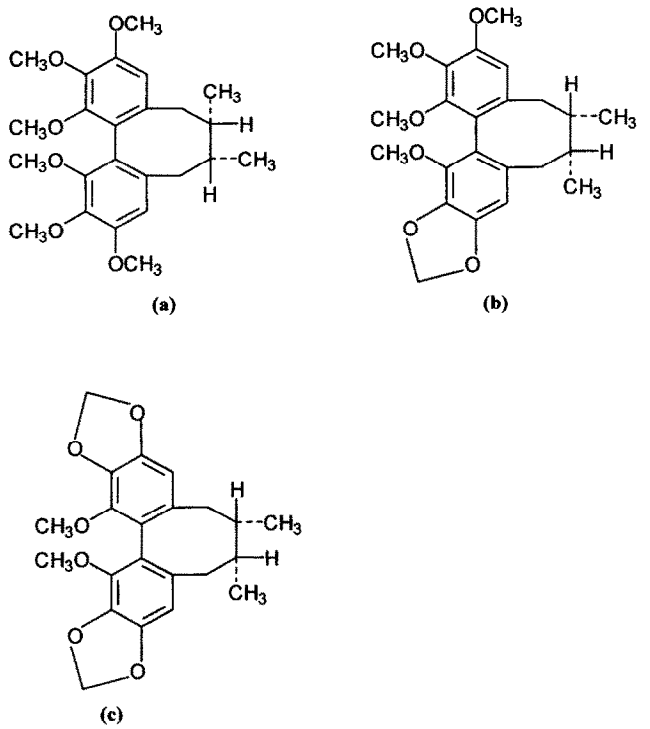

FIG. 1. Structures of schisandrins. (a) schisandrin A; (b) schisandrin B; (c) schisandrin C.

Animal Treatment

Female Balb/c mice (24–26 g) were randomly assigned into groups of five individuals. In the pretreatment groups, animals were treated intragastrically with the schisandrins [suspended in olive oil, 4% (w/v)] for 3 days at a daily dose of 1 mmol/kg. Control animals were administered the olive oil (0.1 mL/10 g). Twenty-four hours after the last dosing, animals were administered an oral dose of CCl_4 [1% (v/v) in olive oil] at 0.1 mL/kg. Control animals were given the olive oil only. Animals were sacrificed 24 hr after the intoxication.

Sample Preparation

Plasma samples were obtained by centrifuging the whole blood at 2,000 × *g* at 4°C. Hepatic tissue samples were excised and rinsed with ice-cold homogenizing buffer (50 mM Tris, 0.1 mM EDTA, pH 7.6). Tissue homogenate was prepared by homogenizing 1 g of hepatic tissue sample in 10 mL ice-cold homogenizing buffer with two 10-sec bursts of a tissue disintegrator (Ultra Turax T25, Ika-Labortechnik, Staufen) at 135,000 rpm. Hepatic mitochondrial fraction was prepared by differential centrifugation in isotonic buffer (0.25 mM sucrose, 0.1 mM EDTA, 5 mM Tris, pH 7.4) as previously described [4].

Biochemical Analysis

Plasma alanine aminotransferase (EC 2.6.1.2) (ALT) activity was measured using an assay kit from Sigma Chemical Co. The GSH level was measured by an HPLC method [5], as modified from Reed *et al.* [6]. GRD activity was determined as described by Godin and Garnett [7].

TABLE 1. The effect of schisandrin pretreatment on CCl_4-induced hepatotoxicity in mice

	Plasma ALT (U/L)	
	Non-CCl_4	CCl_4-treated
CON	10.9 ± 1.48	12801 ± 1030[a]
Sch A	12.9 ± 0.61	14168 ± 1660[a]
Sch B	14.5 ± 0.62	55 ± 6[abc]
Sch C	11.1 ± 0.56	155 ± 31[ab]

Values given are the mean ± SEM, with n = 5.
[a] Significantly different from the non-CCl_4 CON group.
[b] Significantly different from the CCl_4-treated CON group.
[c] Significantly different from the Sch C-pretreated non-CCl_4 or CCl_4-treated group.

Statistical Analysis

Data were analyzed by one-way ANOVA followed by Duncan multiple range test to detect intergroup differences. Significant difference was determined when $P < 0.05$.

RESULTS AND DISCUSSION

It is well established that the pathogenesis of CCl_4-induced hepatic damage involves reactive oxidant species arising from the metabolism of CCl_4 [8]. As shown in Table 1, CCl_4 treatment caused hepatocellular damage in mice, as indicated by a drastic increase in plasma ALT activity. While treating mice with schisandrins at a daily oral dose of 1 mmol/kg for 3 days did not change the plasma ALT activity, Sch B, or Sch C pretreatment at the same dosage regimen almost completely prevented CCl_4 toxicity, as evidenced by the significant decrease (99.5 or 98.7%, respectively) in plasma ALT activity. Sch B pretreatment seemed to be more effective than that of Sch C in protecting against CCl_4 hepatotoxicity, whereas Sch A pretreatment did not produce any detectable effect.

Treating mice with Sch B or Sch C did not produce a significant difference in hepatic mitochondrial GSH level when compared with the controls (Table 2). Following CCl_4 intoxication, the hepatic mitochondrial GSH level was drastically depleted by 72.7%. Sch B or Sch C pretreatment was able to increase the hepatic mitochondrial GSH level in CCl_4-treated mice, with the resultant GSH level being higher than that of the non-CCl_4 control. The mitochondrial GSH stimulatory activity of Sch B was apparently higher than that of Sch C, whereas Sch A pretreatment did not produce any significant effect. The maintenance of mitochondrial glutathione redox status is a crucial determinant for cell survival, particularly under conditions of increased oxidative stress as in the case of CCl_4 intoxication [9]. Our results indicate that the hepatoprotection afforded by Sch B or Sch C pretreatment was paralleled by the increase in mitochondrial GSH level. The

TABLE 2. The effect of schisandrin treatment on hepatic mitochondrial GSH status

	GSH (nmol/mg protein)	GRD (mU/mg protein)
Non-CCl_4		
CON	5.83 ± 0.18	13.9 ± 0.55
Sch A	5.48 ± 0.12	17.4 ± 0.49[a]
Sch B	6.08 ± 0.20	35.7 ± 1.80[ac]
Sch C	6.58 ± 0.03	25.9 ± 1.06[a]
CCl_4-treated		
CON	1.59 ± 0.20[a]	8.5 ± 0.81[a]
Sch A	2.12 ± 0.57[a]	13.8 ± 0.63[b]
Sch B	9.84 ± 0.21[abc]	37.1 ± 1.12[abc]
Sch C	8.12 ± 0.35[ab]	22.0 ± 0.32[ab]

Values given are the mean ± SEM, with $n = 5$.
[a] Significantly different from the non-CCl_4 CON group.
[b] Significantly different from the CCl_4-treated CON group.
[c] Significantly different from the Sch C-pretreated non-CCl_4 or CCl_4-treated group.

extent of mitochondrial GSH enhancement in CCl_4-treated animals correlated well with the degree of hepatoprotection afforded by schisandrin pretreatment. The inability of Sch A pretreatment to substantially increase the hepatic mitochondrial GSH level consistently resulted in a failure to protect against CCl_4 hepatotoxicity (Tables 1 and 2). Detoxification of reactive intermediates arising from the one-electron reduction of CCl_4 in the mitochondrion can lead to the depletion of mitochondrial GSH [10]. Because mitochondria do not contain the enzymes necessary for GSH synthesis [11], the increase in mitochondrial GSH level, as observed in the present study, must be mediated either by enhancing the GRD-catalyzed GSH regeneration from its oxidized form [12] or by facilitating the import of cytosolic GSH through a receptor-mediated mechanism [13]. With regard to the former pathway, the mitochondrial GSH enhancing effects of Sch B and Sch C were found to be associated with significant increases in mitochondrial GRD activity in both control (156.8 and 86.3%) and CCl_4-treated (336.5 and 158.8%) animals when compared with the respective control group (Table 2). In contrast, the impairment in GRD activity (38.8%) was associated with a decrease in the mitochondrial GSH level in CCl_4-intoxicated animals. The relatively small increase in GRD activity (62.3%) was only coupled with a slight and insignificant increase in the mitochondrial GSH level in Sch A-pretreated and CCl_4-intoxicated mice when compared with the CCl_4-treated control (Table 2). In addition, the ability of Sch B or Sch C pretreatment to increase the mitochondrial GSH level over the non-CCl_4 control value also suggests the possibility of enhanced GSH influx into the mitochondria from the cytosolic compartment.

Regarding the structure–activity relationship, our results indicate that the methylenedioxy group containing dibenzocyclooctadiene derivatives, namely Sch B and Sch C (but not Sch A), was able to increase the hepatic mitochondrial GSH level and, hence, protect against CCl_4 hepatotoxicity in mice. This finding indicates that the methylenedioxy group of the dibenzocyclooctadiene skeleton is an important structural determinant in mitochondrial GSH stimulatory activity. Apparently, the possession of one methylenedioxy group in the molecule, as in the case of Sch B, offers more potent activity. However, further in-depth investigation is required to determine whether one or two methylenedioxy groups is optimal for hepatic mitochondrial GSH stimulatory activity.

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