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Effect of α-hederin on hepatic detoxifying systems in mice[1]

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KEY WORDS α-hederin; liver; glutathione; metallothionein; antioxidants

AIM: To examine whether α-hederin (Hed) modulates hepatic detoxifying systems as a means of hepatoprotection. **METHODS:** Mice were iniected Hed 10 and 30 $\mu mol \cdot kg^{-1}$ sc for 3 d, and liver cytosols were prepared 24 h after the last dose to study antioxidant enzymes and nonenzymatic defense components. **RESULTS:** Hed increased liver glutathione (GSH) content (20 %), but had no effect on GSH peroxidase, GSH reductase, and GSH *S*-transferase. The activities of superoxide dismutase and quinone reductase were unaffected by Hed treatment. At the high dose of Hed, catalase activity was decreased by 20 %. Hepatic content of metallothionein was dramatically increased (50-fold), along with elevations of hepatic Zn and Cu concentrations (25 % - 80 %). Hed also increased ascorbic acid concentration (20 %), but no effect on α-tocopherol in liver. **CONCLUSION:** Hed enhanced some nonenzymatic antioxidant components in liver, which play a partial role in Hed protection against hepatotoxicity produced by some chemicals.

α-Hederin (Hed), a triterpenoid saponin found in *Lonicera fulvotomentosa* Hsu *et* A C Cheng (Caprifoloaceae)[1,2], protected mice against hepatotoxicity produced by CCl_4, paracetamol, bromobenzene, furosemide, and thioacetamide[3], as well as by cadmium[4], phalloidin, colchicine, and *D*-galactosamine plus endotoxin[5]. Hed suppressed hepatic cytochrome P-450 enzymes[2,3]. The suppression may play a partial role in protecting mice against hepatotoxicants that require P-450 bioactivation. However, for the hepatotoxicants that do not need bioactivation, other mechanisms of protection must also be involved.

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Some hepatotoxicants produce liver injury by generating reactive oxygen species and free radicals[6]. The defense mechanisms that organisms have against oxidative stress consist of ① prevention of "promary" damage by low-molecular-weight antioxidants (eg, GSH, ascorbic acid, α-tocopherol, uric acid, and metallothionein), ② prevention or limitation of "secondary" damage by detoxifying enzymes (eg, GSH-peroxidase, GSH-reductase, GSH *S*-transferase, catalase, superoxide dismutase and quinone reductase, and ③ enhancement of repair process (eg, lipid, protein, and DNA repair enzymes)[7]. This study was to examine effects of Hed on hepatotoxicant-detoxifying systems in mouse liver.

MATERIALS AND METHODS

α-Hed was obtained from Aldrich Chemical Co (Milwaukee WI, USA). All other chemicals were obtained from Sigma Chemical Co (St Louis MO). CF-1 mice ♀, (n = 6-8 per group), weighing 25-30 g were obtained from Harlan Lab (Indianapolis IN), and housed in an AAALAC accredited facility.

Mice were injected sc Hed (10 and 30 $\mu mol \cdot kg^{-1}$, =7.5 and 22.5 $mg \cdot kg^{-1}$) or vehicle (2 % Tween-80 in saline) daily for 3 d. Mice were decapitated 24 h after the last dose, and livers were removed.

Antioxidant enzyme assays Livers were homogenized in Tris-HCl 50 $mmol \cdot L^{-1}$ (pH 7.4) containing KCl 150 mmol $\cdot L^{-1}$ at 4 ℃, and the 105 000 × g supernatants were used as cytosols. Cytosolic selenium-dependent and-independent GSH peroxidase, GSSG reductase, GSH *S*-transferase, Zn, Cu-superoxide dismutase, quinone reductase, catalase, and protein were assayed as previously described[8].

Nonenzymatic antioxidant component assays Another portions of liver were used to measure concentrations of GSH, metallothionein, ascorbic acid, α-tocopherol, Zn, and Cu as previously described[8].

RESULTS

Antioxidant enzymes Both selenium-dependent and selenium-independent GSH peroxidase were unaffected by Hed treatments. Hed treatment had

no apparent effect on activities of GSSG reductase and GSH S-transferases towards 1-chloro-2,4-dinitrobenzene (DNCB), 3,4-dichloro-nitrobenzene (DCNB), 1,2 epoxyl-3-(*p*-nitrophenoxy) propane (ENPP). Superoxide dismutase (Zn, Cu-SOD), or cytosolic quinone reductase were also unaltered. However, high dose of Hed (30 μmol · kg^{-1}) decreased catalase activity by 20 % (Tab 1).

Nonenzymatic antioxidant components Hed treatment increased hepatic GSH content by 20 %. Consistent with previous observations, Hed produced a dose-dependent elevation of hepatic metallothionein levels up to 50-fold. Hepatic Zn and Cu concentrations were increased 80 % and 30 %, respectively at the high dose of Hed. Hed also increased hepatic concentrations of ascorbic acid (20 %). but had no effect on α-tocopherol (vitamin E) (Tab 1).

DISCUSSION

Theoretically, the hepatoprotection by Hed could be due to alterations in absorption, distribution, biotransformation, and elimination of hepatotoxicants, alterations in cellular detoxifying mechanisms, as well as alterations in cellular repair and regenerating processes[9]. GSH is the most important nonprotein thiol in living systems and is involved in numerous biochemical pathways within cells. GSH plays a key role in liver detoxication reactions, due to its role in maintaining the structural integrity of cell and organelle membranes, and its ability to form conjugates with reactive xenobiotic metabolites[10,11]. GSH is essential in the protection against hydrogen peroxide and organic hydroperoxides via GSH peroxidase. Depletion of GSH to 20 % – 30 % of control levels will impair the cell's defense against toxicants, leading to cell injure[11].

In the present study, Hed treatment increased liver GSH content by approximate 20 %, which may play a role, at least in part, in enhancing cellular defense mechanisms. However, both Se-dependent and Se-independent GSH peroxidase, glutathione reductase and GSH S-transferases were basically unchanged by Hed treatment of normal mice. Whether Hed treatment maintains GSH system homeostasis during pathological processes requires further investigation.

The superoxide anion radical is produced by the addition of one electron to molecular oxygen. Some hepatotoxicants exert toxic effects via the generation of superoxide anion through redox cycling[6]. Superoxide dismutase is a family of metalloenzymes which is known to accelerate dismutation of

Tab 1. Effects of α-hederin on hepatic antioxidant enzymes and nonenzymatic antioxidant components. $n = 6-8$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* 0 μmol·kg^{-1}.

α-Hederin (μmol·kg^{-1})	0	10	30
GSH peroxidase (μmol·min^{-1}/g protein)			
Se-dependent	660 ± 60	690 ± 63[a]	641 ± 127[a]
Se-independent	139 ± 36	137 ± 38[a]	105 ± 35[a]
GSG reductase (μmol·min^{-1}/g protein)	71 ± 6	71 ± 6[a]	79 ± 10[a]
GSG S-transferase (μmol·min^{-1}/g protein)			
DCNB	1 130 ± 167	1 150 ± 164[a]	1 270 ± 196[a]
DNCB	1 190 ± 186	1 170 ± 194[a]	1 180 ± 174[a]
ENPP	7.1 ± 1.5	6.90 ± 1.5[a]	8.60 ± 2.1[a]
Superoxide dismutase (ZnCu-SOD, kU/g protein)	12 ± 3	13.9 ± 2.2[a]	10.9 ± 2.3[a]
Quinone reductase (μmol·min^{-1}/g protein)	600 ± 90	498 ± 168[a]	518 ± 96[a]
Catalase (U/g liver)	44 ± 7	38 ± 8[a]	36 ± 8[b]
GSH (μmol/g liver)	11.1 ± 1.2	10.9 ± 1.6	13.6 ± 1.6[b]
Metallothionein (μg/g liver)	3.5 ± 0.5	31 ± 8[b]	181 ± 70[b]
Zinc (nmol/g liver)	456 ± 48	498 ± 51[a]	817 ± 90[b]
Copper (nmol/g liver)	147 ± 24	170 ± 27[a]	186 ± 38[b]
Ascorbic acid (μg/g liver)	142 ± 18	149 ± 15[a]	172 ± 24[b]
α-Tocopherol (μg/g liver)	5.6 ± 0.7	5.5 ± 0.5[a]	5.5 ± 0.6[b]

superoxide radical to hydrogen peroxide and molecular oxygen[8], and the hydrogen peroxide produced is further removed by catalase. Quinone reductase, another antioxidant enzyme, catalyzes two-electron transfers to several quinone compounds with formation of relatively stable hydroquinones, thus reducing superoxide anion production via the quinone redox cycle. In the present study, Hed treatment had no apparent effect on the activities of superoxide dismutase or quinone reductase, and at the high dose, decreased the activity of catalase. It appears that the protection by Hed may not be mediated by these antioxidant enzymes.

For nonenzymatic components, α-tocopherol is a lipophilic, while ascorbate is a water-soluble antioxidant[7]. In the present study, treatment of mice with Hed slightly increased hepatic ascorbate concentration, without affecting hepatic α-tocopherol content.

Metallothionein, a low-molecular weight, cysteine-rich protein, is also an important nonenzymatic antioxidant component[12]. Hed treatment increased hepatic metallothionein up to 50-fold, which plays an important role in Hed protection against cadmium-induced liver injury[4]. The sulfhydryl-rich group in MT may function similarly to GSH to provide a neutrophilic sink for reactive toxic metabolites or free radicals, and thus may play a role, at least in part, in Hed protection against radical-derived tissue damage. Along with increased MT, hepatic Zn and Cu contents were also increased. The increased Zn and Cu can provide metals for Zn, Cu-SOD and ceruloplasmin. Both enzymes are superoxide anion scavengers. Zn itself also plays a role as an antioxidant by protecting sulfhydryl groups and inhibiting reactive oxygen species produced by transition metals[13]. In summary, treatment of mice with Hed increased some cellular defense mechanisms, such as GSH, metallothionein, ascorbic acid and Zn. It appears that the hepatoprotective effects of Hed may be due, at least in part, to alterations in these nonenzymatic components in the mouse liver.

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REFERENCES

1 Mao Q, Jia XS. Studies on the chemical constituents of *Lonicera fulvotomentosa* Hsu *et* S C Cheng. Acta Pharm Sin 1989; **24**: 269 - 74.

2 Shi JZ, Liu GT. Effect of α-hederin and sapindoside B on hepatic microsomal cytochrome P-450 in mice Acta Pharmacol Sin 1996; **17**: 264 - 6.

3 Liu J, Liu YP, Bullock P, Klaassen CD. Suppression of liver cytochrome P 450 by α-hederin: relevance to hepatoprotection. Toxicol Appl Pharmacol 1995; **134**: 124 - 31.

4 Liu J, Choudhuri S, Liu YP, Kreppel H, Andrews GK, Klaassen CD. Induction of metallothionein by α-hederin. Toxicol Appl Pharmacol 1993; **121**: 144 - 51

5 Liu YP, Liu J, Klaassen CD The protective effects of oleanolic acid and α-hederin on chemical-induced acute necrotic liver injury in mice. Toxicologist 1991; **11** : 610

6 Freeman BA, Grapo JD. Free radicals and tissue injury. Lab Invest 1982; **47**: 412 - 26.

7 Bast A, Haenen GMMH, Doelman JA. Oxidants and antioxidants: state of the art. Am J Med 1991; **91** Suppl 3C: 2S - 13S.

8 Liu J, Liu YP, Parkinson A, Klaassen CD. Effect of oleanolic acid on hepatic toxicant-activating and detoxifying systems in mice. J Pharmacol Exp Ther 1995; **275**: 768 - 74

9 Farber JL, Gerson RJ. Mechanism of cell injury with hepatotoxic chemicals. Pharmacol Rev 1984;**36**: 71S - 75S

10 Klaassen CD, Bracken WM, Dudley RE, Goering PL, Hazelton GA, Hjelle J. Role of sulfhydryls in the hepatotoxicity of organic and metallic compounds. Fundam Appl Toxicol 1985; **5** : 806 - 15.

11 Reed DJ. Glutathione: toxicological implications. Annu Rev Pharmacol Toxicol 1990; **30**: 603 - 31

12 Sato M, Bremner I. Oxygen free radicals and metallothionein. Free Rad Biol Med 1993; **14**: 325 - 37

13 Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. Free Rad Biol Med 1990; **8**: 281 - 91.

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α-常春藤皂苷对小鼠肝脏解毒系统的作用[1]

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关键词 α-常春藤皂苷；肝；谷胱甘肽；金属硫蛋白；抗氧化剂

目的：研究 α-常春藤皂苷(Hed)对肝解毒系统的作用。**方法**：小鼠 sc Hed 10 - 30 μmol·kg^{-1}三日，然后检测肝脏抗氧化损伤的酶类及非酶成份。**结果**：Hed 增加肝脏谷胱甘肽含量 20 %，但对谷胱

甘肽氧化酶、还原酶和转移酶类均无明显作用. Hed诱导肝金属硫蛋白50倍，并同时增加肝脏锌(80 %)和铜(30 %)含量. Hed对超氧化物歧化酶，蔗酮还原酶无明显影响，大剂量时降低过氧化氢酶. Hed还增加肝脏抗坏血酸含量20 %，但对维生素E含量无明显影响. 结论：Hed的保肝作用至少在某一方面是由于诱导肝脏非酶类的抗氧化损伤物质.

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Binding conformers searching method for ligands according to the structures of their receptors and its application to thrombin inhibitors[1]

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KEY WORDS molecular conformation; phosphonopeptides; thrombin receptor; ligands; structure-activity relationship; molecular mechanics

AIM: To develop a method of finding binding conformers for ligands according to the three-dimensional structures of their receptors. **METHODS:** Combining the systematic search method of ligand with the molecular docking approach of ligand fitting into its receptor, we developed a binding conformer searching method for ligands. **RESULTS:** The binding conformers of phosphonopeptidyl thrombin inhibitors were recognized. The binding (interaction) energies between these inhibitors and thrombin were calculated with molecular mechanical method. **CONCLUSION:** Both of the total binding energies and steric binding energies have good correlations with the inhibitory activities of these thrombin inhibitors, demonstrating that our approach is reasonable. It can also be used to explain the inhibition mechanism of thrombin interacting with these inhibitors.

The interaction of ligands with their receptor macromolecules are central to all of biological processes, because that tells us the binding fashion of the ligands to their receptors, and from the interaction fashions, one can design novel ligands which might bind to the receptors tightly[1]. Nowadays, there is accumulating evidence that ligand-receptor non-covalent interactions can be modeled and simulated with relatively simple molecular mechanical approaches[2]. Among these approaches, the key difference, of course, is the sampling problem, since one should, in principle, consider the many alternative conformational states of the macromolecule, the ligands, and the complexes of them[2]. But how to recognize binding (bioactive) conformer of a ligand when which interacts with the receptor is still an unsolved problem, especially for the ligand with a large number of freedoms of flexibility. Our present approach attempts to go one step further to solve this kind of problem.

In this approach, we combined the systematic search method of ligand with the molecular docking approach[3] of ligand fitting into its receptor to try to find the binding conformer on the basis of the 3D structure of the active site of a receptor. Once the binding conformer of a ligand has been found, one can perform the calculation of the ligand interacting with the receptor, and design more potent molecules to bind to the receptor.

Thrombin, a trypsin-like serine protease, is the final enzyme in the blood coagulation cascade[4], and is an ideal target for the development of an anticoagulant protease inhibitors[5]. Modeled on the "fibrinogen-like" sequence *D*-Phe-Pro-Arg, a

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