BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 May; 13 (3) : 213-217

Protective effects of fulvotomentosides on cadmium-induced hepatotoxicity

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ABSTRACT Fulvotomentosides (Ful) is the total saponins of *Lonicera fulvotomentosa*. In the present study, we examined the effects of Ful on cadmium ($CdCl_2$)-induced acute liver injury in mice. Ful pretreatment (150 mg · kg^{-1}, sc · 3 d) remarkably decreased $CdCl_2$ (3.7 mg Cd · kg^{-1}, iv)-induced liver damage as indicated by serum activities of alanine aminotransferase and sorbitol dehydrogenase. Distribution of Cd to 12 organs and hepatic subcellular fractions was determined 2 h after Cd challenge. Ful pretreatment did not produce a marked shift in the distribution of Cd to various organs, but markedly altered the hepatic subcellular distribution of Cd, with more Cd bound to metallothionein (MT) in the cytosol, less in the nuclear, mitochondrial, and microsomal fractions. Ful pretreatment produced a dose-dependent increase in hepatic MT as determined by the Cd · hemoglobin assay. In conclusion, Ful protected against Cd hepatotoxicity by inducing MT, which binds Cd in the cytosol and lowers the amount of Cd available to other critical organelles and proteins.

KEY WORDS fulvotomentosides; cadmium poisoning; liver; alanine aminotransferase; iditol dehydrogenase; tissue distribution; metallothionein

Cadmium (Cd) is an environmental pollutant which presents a potential threat to human beings[1]. Acute exposure to Cd causes severe liver damages as indicated by marked elevation in serum alanine aminotransferase (ALT / GPT) and sorbitol dehydrogenase (SDH) activities, as well as widespread liver congestion and necrosis[1,2].

Fulvotomentosides (Ful) is the total saponins isolated from *Lonicera fulvotomentosa*[3], an herbal drug traditionally used for infectious diseases. Ful has been shown to have anti-inflammatory[4] and protective effects against some hepatotoxicants[5]. The aim of the present paper is to examine the protective effects of Ful against cadmium ($CdCl_2$) hepatotoxicity.

Received 1992 Jan 31 Accepted 1992 Feb 12
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MATERIALS AND METHODS

Chemicals and mice Ful was extracted from *Lonicera fulvotomentosa* by Guizhou Institute of Chinese Traditional Medicine[3]. Ful is composed of 5 triterpenoid saponins, of which three have been identified as fulvotomentoside A, α-hederin, and sapindoside B. $CdCl_2$ was obtained from Fisher Scientific Co, USA, and $^{109}CdCl_2$ (carrier-free) was obtained from New England Nuclear Co, USA. All chemicals were dissolved in saline. Male CF-1 mice ($26 \pm s$ 2 g) were housed in plastic cages and exposed to a 12-h light / dark cycle. Food (Purina Lab rodent chow) and tap water were provided *ad lib.*

Evaluation of hepatotoxicity Mice received either saline (10 ml · kg^{-1}, sc) or Ful (150 mg · kg^{-1}, sc) 60, 36, and 12 h prior to injection of a hepatotoxic dose of $CdCl_2$ (3.7 mg Cd · kg^{-1}, iv). Twelve hours following iv $CdCl_2$, mice were decapitated, and blood was collected. Serum activities of SDH[6] and ALT / GPT[7] were measured as indices of hepatotoxicity.

Organ distribution of ^{109}Cd The distribution of ^{109}Cd to various organs was determined 2 h following iv $^{109}CdCl_2$ (3.5 mg Cd · kg^{-1}, 370 kBq / mg Cd). The concentrations of ^{109}Cd in liver, kidney, spleen,

lung, heart, pancreas, stomach, intestine, muscle, bone, brain, and blood were determined by gamma scintillation. The femur, soleus, and initial 15-cm segment of intestine were used as representative of bone, muscle, and intestine, respectively[8].

Subcellular distribution of ^{109}Cd Mice were pretreated as described above. A portion of liver was homogenized in (1 : 4, wt : vol) Tris buffer (sucrose 0.25 mol · L^{-1}, Tris-acetate 10 mmol · L^{-1}, pH 7.4). Various fractions were prepared by differential centrifugation at 4℃ [8]. The resultant pellets were defined as nuclei (600 × *g*, 10 min), mitochondria (10 000 × *g*, 10 min), microsomes (100 000 × *g*, 65 min), and cytosol (100 000 × *g* supernatant). The distribution of ^{109}Cd in the hepatic cytosolic fraction was chromatographed on Sephadex G-75 gel filtration. Elution was performed with Tris-acetate 10 mmol · L^{-1}, (pH 7.4) at a flow rate of 30 ml · h^{-1} at 4℃, and seventy 5-ml fractions were collected. The amount of ^{109}Cd in subcellular fractions was measured as described above.

Metallothionein (MT) induction in liver Mice were pretreated with saline (10 ml · kg^{-1}, sc) or Ful (15, 150, and 450 mg · kg^{-1}, sc) for 3 d. Twenty-four hours after the last dose, livers were excised and homogenized as previously described in Tris-HCl buffer 10 mmol · L^{-1}, followed by centrifugation (10 000 × *g*, 10 min). Resultant supernatant was centrifuged at 100 000 × *g* for 65 min at 4℃. The MT concentration in hepatic cytosol was quantitated by the Cd / hemoglobin assay[9].

Statistics Comparison between control and treatment groups was made by *t* test.

RESULTS

Prevention of $CdCl_2$ hepatotoxicity Marked elevation of serum ALT and SDH activities were observed in mice 12 h following iv $CdCl_2$ (Fig 1). The activities of ALT and SDH were 35- and 21-fold higher, respectively, than that of controls. Ful pretreatment attenuated the marked increases of ALT and SDH activities produced by $CdCl_2$; ALT and SDH activities were decreased by 96 and 85%, respectively. ALT and SDH activities in mice receiving Ful pretreatment + saline challenge were not different from that of controls.

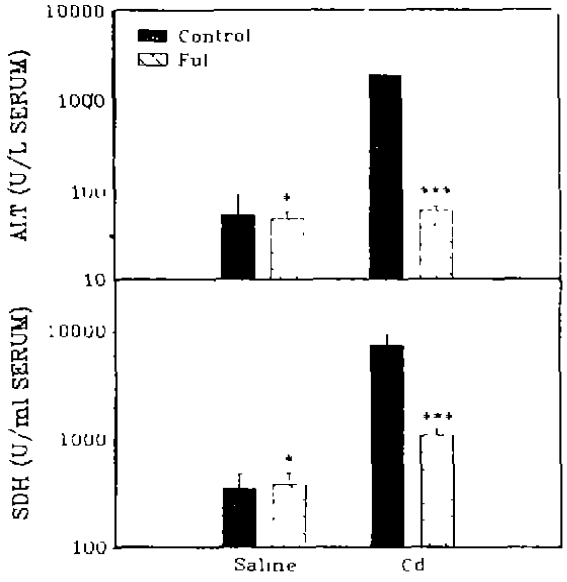

Fig 1. Effects of Ful pretreatment (150 mg · kg^{-1}, sc × 3 d) on serum ALT and SDH activities following saline ($n=5$) or Cd (3.7 mg · kg^{-1}, iv) challenge ($n=13-18$). $\bar{x}\pm s$. * $P>0.05$. *** $P<0.01$.

Distribution of ^{109}Cd Pretreatment with Ful resulted in an 11% increase of ^{109}Cd in liver and a small decrease of ^{109}Cd in kidney, testes, bone, and blood. There were no changes in ^{109}Cd distribution to spleen, intestine, stomach, pancreas, heart, lung, brain, and muscle as a result of Ful pretreatment (Tab 1).

Pretreatment with Ful decreased the amount of ^{109}Cd in mitochondrial and microsomal fractions 20%. In contrast, Ful

Tab 1. Effect of Ful pretreatment on the distribution of ^{109}Cd (μg / g tissue) to major organs. $n=6-8$. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$.

Tissue	Control	Ful-pretreatment
Liver	31.3 ± 2.26	35.5 ± 2.65**
Intestine	14.8 ± 1.83	12.9 ± 1.29*
Kidney	12.5 ± 1.44	10.5 ± 1.52**
Pancreas	9.83 ± 0.35	9.07 ± 0.94*
Stomach	4.54 ± 0.49	3.98 ± 0.56*
Heart	2.88 ± 0.37	2.49 ± 0.12*
Spleen	2.35 ± 0.30	2.02 ± 0.42*
Lung	1.62 ± 0.18	1.37 ± 0.13*
Bone	1.26 ± 0.12	0.93 ± 0.14**
Testes	0.72 ± 0.06	0.59 ± 0.03**
Blood	0.35 ± 0.05	0.27 ± 0.07**
Muscle	0.32 ± 0.03	0.30 ± 0.03*
Brain	0.16 ± 0.01	0.15 ± 0.01*

pretreatment caused an increase of ^{109}Cd in the cytosol (170% of control) (Fig 2). When the data were expressed as % of the total ^{109}Cd within the liver (lower panel), the differences in all fractions were more evident.

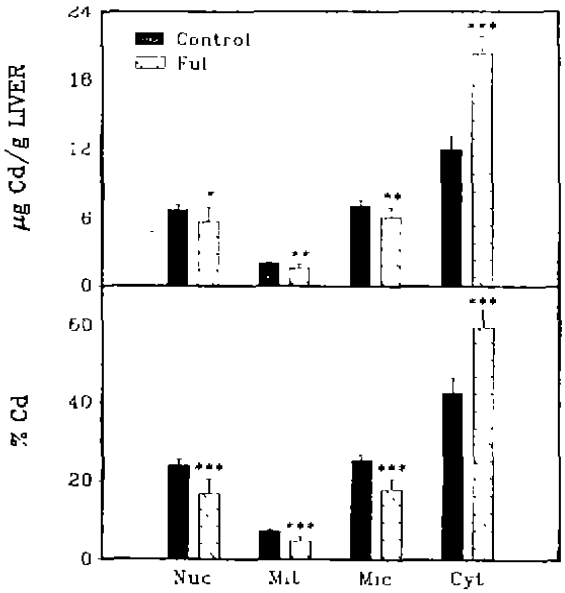

Fig 2. Effect of Ful pretreatment (150 mg · kg^{-1}, sc > 3 d) on hepatic subcellular distribution of $^{109}CdCl_2$ (3.5 mg · kg^{-1}, 370 kBq / mg Cd, ip). Lower panel shows the % of total Cd in homogenate. $n=6$. $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

The distribution of Cd within hepatic cytosol is shown in Fig 3 by representative gel-filtration elution profiles. In control mice, the majority of ^{109}Cd in the cytosol was associated with high-molecular-weight proteins (HMW, fraction № 10–23); however, following Ful pretreatment, the majority of ^{109}Cd eluted with the low-molecular-weight protein, metallothionein (MT, fraction № 31–46). Pretreatment with Ful did not alter the distribution of ^{109}Cd to HMW proteins but markedly increased its binding to MT. When the data were expressed as % of total ^{109}Cd within the cytosol (Fig 4), 84% was associated

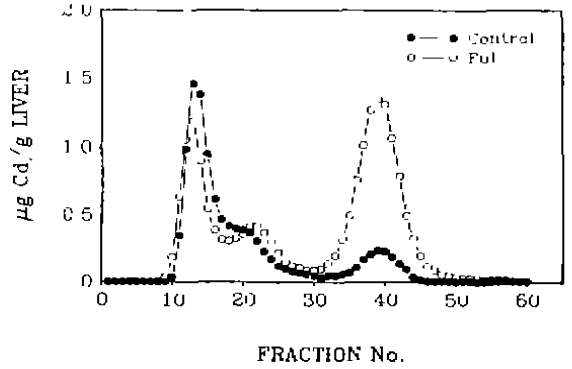

Fig 3. Representative gel-filtration elution profiles of Cd in hepatic cytosol from control or Ful-pretreated mice. Fractions № 10–23 and 31–46 were considered to be high-molecular-weight (HMW) protein and metallothionein (MT), respectively.

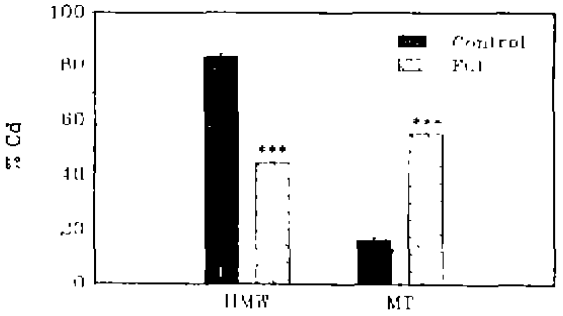

Fig 4. Compilation of gel-filtration of Cd in hepatic cytosol. $n=6$, $\bar{x}\pm s$. $^{***}P<0.01$.

with HMW proteins and only 16% was bound to MT in control. In contrast, Ful pretreatment increased the amount of ^{109}Cd bound to MT to 55% and reduced the ^{109}Cd associated with HMW to 45%.

Induction of hepatic MT Ful pretreatment produced a dose-related increase in hepatic MT. MT concentrations in liver increased 5-, 20-, and 70-fold after sc Ful 15, 150, and 450 mg · kg^{-1} · d^{-1} for 3 d, respectively (Fig 5).

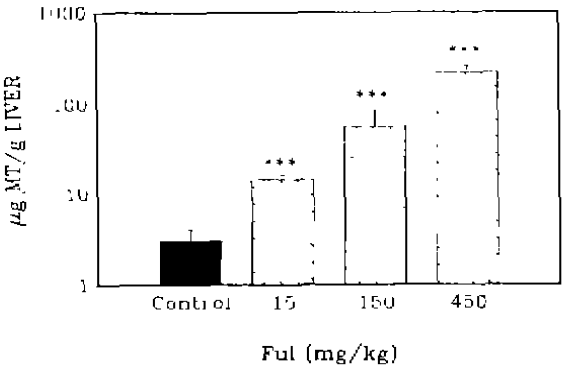

Fig 5. Hepatic MT induction following Ful pretreatment (15–450 mg · kg^{-1}, sc × 3 d). $n=5$. $\bar{x}\pm s$. $^{***}P<0.01$.

DISCUSSION

The ability of Ful to protect against Cd-induced toxicity may theoretically result from a decreased distribution of Cd to the target organ of acute Cd toxicity, the liver. However, in the present study, we did not observe a decrease in distribution of Cd to the liver following Ful pretreatment. In fact, more of the Cd accumulated in the liver of Ful-pretreated mice. Therefore, protection is not due to decreased Cd accumulation in the liver.

An alternative explanation for the protection is that Ful pretreatment may alter the subcellular distribution of Cd. The present study supports this theory. Following Ful pretreatment, more Cd was found in the liver cytosol, and less was present in the mitochondrial, microsomal, and nuclear fractions. The majority of Cd in the cytosol of Ful-pretreated mice was associated with MT, with less Cd bound to HMW proteins compared to controls. This altered subcellular distribution of Cd in the liver is extremely important, because MT sequesters Cd in the cytosol and renders it nontoxic[8,10]. This concomitantly decreased the amount of Cd available to critical organelles and proteins, which are suggested to be targets of Cd toxicity[11-13].

Ful pretreatment produced a dose-related induction of hepatic MT. MT, a cysteine-rich and metal-binding protein, has been proposed to play an important role in the detoxification of heavy metals, as well as free radicals[8,14,15]. MT is known to be induced by metals, hormones, and various stresses[14,15]. So far there is no information on induction of MT by triterpenoid saponins such as Ful. MT induction by Ful explains the mechanism of the protection against Cd, but also may be responsible for some other pharmacological effects produced by Ful.

In summary, Ful pretreatment markedly protects against Cd-induced hepatotoxicity. This protection appears to be due to an increase of MT in the liver, thus altering the subcellular distribution of Cd, with more Cd localizing in the cytosol bound to MT and less associated with other critical organelles and proteins.

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黄褐毛忍冬总皂甙对镉所致急性肝损伤的保护作用

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提要 黄褐毛忍冬总皂甙(fulvotomentosides: Ful)对镉(Cd)所致小鼠急性肝损伤有明显保护作用. 其作用机制在于 Ful 诱导肝脏合成大量金属硫基蛋白(metallothionein, MT). MT 结合 Cd 于细胞浆中, 从而减少 Cd 在核、线粒体、微粒体及细胞浆中高分子蛋白质中的分布, 由此减轻 Cd 对肝细胞的毒性.

关键词 黄褐毛忍冬总皂甙; 镉中毒; 肝; 丙氨酸转氨酶; 艾杜醇脱氢酶; 组织分布; 金属硫蛋白

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