BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 May; 13 (3): 209-212

Protective effects of fulvotomentosides on acetaminophen-induced hepatotoxicity

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ABSTRACT Fulvotomentosides (Ful) is the total saponins of *Lonicera fulvotomentosa*. In the present study, we examined the effect of Ful on acetaminophen (AA)-induced hepatotoxicity in mice. Ful pretreatment (75-225 mg · kg^{-1}, sc × 3 d) significantly decreased AA (500 mg · kg^{-1}, ip)-induced liver damage as indicated by serum activities of alanine aminotransferase and sorbitol dehydrogenase. Ful pretreatment (225 mg · kg^{-1}, sc × 3 d) decreased hepatic cytochrome P-450, cytochrome b_5, and NADPH-cytochrome c reductase by approximately 15-20%. Microsomes from Ful-pretreated mice, incubated *in vitro* with AA, produced less AA-glutathione. A 28% increase in urinary excretion of AA-glucuronide was observed in Ful (150 mg · kg^{-1}, sc × 3 d) pretreated mice. Ful pretreatment had no influence on liver UDP-glucuronic acid concentration, but increased hepatic glucuronyltransferase activity towards AA. In summary, Ful pretreatment protects against AA-induced hepatotoxicity. One of the mechanisms for this protection appears to be the decreased AA toxic activation via P-450, as well as increased detoxication via glucuronidation of AA.

KEY WORDS fulvotomentosides; acetaminophen; liver; toxicology; cytochromes; drug metabolic detoxication

Fulvotomentosides (Ful) is the total saponins isolated from *Lonicera fulvotomentosa*[1], a herbal drug traditionally used for infectious diseases. Ful has been shown to have anti-inflammatory effects[2] and hepatoprotective effects against some hepatotoxicants[3]. The aim of this study is to examine the protective effects of Ful against acetaminophen (AA) induced hepatotoxicity in mice.

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 Traditional Medical Institute, China[1]. Ful is composed of 5 triterpenoid saponins, of which three have been identified as fulvotomentoside A, α-hederin, and sapindoside B. Acetaminophen (AA) and UDP-glucuronic acid (UDP-GA) are obtained from Sigma (St Louis MO, USA). [^{3}H]diethyl-stilbestrol (DES) was purchased from Amersham (Arlington Heights IL, USA). All other chemicals were of reagent grade. CF-1 ♂ mice 26 ± *s* 2 g were housed in plastic cages in groups of 5 and exposed to a 12-h light / dark cycle. Food and tap water were provided *ad lib*.

Hepatotoxicity studies Mice received either saline (10 ml · kg^{-1}, sc) or Ful (75-225 mg · kg^{-1}, sc) 72, 48, and 24 h prior to injection of a hepatotoxic dose of AA (500 mg · kg^{-1}, ip). Twenty-four hours after injection of AA, blood was collected. The serum activities of sorbitol dehydrogenase (SDH)[4] and alanine aminotransferase (ALT / GPT)[5] were measured as indices of hepatotoxicity.

Cytochrome P-450 studies Mice received either saline or Ful (225 mg · kg^{-1}, sc) for 3 d. Liver microsomes were prepared. Cytochrome P-450 was determined from the CO difference spectrum of dithionite reduced microsomes based on an extinction coefficient of 91 mM^{-1} cm^{-1}. Cytochrome b_5 was

determined from the difference spectrum of NADH–reduced *vs* oxidized microsomes, based on an extinction coefficient of 185 mM^{-1} cm^{-1} [6]. NADPH–cytochrome c reductase was determined from its absorbance at 550 nm, based on an extinction coefficient of 19.1 mM^{-1} cm^{-1} [7].

Urinary excretion studies[8] Mice were pretreated with Ful (150 mg · kg^{-1}, sc × 3 d) or saline as described above. AA was dissolved in saline and injected into the tail vein at a dosage of 1 mmol · kg^{-1}. Urine was collected for 2 h. AA and its metabolites in urine were assayed by HPLC[9]. AA and its metabolites were eluted with 1.5% aqueous acetic acid : methanol (90 : 15) at a flow rate of 1.5 ml · min^{-1}. The elution of the metabolites was monitored at 254 nm. The concentration of AA metabolites were calculated from an AA standard curve as previously described[8,9]. In order to remove proteins from the samples, urine was diluted 100–fold with methanol and centrifuged before analysis

Microsomal formation of AA–GSH[10] Hepatic microsomes were incubated at 37℃ for 30–60 min with AA 10 mmol · L^{-1}, GSH 2.5 mmol · L^{-1}, and a NADPH regenerating system. At the end of incubation, 100 μl incubation mixtures were mixed with 200 μl methanol and centrifuged. The supernatant was analyzed by HPLC for AA–GSH as described above.

Hepatic UDP–GA and UDP–GT Hepatic UDP–glucuronic acid (UDP–GA) content was quantitated via a UDP–GT catalyzed reaction with [^{3}H]DES as the substrate[11]. The UDP–glucuronosyl–transferase (UDP–GT) activity towards AA was measured via the formation of AA–glucuronide *in vitro*[10].

Statistics Comparisons between saline and Ful pretreatment were made by *t* test.

RESULTS

Protection against AA hepatotoxicity AA (500 mg · kg^{-1} ip) caused 23% of the mice to die, and marked elevation of serum ALT (30–fold) and SDH (25–fold) levels in saline–pretreated mice. Ful pretreatment produced a dose–dependent reduction of the elevated serum ALT and SDH activities produced by AA, decreased the mortality at the low dose, and prevented the mortality at the dosages of 150 and 225 mg · kg^{-1} (Tab 1).

Tab 1. Effect of Ful pretreatment (sc, × 3 d) on acetaminophen (500 mg · kg^{-1})–induced liver injury in mice. $\bar{x} \pm s$. $^{**}P < 0.05$.

Ful / mg · kg^{-1}	n	Mortality / %	serum ALT / U · L^{-1}	serum SDH / U · ml^{-1}
0	13	23	6 720 ± 3 460	12 700 ± 5 700
75	10	10	3 250 ± 2 770**	6 410 ± 4 490**
150	10	0	2 710 ± 3 200**	5 140 ± 6 840**
225	10	0	1 160 ± 1 900**	1 800 ± 2 280**

Inhibition of cytochrome P–450 Ful pretreatment (225 mg · kg^{-1}, sc × 3 d) decreased the total amount of liver cytochrome P–450 by 20%, cytochrome b_5 by 22%, and the rate of NADPH–cytochrome c reductase by 17% (Tab 2).

Tab 2. Effect of Ful pretreatment (225 mg · kg^{-1}, sc × 3 d) on cytochrome P–450, b_5, and NADPH–cytochrome c reductase in mice. $n = 6$, $\bar{x} \pm s$. $^{**}P < 0.05$.

	P–450, nmol / mg protein	b_5, nmol / mg protein	Cyt–c reductase, nmol · min^{-1} / mg protein
Control	7.98 ± 1.32	2.09 ± 0.44	647 ± 169
Ful	6.32 ± 0.69**	1.64 ± 0.05**	534 ± 39**

Urinary excretion of AA and AA–metabolites AA–glucuronide (AA–Glu) was the major AA–metabolite excreted into urine, which comprised 63% of the urinary excretion of AA and its metabolites in control mice and 74% in the Ful–pretreated mice. In contrast,

AA–cysteine, the hydrolyzed product of AA–glutathione, was decreased by 39%. AA–sulfate, AA, and AA–mercapturate were not different from that of controls (Fig 1).

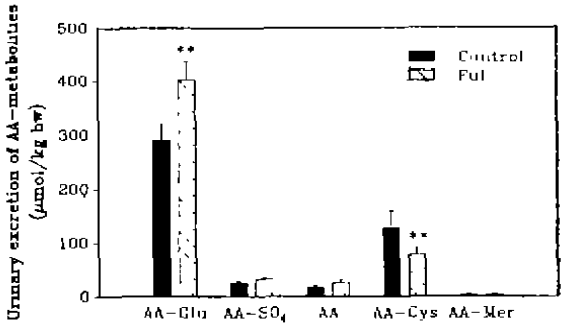

Fig 1. Effect of Ful pretreatment on urinary excretion of AA, AA–glucuronide, AA–sulfate, AA–cysteine, and AA–mercapturate. Urine was collected for 2 h after iv AA 1 mmol · kg^{-1}. $\bar{x}\pm s$ of 8 mice. $^{**}P<0.05$.

Microsomal formation of AA–GSH, hepatic UDP–GA, and UDP–GT Liver microsomes from Ful pretreated mice, incubated *in vitro* with AA, produced less reactive intermediates, as indicated by 23% reduction of AA–GSH adduct formation. Although Ful pretreatment did not significantly increase the UDP–GA concentration in the liver, it enhanced the hepatic UDP–GT activity towards AA (Tab 3).

Tab 3. Effect of Ful pretreatment (150 mg kg^{-1}, sc · 3 d) on AA–GSH fromation, hepatic UDP–GA concentration, and UDP–GT activity in mice. $n=7$–8. $\bar{x}\pm s$. $^{**}P<0.05$.

	AA–GSH, pmol · min^{-1} · mg protein	UDP–GA, nmol / g liver	UDP–GT, nmol · min^{-1} · mg protein
Control	522 ± 57	405 ± 57	2.10 ± 0.23
Ful	390 ± 26**	370 ± 71	2.42 ± 0.19*

DISCUSSION

The analgesic drug AA is safe in the therapeutic range, but an overdose causes severe hepatotoxicity in both animals and humans[12]. The present study showed that Ful pretreatment produced a dose–dependent protection against AA–induced liver injury in mice, as evidenced by decreased mortality, serum ALT, and SDH activities.

It is known that the hepatotoxic effect of AA is caused by *N*–acetyl–*p*–benzoquinoneimine (NAPQI), a cytochrome P–450 mediated intermediary metabolite of AA[13]. NAPQI can react with sulfhydryl groups such as GSH and protein thiols. The covalent binding of NAPQI to cell protein is considered the initial step in a chain of events eventually leading to cell necrosis[14]. Because NAPQI is produced via P–450 activation, treatments that inhibit cytochrome P–450 could decrease AA–induced hepatotoxicity[15]. In this study, cytochrome P–450, b_5, and the activity of NADPH–cytochrome c reductase were decreased in Ful–pretreated liver microsomes. In addition, two indices of AA toxic metabolic activation, the microsomal formation of AA–GSH *in vitro* as well as urinary excretion of AA–Cys[8,10], were also decreased. Therefore, it appears that the decreased AA hepatotoxicity by Ful may be due, in part, to the decreased reactive metabolites of AA.

In contrast to the toxic activation of AA via the cytochrome P–450 metabolic pathway, glucuronidation and sulfation of AA represent metabolic detoxification pathways[8,12,14]. Glucuronidation of AA is especially important, because it accounts for 70–80% of AA metabolites[14]. Enhanced glucuronidation of AA can protect against AA–hepatotoxicity[10]. In the present study, the urinary excretion of AA–glucuronide was increased, suggesting that Ful pretreatment enhanced the glucuronidation of AA. To further examine the effect of Ful on the glucuronidation of AA, the co–substrate UDP–GA and the rate–limiting enzyme UDP–GT were examined. Ful pretreatment had no significant effect on liver UDP–GA

concentration. However, it increased hepatic UDP-GT activity towards AA, and thus increased AA detoxification.

In summary, Ful pretreatment protects mice against AA hepatotoxicity. The mechanism of protection appears to be due, at least in part, to increased AA-detoxification by glucuronidation, as well as inhibition of AA toxic activation by cytochrome P-450.

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黄褐毛忍冬总皂甙对醋氨酚所致肝损伤的保护作用

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提要 黄褐毛忍冬总皂甙(fulvotomentosides, Ful)对醋氨酚(扑热息痛)所致小鼠急性肝损伤有明显保护作用. 其作用机制可能通过: 1 抑制细胞色素 P-450 药酶代谢系统, 从而减少醋氨酚毒性代谢产物; 2 诱导肝脏葡萄糖醛酸结合酶活性, 增加醋氨酚葡萄糖醛酸结合代谢, 从而加强醋氨酚在体内的解毒代谢.

关键词 黄褐毛忍冬总皂甙; 醋氨酚; 肝; 毒理学; 细胞色素类; 药物代谢解毒

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