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Effect of α-hederin and sapindoside B on hepatic microsomal cytochrome P-450 in mice[1]

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KEY WORDS α-hederin; sapindoside B; liver; cytochrome P-450; phenobarbital

AIM: To study the relation between the effect of α-hederin (Hed) and sapindoside B (Sap B) on cytochrome P-450 and their hepatoprotection. **METHODS:** Mice were given sc Hed, Sap B, as well as a mixture of Hed + Sap B (1 : 1.5), and hepatic microsomal cytochrome P-450 was examined. **RESULTS:** Hed 20 mg·kg^{-1}, Sap B 20 mg·kg^{-1} and Hed + Sap B 20 mg·kg^{-1} sc, reduced hepatic P-450 content by 40 %, 55 % and 50 %, respectively. The effect of the saponins on P-450 was reversible, as P-450 level returned to normal after 3 d. Phenobarbital (Phe) 50 mg·kg^{-1} ip increased P-450 2.5-fold, the Phe-induced P-450 was inhibited 50 % by Hed + Sap B. No inhibitory effect was seen when liver microsomes were incubated with Hed + Sap B *in vitro*. **CONCLUSION:** The hepatoprotective effects of Hed and Sap B were at least in part, due to its suppressive effect on liver cytochrome P-450.

The protective effect of fulvotomentosides (Ful) against CCl_4-, and acetaminophen-induced liver injuries in mice was observed[1,2]. Ful extracted from the flowers of *Lonicera fulvotomentosa* Hsu *et* S C Cheng (Caprifoliaceae) contains 5 triterpene saponins[3]. α-Hederin (Hed) and sapindoside B (Sap B) are the 2 major saponins. Hed mp: 259 - 262 ℃, 3-*O*-α-*L*-rhamnopyanosyl-(1 - 2)-α-*L*-arabanopyranosyl-hederagenin. Sap B mp: 222 - 225 ℃, 3-*O*-β-*D*-xylpyranosyl-(1 - 3)-α-*L*-rhamnopyranosyl-(1 - 2)-α-*L*-arabanopyranosyl-hederagenin. Hed and Sap B were effective in reducing CCl_4-, and acetaminophen-induced liver injury in mice, while the other 3 components were ineffective (in press). Many hepatotoxicants need metabolic activation, usually by hepatic cytochrome P-450 enzymes, to produce toxic metabolites, which cause liver injury[4]. Any treatment that suppresses liver cytochrome P-450 protects against hepatotoxicity produced by some chemicals[5]. Ful suppressed hepatic cytochrome P-450, which was thought to play a role in protecting against acetaminophen-induced liver injury[2]. This study was to determine whether Hed and Sap B, could suppress P-450 as a means of heptoprotection.

MATERIALS AND METHODS

Kunming strain mice ♂, weighing 20 - 24 g were obtained from the Animal Center of Chinese Academy of Medical Sciences. Hed and Sap supplied by Pharmacist Ms JIA Xian-Sheng of our Institute (purities > 98 %) were mixed in a proportion of 1 : 1.5 as a suspension in 5 % Tween-80. The suspension was injected sc. NADP was from Sigma Co.

Mice were injected sc Hed + Sap B, Hed, or Sap B at dosages of 10 and 20 mg·kg^{-1}, 2 or 3 times at 8-h interval. The mice were fasted overnight, and decapitated after 24 h. In another group of mice, phenobarbital (Phe) 50 mg·kg^{-1} was injected ip with or without Hed and Sap B to determine their effect on Phe-induced P-450 enzymes.

The liver homogenate was made as 20 % in TMS buffer (Tris-HCl 0.05 mol·L^{-1}, sucrose 0.2 mol·L^{-1}, $MgCl_2$ 3 mmol·L^{-1}, pH 7.5). The suspension was centrifuged at 10 000 × *g* for 20 min. The supernatant was centrifuged at 105 000 × *g* for 60 min. The pellet was resuspended in TMS buffer. Microsomal protein[6] and P-450[7] were assayed.

***In vitro* study** The microsomal protein (1.5 mg) was incubated with Hed + Sap B (60 μg, dissolved in Me_2SO) at 37 ℃ with or without NADP 0.5 mmol·L^{-1} for 0, 1, and 2 h. P-450 was determined spectrophotometrically at 400 - 500 nm.

RESULTS

Effect in mice The mixture of Hed + Sap B

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produced a concentration-dependent inhibition of P-450. Hed + Sap B (20 and 10 mg·kg^{-1} sc) decreased the P-450 content by 50 % and 25 %, respectively. Hed 20 mg·kg^{-1} and Sap B 20 mg·kg^{-1} decreased P-450 content by 50 % and 60 %, respectively (Tab 1).

Tab 1. Effect of α-hederin (Hed), sapindoside B (Sap B), Hed + Sap B (1∶1.5 mixture), sc twice and phenobarbital (Phe) ip on hepatic microsomal cytochrome P-450. $\bar{x} \pm s$. $^cP<0.01$ *vs* normal. $^fP<0.01$ *vs* Phe.

Group/mg·kg^{-1}	Mice	P-450, μmol/g protein
Normal	6	0.24 ± 0.04
Hed + Sap B 20	6	0.12 ± 0.04^c
Hed + Sap B 10	5	0.18 ± 0.08^c
Normal	7	0.30 ± 0.04
Hed 20	5	0.14 ± 0.08^c
Sap B 20	5	0.10 ± 0.03^c
Normal	6	0.39 ± 0.03
Hed + Sap B 20	6	0.28 ± 0.09c
Phe 50	6	1.01 ± 0.17
Phe + Hed + Sap B	6	0.44 ± 0.12^f

Time course of P-450 suppression by Hed + Sap B is shown in Fig 1. The P-450 content was not altered until 8 h after Hed + Sap B administration, decreased by 60 % at 24 h, and gradually returned to normal after 3 d (Fig 1).

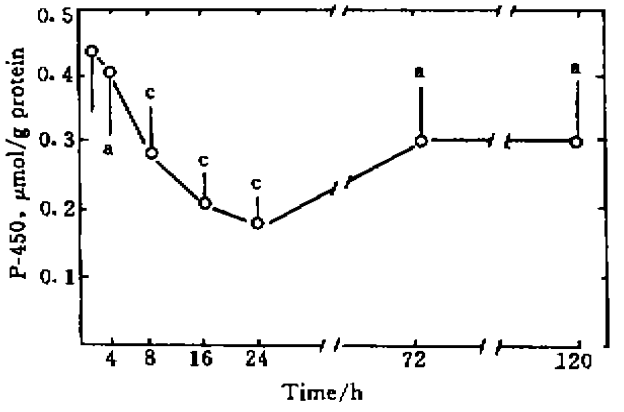

Fig 1. Hepatic microsomal cytochrome P-450 after sc α-hederin (Hed) + sapindoside B (Sap B) (1∶1.5) 20 mg·kg^{-1} twice in mice. $n = 5-6$, $\bar{x} \pm s$. $^aP>0.05$, $^cP<0.01$ *vs* normal.

Effect of Hed + Sap B on Phe-induced P-450 in mice Phe 50 mg·kg^{-1} increased P-450 content 2.5-fold, while Hed + Sap B produced 30 % decrease in P-450, similar to that seen in Tab 1 and Fig 1. When Hed + Sap B was given simultaneously with Phe, the Phe-induced P-450 was suppressed, P-450 content was 45 % of Phe group (Tab 1).

Effect *in vitro* The P-450 changed after incubation of liver microsomes with Hed + Sap B for 1 and 2 h. The P-450 content was decreased with the incubation time. However, there was no difference between control and Hed + Sap B group ($P > 0.05$). No absorption at 455 nm was monitored, indicating that Hed + Sap B did not form a complex with P-450 (Tab 2).

Tab 2. Change of hepatic microsomal cytochrome P-450 after liver microsome was incubated with Hed + Sap B (1∶1.5 mixture 60 μg) *in vitro*. $n = 5$. $\bar{x} \pm s$. $^aP>0.05$ *vs* control.

Group	Time/h	P-450, μmol/g protein
Control	0	0.385 ± 0.016
Hed + Sap B	0	0.37 ± 0.06^a
Control	1	0.231 ± 0.016
Hed + Sap B	1	0.20 ± 0.03^a
Control	2	0.18 ± 0.06
Hed + Sap B	2	0.231 ± 0.016^a

DISCUSSION

The present study demonstrated that 2 major components of Ful, Hed and Sap B suppressed P-450 enzymes in mice when used alone or in combination. The effect was reversible. Phe-induced P-450 enzymes were also decreased by Hed + Sap B.

The suppressive effects of Hed + Sap B did not appear to be due to the divert inhibition and the formation of complex. The study further identified that Hed and Sap B were the active ingredients of Ful in the inhibition of P-450 in mice.

The P-450 suppression by Hed and Sap B has obvious impact on their hepatoprotective effect, as hepatotoxicity of CCl_4 is mediated by P-450-mediated metabolites, trichloromethyl radical[8], similarly, the hepatotoxicity of acetaminophen is mediated by NAPQI[9], P-450-mediated metabolites. Therefore, the P-450 inhibition by Hed, and

Sap B reduced reactive metabolites, thus, alleviated toxic insult.

In addition to P-450 suppression, other mechanisms may contribute to hepatoprotection by Hed and Sap B. For example, Hed protects against Cd liver injury by increased metallothionein[10]. Other mechanisms for Hed + Sap B protecting against chemical-induced liver injury need further investigation.

In summary, the present study demonstrated the suppressive effect of Hed + Sap B on the P-450, and this effect appeared to be one of the mechanisms for Hed + Sap B protecting against some hepatotoxicants.

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α-常春藤皂甙和无患子皂甙 B 对小鼠肝微粒体细胞色素 P-450 的作用

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关键词 α-常春藤皂甙; 无患子皂甙 B; 肝; 细胞色素 P-450 类; 苯巴比妥

目的: 研究 α-常春藤皂甙(Hed)和无患子皂甙 B (Sap B)对小鼠肝细胞色素 P-450 的影响与保肝作用的关系. **方法**: 给小鼠 sc Hed, Sap B 和 Hed+Sap B (1:1.5), 检测小鼠肝微粒体细胞色素 P-450 的含量. **结果**: Hed 20 mg·kg^{-1}, Sap B 20 mg·kg^{-1}和 Hed+Sap B 20 mg·kg^{-1}使肝细胞色素 P-450 分别降低了 40 %, 55 %和 50 %. 这种抑制作用在 3 d 后基本恢复正常 苯巴比妥 ip 50 mg·kg^{-1}使小鼠肝细胞色素 P-450 增加 2 5 倍, Hed+Sap B 使苯巴比妥诱导的 P-450 降低了 50 %, 小鼠肝微粒体体外与 Hed+Sap B 共孵对 P-450 没有影响. **结论**: Hed 和 Sap B 的保肝作用至少在某一方面是由于降低了肝细胞色素 P-450 而产生的

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