A STUDY ON EFFECTS OF GLUTATHIONE S-TRANSFERASE FROM SILKWORM ON CCL_4-INDUCED MOUSE LIVER INJURY

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ABSTRACT

To assess the hepatoprotective activity of Glutathione S-transferase(GSTsw), extracted and purified from silkworm, in experimental acute mice liver injury and explore mechanisms. Mice were divided into five groups: control group, carbon tetrachloride (CCl_4) group, and three treatment groups that received CCl_4 and GSTsw at doses of 0.083mg•g^{-1}, 0.0415 mg•g^{-1} and 0.0207 mg•g^{-1} for 3 days. ALT in serum, GST, SOD and T-AOC in liver tissue homogenate, and changes in liver pathology in the five groups were studied. CCl_4 administration led to pathological and biochemical evidence of liver injury as compared to untreated controls. GSTsw administration led to significant protection against CCl_4-induced changes in liver pathology. It was also associated with significantly lower serum ALT levels, higher GST SOD and T-AOC level in live tissue homogenate. Thus, GSTsw showed protective activity against CCl_4-induced hepatotoxicity in mice.

Keywords: Glutathione S-transferase, Bombyx mori, liver injury, carbon tetrachloride.

INTRODUCTION

Glutathione S-transferases (GSTs, E.C.2.5.1.18) are a complex multigene family of enzymes that possess many biological functions, the most important of which is detoxification (Wolkoff, 1980). They result in the synthesis of mercapturic acids and represent an important excretory route for xenobiotics including carcinogens, toxins, and drugs. These enzymes catalyze the conjugation of electrophilic molecules with reduced glutathione (GSH), and generally make the resulting products more water soluble and excretable (Boyland and Chasseaud, 1969). The GSTs have long been demonstrated to be involved in intracellular transport of hormones, endogenous metabolites and exogenous chemicals and in the protection from oxidative damage and oxidative stress (Pickett and Lu, 1989, Enayati *et al*., 2005). It is also involved in non-substrate binding to such substances as bilirubin and bile acids and has an important role in hepatic anion transport (Meyer *et al*., 1985).

The silkworm (*Bombyx mori*) as a kind of insect develops adaptations to protect it against potentially toxic compounds, such as insecticides. GSTs in insects, together with esterases and cytochrome P450 dependent monooxygenases, are focused on the metabolic detoxification for alkylating agents, herbicides and insecticides (Clark, 1990). The GST isoenzymes catalyse the conjugation of glutathione to a wide range of compounds of which 1-chloro-2-4-dinitrobenzene is most frequently used as the substrate to measure the GST ctivity (Mannervik and Danielson, 1988).

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GST is mainly distributed in fat body of silkworm (Chien and Dauterman, 1991) similar to the human liver. GSH has been use to cure liver disease, such as acute liver injury caused by toxins, and obtained good effects (Gorla N, 1983). Since GSTs catalyse GSH to some toxic compounds, we guessed that GSTsw administration may improve liver biotransform function to reduce toxicity.

MATERIALS

Reagents

All the chemicals used in this study were of analytical grade. Carbon tetrachloride (CCl_4) was obtained from the Yangtze River chemical plant (China). Superoxide dismutase (SOD), Glutathione-S-transferase (GST), alanine transaminase (ALT) and total antioxidant activity (T-AOC) KITs were purchased from Nanjing bio-engineering institute (China).

GST from silkworm

Glutathione S-transferase from silkworm (GSTsw) was supplied by silkworm physiological lab, Institute of sericulture, Chinese Academy of Agricultural Sciences. Gui *et al*. have documented the extraction and purification method (Hou *et al*., 2008).

Animals

Animals used for study were obtained from Jiangsu University laboratory animal Center. Kunming mice weighing 20±2g each, equal male and female, were housed in polypropylene cages after acclimatization for a period of one week in a new environment. Mice were kept at an ambient temperature and had free access to food and water.

METHODS

Experimental design

Mice were randomly divided into five groups of eight each: the normal group (A), the toxic control group received CCl_4 (B) and GSTsw intervention group (C, D, and E). AT 5:00 p.m., group A and B were intraperitoneal injection of saline 0.5 ml, group C, D and E were intraperitoneal administration GSTsw(diluted in sterile saline) in a dose of 0.083mg•g^{-1} body weight, 0.0415 mg•g^{-1} body weight and 0.0207 mg•g^{-1} body weight respectively, for a total of three days. 4h after the end of administration, group A was intraperitoneal injection of olive oil in a dose of 0.01ml•g^{-1} body weight, group B, C, D and E were received intraperitoneal injection of 0.1% CCl_4 dissolved in olive oil in a dose of 0.01ml•g^{-1} body weight (Xu Shuyun, 2002).

Sample collection

Mice were fasted for 16 h after intraperitoneal injection of CCl_4 and were sacrificed, and blood was collected and separated for serum, stored at -20 for further laboratory tests. The left lobe liver was cut and fixed in10% formalin for 24 hours, sliced and stained with haematoxylin and eosin, and observed under light microscope. The right lobe of liver was homogenated for analyzing SOD, T-AOC, and GST activity.

Biochemical detection

The activity of ALT in Serum, SOD, T-AOC and GST in liver homogenate were assayed based upon the method provided by KITs.

STATISTICAL ANALYSIS

Data was presented as mean (x) ±Standard Deviation (SD), and was analyzed with SPSS 13.0 software. The differences among different groups were analyzed using one-way analysis of variance (ANOVA), differences were considered to be statically significant at $P<0.05$.

RESULTS

Serum ALT

ALT is at a lower level in normal animal serum. CCl_4 can damage the hepatocytes membrane structure, which results in leakage of ALT, and activity in serum increase. Group B administrated CCl_4 leaded to significant ($p<0.01$) rise of ALT activity when compared to the control group as shown in table 1. Group C intraperitoneally injected in a large dose of GSTsw was seen to lower significantly ALT activity ($p<0.01$), and to restore to normal level ($P>0.05$), however, medium and small doses of GSTsw did not have this effect.

Liver homogenate biochemical

GSTsw has vital effect on T-AOC, GSH and SOD activity in mouse liver homogenate as shown in table 1. Significant ($p<0.01$) reduction in T-AOC, GST and SOD activity was observed in CCl_4 treated rats compared with normal group. A large dose of GSTsw were shown significant ($p<0.01$) increase in activity of T-AOC, GST and SOD when compared to CCl_4 treated mice, low doses did not has significant action ($p>0.05$).

Hepatic lobule, hepatic sinusoid and hepatic cord were normal in Group A as shown in fig. 1, however, central veins of hepatic lobules vasodilatation and congestion, ambitus hepatocyte necrosis, ballooning degeneration of some cell had taken place in Group B treated by CCl_4. Hepatocyte edema, sporadic lymphocyte infiltration, individual hepatocyte regeneration were seen in group C in the intervention of large dose GSTsw, hepatocyte edema and lamellar lymphocyte infiltration were shown in medium dose, and hepatocyte edema, partly hepatocyte ballooning degeneration had taken place in partly hepatocyte in Group E.

DISCUSSION

It is the first study to show the hepatoprotective effects of GSTsw, which was evidenced by the amelioration of biochemical indicators of liver damage and the pathological disturbances caused by CCl_4.

The hepatic damage induced by CCl_4 is well known to be mediated by free radical metabolites, such as trichloromethyl-free radicals (•CCl_3) and trichloromethyl-peroxy radicals (•$OOCCl_3$), which could readily interact with unsaturated membrane lipid to produce lipid peroxidation and/or with other critical cellular

Table 1: Effects of GSTsw on T-AOC, GSH and SOD in liver tissue homogenate

Group	ALT/U·ml^{-1}	T-AOC/U·$mgprot^{-1}$	GST activity/U·$mgprot^{-1}$	SOD activity/U·$mgprot^{-1}$
A	29.42±8.16	2.03±0.16	81.05±11.09	335.16±19.71
B	87.75±27.28**	1.52±0.10**	47.88±6.15**	198.40±25.49**
C	28±9.03##	2.75±0.70##	83.66±11.34##	347.72±31.20##
D	53±12.07**	1.76±0.33**	54.65±4.63**	224.40±65.55#
E	79.5±14.51*	1.47±0.31*	47.21±7.91**	142.15±30.54**

Compared with the group A, *: P <0.05, **: P <0.01; Compared with group B, #: P <0.05, # #: P <0.01

macromolecules, and lead to cell damage [Snyder, Andrews, 1996]. CCl_4 liver toxicity depends on the cytochrome P450 in endoplasmic reticulum which catalytic reduction dehalogenation of CCl_4 to radical instability •CCl_3. It was take place that covalent binding reaction with macromolecular material in the cells, follow with the cell structure damage, enzyme activity decline and cell damage. •CCl_3 and oxygen can also form a high-activity•$OOCCl_3$, and lipid peroxidation reaction occurs, results in the destruction of poly-unsaturated fatty acids. In particular with the combination of lecithin, affect the permeability of cell membranes.

When the liver function damage, a number of blood biochemical indicators increase, such as ALT, AST, alkaline phosphatase, bilirubin, and so on. In this indicators system, ALT as more sensitive and higher specificity is commonly used for liver function evaluation, especial for acute liver damage. When hepatocyte damage, cell membrane stability decline and permeability increase, result in an outflow ALT; more seriously, some of hepatocyte necrosis when the cells lose their calcium

Histopathological changes

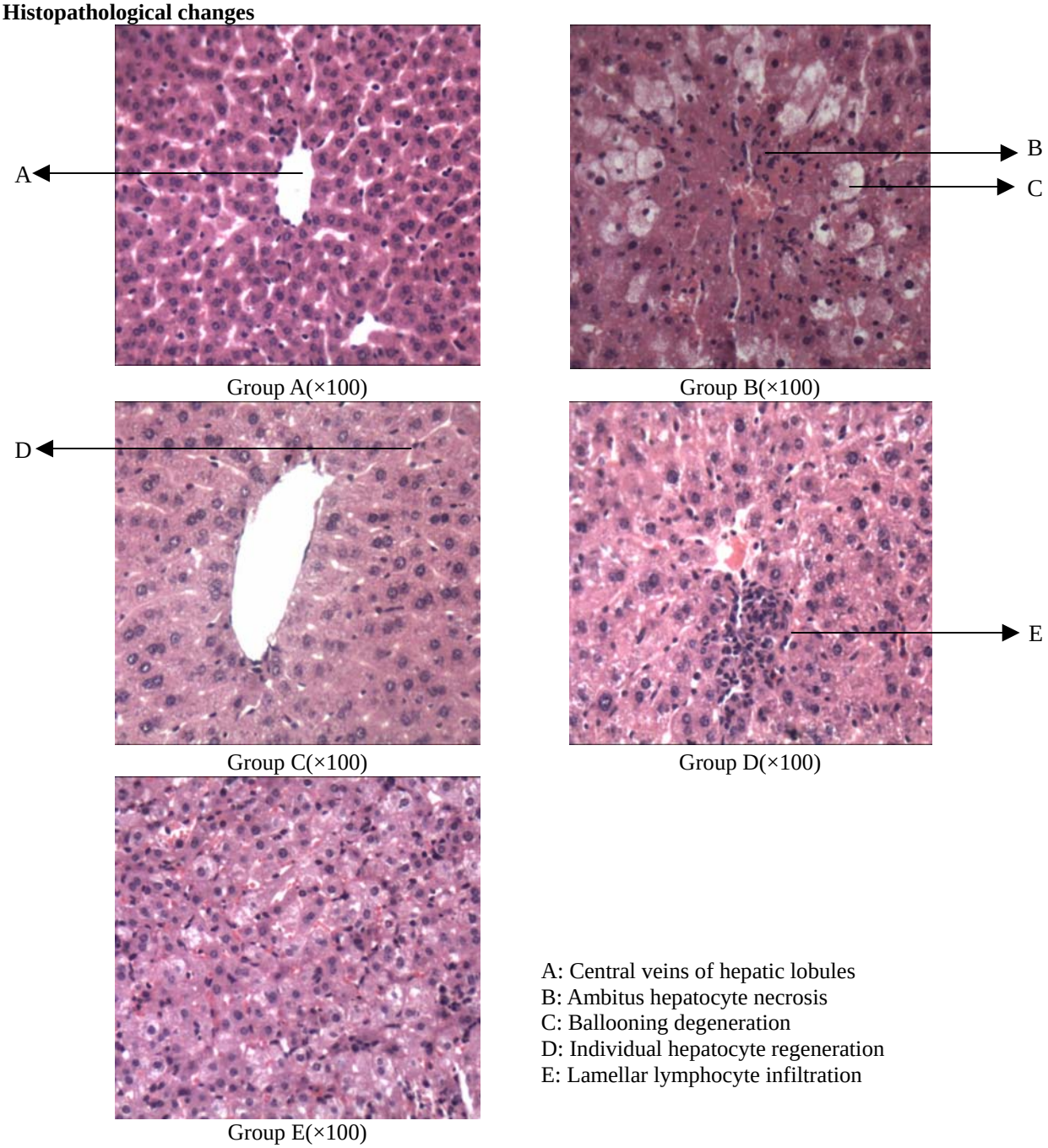

Fig. 1: Histopathological analysis of mice liver.

balance and stability within the environmental damage (Rarombi 2000, Recknagel *et al.*, 1989), a large amount of ALT is released into circulation. All of which lead to serum ALT activity in serum increase. The main purpose of this study is to evaluate the effect of GST on mice acute liver injury induced by CC4, so a single ALT used as indicators for evaluation.

In this experiment, ALT was lower in normal group, increased in group B treated by CCl4. The leakage of large quantities of enzymes into the blood stream was matched with massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver as shown in liver pathological results. It indicated that free radical induced by CCl_4 has lead to damage of hepatocyte membrane and hepatocyte necrosis. ALT in group C intervented by GSTsw was normal as group A. and was high in group D and E as group B. it suggested that GSTsw intervention reduced the liver damage severity, which was consistent with histopathological observation. It implied that a large dose of GSTsw has good hepatocyte protection.

The cleavage of CCl_4 leads to the formation of highly unstable free radicals, to initiate peroxidation (Recknagel *et al.*, 1989). Reactive oxygen species, including superoxide, hydroxyl radicals and hydrogen peroxide, are generated and react with biological molecules, eventually damage membranes and other tissues (Vuillaume, 1987). Thus, the inhibition of the generation of free radicals retards CCl_4-induced lipid peroxidation. Antioxidant enzymes SOD represent one protection against oxidative tissue-damage (Halliwell and Gutteridge, 1990). SOD converted O_2 into H_2O_2. GPx and catalase metabolize H_2O_2 to non-toxic products.

GST as a cell detoxification system is an important component of the degradation of oxygen metabolites to protect the cells. GST exist in all eukaryotic and prokaryote, distributed in the cytoplasm, and particles of mitochondria (Laughlin *et al*, 1996; Pemble *et al*, 1996). GSH as substrate of GST is the high expression in mammalian liver, which accounts for four percent of the total soluble protein (Eaton and Bammler, 1999). So GST plays a very important role in protecting mice liver form toxins damage.

Large dose administration of CCl_4 can causes a large number of liver cell necrosis, results in down-regulation of product of antioxidant enzymes, accordingly GST and SOD activity decrease, follow with decline in total antioxidant capacity, which was occurred in this experiment. Administration of GSTsw resulted in the increase of T-AOC, GST and SOD activity in liver tissue, which prevented the liver cells against the CCl_4-indeuced damage. It appeared that large dose administration of GSTsw significantly protected hepatocytes against damage caused by CCl_4. So, it suggested that another potential hepatoprotective activity of GSTsw against hepatic damage induced by CCl_4 could be due to its increasing antioxidant capacity.

Further study should be carried on revealing the concrete molecular mechanism of GSTsw reversing CCl_4-treated hepatocytes damage.

CONCLUSIONS

Intraperitoneal injection of GSTsw can reverse liver injury induced by CCl_4, decline ALT activity in serum, and increase GST, SOD and T-AOC activity in liver. It appeared dose-dependent, the higher the effect better was. Further research, including of more in-depth mechanism, will be beneficial to the development of GSTsw as a new drug or health product.

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