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Structure-activity relationship of schisandrins in enhancing liver mitochondrial glutathione status in CCl_4-poisoned mice

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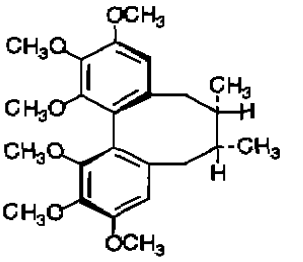
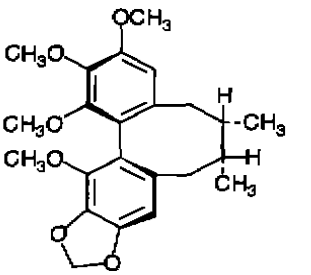
KEY WORDS *Schisandra chinensis*; dibenzocyclooctadienes; liver mitochondria; glutathione; glutathione reductase; carbon tetrachloride poisoning; structure-activity relationship

AIM: To explore whether the methylenedioxy group and cyclooctadiene ring of the dibenzocyclooctadiene skeleton of schisandrins (Sch) play a role in the liver mitochondrial glutathione status enhancing activity. **METHOD:** The effects of three dibenzocyclooctadiene derivatives, Sch A, Sch B, Sch C, and a synthetic intermediate of Sch C, (dimethyl biphenyl dicarboxylate, DBD) on carbon tetrachloride (CCl_4)-hepatotoxicity and liver mitochondrial glutathione status were examined in mice. **RESULTS:** Pretreating mice with intragastric Sch B, Sch C, or DBD 1 mmol·kg^{-1}·d^{-1} for 3 d protected against CCl_4-hepatotoxicity. The hepatoprotection afforded by Sch B or Sch C pretreatment was associated with increases in liver mitochondrial reduced glutathione (mtGSH) level and glutathione reductase (mtGRD) activity, an indication of enhanced mitochondrial glutathione status. In contrast, the hepatoprotective action of DBD was not accompanied by any detectable changes in mtGSH level and mtGRD activity. **CONCLUSION:** Both the methylenedioxy group and the cyclooctadiene ring of the dibenzocyclooctadiene molecule are important structural determinants in the enhancement of liver mitochondrial glutathione status.

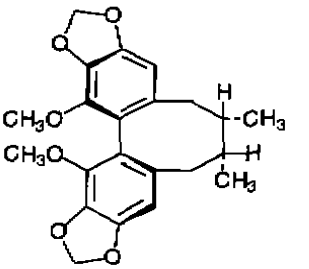
Fructus Schisandrae, the fruit of *Schisandra chinensis*, is a widely used herbal material in traditional Chinese medicine. Previous studies in our laboratory have demonstrated the hepatoprotective effect of schisandrin B (Sch B, a racemate containing γ-schisandrin/Gomisin N), a dibenzocyclooctadiene derivative isolated from *Fructus Schisandrae*, on carbon tetrachloride (CCl_4) toxicity in mice[1]. The hepatoprotection was associated with enhancements in membrane stability[2] and the functioning of hepatic glutathione antioxidant system[1]. It is well established that the pathogenesis of CCl_4-induced hepatic damage involves reactive oxidant species arising from the metabolism of CCl_4[3]. A recent study also indicated that the hepatoprotection afforded by Sch B pretreatment against CCl_4 toxicity was paralleled by the enhancement of liver mitochondrial glutathione status, as assessed by mitochondrial reduced glutathione (mtGSH) level and glutathione reductase (mtGRD) activity[4]. Results obtained from a study examining the hepatoprotective action of dibenzocyclooctadiene



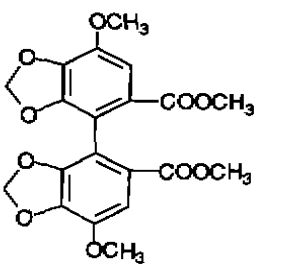
Schisandrin A



Schisandrin B



Schisandrin C



Dimethyl biphenyl dicarboxylate

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derivatives against CCl_4- and galactosamine-induced cellular damage in primary hepatocyte cultures suggest that the methylenedioxy group of the dibenzocyclooctadiene skeleton may play an important role in antihepatotoxic activity[5]. However, it is unclear whether this structure-activity relationship is still valid with regard to the liver mitochondrial glutathione status enhancing activity of schisandrins (Sch). As a preliminary approach to exploring this relationship, we examined the effects of Sch A (deoxyschisandrin), Sch B, and Sch C which differ structurally by the presence or absence of the methylenedioxy group, on CCl_4-hepatotoxicity and hepatic mitochondrial glutathione status in mice. The effect of dimethyl biphenyl dicarboxylate (DBD), a synthetic intermediate of Sch C lacking of a cyclooctadiene ring, was also examined.

MATERIALS AND METHODS

Drugs and chemicals Edetic acid, GSH, and tris [hydroxymethyl] aminomethane (Tris) were purchased from Sigma Chemical Co (St Louis MO, USA). 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 from the mainland of China. Schisandrins (dibenzocyclooctadiene derivatives), including Sch A, Sch B, and Sch C, were racemic mixture that purified from the petroleum ether extract of *Fructus Schisandrae* by silica gel column chromatography[1]. The chemical structures of Sch A, Sch B, and Sch C were confirmed by comparing the silica gel TLC and spectral characteristics (^{1}H- and ^{13}C-NMR and mass spectra) with authentic standards obtained from the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing. The purity of the compounds, as assessed by HPLC, was found to be higher than 95 % (wt/wt). DBD (also a racemic mixture) was manufactured by Guangzhou Qun Xing Pharmaceutical Company, China.

Mice Female Balb/c mice (24 – 26 g) were randomly assigned into groups of 5 mice. In the pretreatment groups, mice were treated intragastrically with Sch or DBD (suspended in olive oil, 4 % (wt/vol) at a dose of 1 mmol $\cdot kg^{-1} \cdot d^{-1}$ for 3 d. Control mice were administered with the olive oil (10 mL $\cdot kg^{-1}$). Twenty-four hours after the last dosing, mice were administered with an oral dose of CCl_4 (1 % vol/vol in olive oil) at 0.1 mL $\cdot kg^{-1}$. Control mice were given the olive oil only. Twenty-four hours after the intoxication, mice were killed.

Sample preparation Plasma samples were obtained by centrifuging the whole blood at 2000 × *g* at 4 ℃. Liver tissue was rinsed with ice-cold homogenizing buffer (Tris 50 mmol $\cdot L^{-1}$, edetic acid 0.1 mmol $\cdot L^{-1}$, pH 7.6). Tissue homogenate was prepared by homogenizing 1 g of liver tissue in 10 mL ice-cold buffer with two 10-s bursts of a tissue disintegrator (Ika Ultra Turax T25) at 135 000 rpm. Liver mitochondrial fraction was prepared by differential centrifugation in isotonic buffer (sucrose 0.25 mmol $\cdot L^{-1}$, edetic acid 0.1 mmol $\cdot L^{-1}$, Tris 5 mmol $\cdot L^{-1}$, pH 7.4)[6].

Biochemical analysis Plasma alanine aminotransferase (AlaAt) activity was measured using an assay kit from Sigma Chemical Co. GSH level was measured by an HPLC method[7], as modified from Reed *et al*[8]. GRD activity was determined[9].

Statistical analysis Results were expressed as $\bar{x} \pm s$ and were analyzed by one-way ANOVA followed by Duncan's multiple range test to detect inter-group differences.

RESULTS AND DISCUSSION

CCl_4 treatment caused hepatocellular damage in mice, as indicated by a drastic increase in plasma AlaAT activity. While treating mice with dibenzocyclooctadiene derivatives at an intragastric of 1 mmol $\cdot kg^{-1} \cdot d^{-1}$ for 3 d did not change the plasma AlaAt activity, Sch B or Sch C pretreatment at the same dosage regimen almost completely prevented the CCl_4 toxicity, as evidenced by the decrease (99.5 % or 98.7 %, respectively) in plasma AlaAt 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. DBD pretreatment also protected against CCl_4-

hepatotoxicity, but to a lesser extent that those of Sch B and Sch C, with a 73.8 % decrease in plasma AlaAt activity (Tab 1).

Tab 1. Effects of ig schisandrin/DBD treatments in enhancing liver mitochondrial GSH status in CCl_4-poisoned mice. $n = 5$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **the non-CCl_4 control.** $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* **CCl_4-treated control.** $^iP < 0.01$ *vs* **Sch C.**

	Plasma AlaAt ($U \cdot L^{-1}$)	mtGSH (μmol/g protein)	mtGRD (U/g protein)
Non-CCl_4			
control	10.9 ± 3.3	5.8 ± 0.4	13.9 ± 1.2
Sch A	12.9 ± 1.4^a	5.5 ± 0.3^a	17.4 ± 1.1^b
Sch B	14.5 ± 1.4^a	6.1 ± 0.5^a	36 ± 4^{bi}
Sch C	11.1 ± 1.2^a	6.58 ± 0.07^a	25.9 ± 2.4^b
DBD	11.9 ± 1.1^a	4.7 ± 0.6^a	17.7 ± 2.4^b
CCl_4-treated			
control	$12\,800 \pm 2\,300^c$	1.6 ± 0.5^c	8.5 ± 1.8^b
Sch A	$14\,200 \pm 3\,700^{cd}$	2.1 ± 1.3^{cd}	13.8 ± 1.4^{ae}
Sch B	55 ± 13^{bf}	9.8 ± 0.5^{cfi}	37.1 ± 2.5^{cfi}
Sch C	155 ± 69^{bf}	8.1 ± 0.8^{cf}	22.0 ± 0.7^{bf}
DBD	$3\,350 \pm 510^{cei}$	1.0 ± 1.1^{cdi}	7.6 ± 1.8^{bdi}

Treating mice with Sch B or Sch C did not produce significant difference in hepatic mtGSH level when compared with the controls (Tab 1). Following the CCl_4 intoxication, the hepatic mtGSH level was drastically depleted by 72.7 %. Sch B or Sch C pretreatment increased the hepatic mtGSH level in CCl_4-treated mice, with the resultant GSH level being higher than that of the non-CCl_4 control. The mtGSH stimulatory activity of Sch B was apparently higher than that of Sch C, whereas Sch A and DBD pretreatments did not produce any significant effects.

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[10]. Our results indicate that the hepatoprotection afforded by Sch B or Sch C pretreatment was paralleled by the increase in mtGSH level. The extent of mitochondrial GSH enhancement in CCl_4-treated mice correlated well with the degree of hepatoprotection afforded by Sch B or Sch C pretreatment. Consistently, the inability of Sch A pretreatment to substantially increase hepatic mtGSH level resulted in the failure in protecting against CCl_4-hepatotoxicity (Tab 1). DBD pretreatment, which did not enhance the mitochodrial glutathione status, could only abate the increase in plasma AlaAt activity to a lesser extent in CCl_4-treated mice when compared with those of Sch B or Sch C.

Detoxification of reactive intermediates arising from the one-electron reduction of CCl_4 in the mitochondrion can lead to the depletion of mtGSH[11]. Because mitochondria do not contain the enzymes necessary for GSH synthesis[12], 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[13] or by facilitating the import of cytosolic GSH through a receptor-mediated mechanism[14]. With regard to the former pathway, the mitochondrial GSH enhancing effects of Sch B and Sch C were found to be associated with increases in mtGRD activity in both control (156.8 % and 86.3 %) and CCl_4-treated (336.5 % and 158.8 %) mice when compared with the respective control group (Tab 1). In contrast, the impairment in mtGRD activity (−38.8 %) was associated with a decrease in mtGSH level in CCl_4-intoxicated mice. The relatively small increase in mtGRD activity (62.3 %) was only coupled with a slight and insignificant increase in mtGSH level in Sch A-pretreated and CCl_4-intoxicated mice when compared with the CCl_4-treated control (Tab 1). In addition, the ability of Sch B or Sch C pretreatment to increase mtGSH level over the non-CCl_4 control value also suggests the possibility of enhanced GSH influx into the mitochondria from the cytosolic compartment. In the absence of enhancement in mitochondrial glutathione status, the plasma AlaAt lowering effect produced by DBD may be attributed to other hepatoprotective action unrelated to hepatoprotection against CCl_4 toxicity. In this regard, DBD pretreatment was found to be unable to protect against CCl_4 or cadmium induced hepatotoxicity in mice, as assessed by morphological measurement on liver necrosis[15].

As regards the structure-activity relationship, our results indicate that the methylenedioxy group containing dibenzocyclooctadiene deriva-

tives, namely Sch B and Sch C (but not Sch A), were able to increase the hepatic mtGSH level and mtGRD activity, and hence protected against CCl_4-hepatotoxicity in mice. This finding indicates that the methylenedioxy group of the dibenzocyclooctadiene skeleton is an important structural determinant in the mitochondrial glutathione status enhancing 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 liver mitochondrial glutathione status enhancing activity. On the other hand, DBD, which possesses two methylenedioxy groups but with no cyclooctadiene ring structure, did not stimulate mtGSH or mtGRD activity. This suggests the important role of the cyclooctadiene ring structure of dibenzocyclooctadiene molecule in enhancing mitochondrial glutathione status.

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五味子素对四氯化碳染毒小鼠促进肝线粒体谷胱甘肽抗氧化状态的构效关系

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关键词 五味子; 二苯环辛二烯类; 肝线粒体; 谷胱甘肽; 谷胱甘肽还原酶; 四氯化碳中毒; 结构-活性关系;

目的: 研究五味子素二苯环辛二烯分子中甲二氧基团和环辛二烯对促进肝线粒体谷胱甘肽抗氧化状态所起的作用. **方法**: 利用四氯化碳染毒小鼠测试服食五味子甲素(Sch A), 乙素(Sch B), Sch C 和联苯双酯(DBD)对肝线粒体的抗氧化作用. **结果**: 小鼠服食 Sch B, Sch C 或 DBD (每天 1 mmol·kg^{-1}, 连续三天)后对四氯化碳损伤的肝有保护作用. Sch B 和 Sch C 对肝的保护作用和增加肝线粒体 GSH 水平和 GSH 还原酶活性有互连关系. 后者对肝线粒体的谷胱甘肽抗氧化状态有增强作用. DBD 不能产生类似作用. **结论**: 二苯环辛二烯类分子的甲二氧基团和环辛二烯对促进肝线粒体谷胱甘肽抗氧化状态有决定性影响.