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PROTECTIVE EFFECT OF SCOPARONE AGAINST ACETAMINOPHEN INDUCED LIVER TOXICITY IN MICE

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ABSTRACT: *Protective effect of scoparone against the acetaminophen inducible hepatic toxicity in mice was investigated. Scoparone (5mg/kg) was administered intraperitoneally to mice daily for 5 days. Scoparone pretreatment before the administration of acetaminophen has blocked subsequent increases in liver to body weight ratio. When biological changes were measured, scoparone protects against acetaminophen inducible hepatotoxicity in mice as evidenced by the decreased formation of lipid peroxide, lowered serum transaminase activity and the decreased level of serum acetaminophen. In conjuction with the results of Huh (Arch. Pharm. Res. 10, 165(1987)), these results suggest that the most likely mechanism for the observed protective effects of scoparone against the acetaminophen-induced hepatotoxicity is the induction of hepatic microsomal UDP-glucuronyltransferase activity.*

Keywords: *Scoparone, liver weight, malondialdehyde, serum alanine and aspartate transaminases, UDP-glucuronyltransferase.*

INTRODUCTION

Scoparone is a coumarin derivative and is biologically active component of Artemesiae capillaris flos(Masaki *et al.*, 1976; Joji *et al.*, 1982). It is well known to have many medicinal properties, such as hypotensive action, choleretic and antiinflammatory properties(Thakur *et al.*, 1978; Zutshi *et al.*, 1978; Joji *et al.*, 1982). We have previously reported that scoparone induced the activity of hepatic microsomal UDP-glucuronyltransferase which plays an essential role in the conjugative elimination of toxic substances (Huh *et al.*, 1987).

Acetaminophen is used increasingly as a substitute for aspirin for its analgesic and antipyretic properties(Walker *et al.*, 1985). Unlike aspirin, however, it has only minimal gastrointestinal side effects and does not cause methemoglobinemia as does its analog, phenacetin(Raheja *et al.*, 1982). Acetaminophen, although a safe drug in clinical use,

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produces severe liver damage when overdosed(Fischer *et al.*, 1981; Wendel *et al.*, 1986). In addition, acetaminophen is metabolized in the liver predominantly to glucuronic acid and sulfate conjugates of the parent drug(Abernethy *et al.*, 1983; Poulsen *et al.*, 1985). In the present work, we have studied the protective effect of scoparone against the hepatic necrosis caused by acetaminophen as the model chemical for induction of hepatic lesions.

MATERIALS AND METHODS

Materials

Acetaminophen, uridine 5′-diphosphoglucuronic acid(UDPGA) and bovine serum albumin(BSA) were purchased from Sigma Chemical Company, scoparone from Aldrich Chemical Co., Na salt of thiobarbituric acid and p-nitrophenol from Nakarai Chemical Co., and olive oil from Fluka Chemical Co. All other reagents were of reagent grade commercially available.

Animals

Male ICR-mice weighing 20 to 25g were used for these experiments. They were divided into 4 groups of 6 mice each. Mice of group 1 received olive oil intraperitoneally(ip.) daily for 5 days; mice of group 2 received scoparone(5mg/kg in olive oil) ip. daily for 5 days; those of group 3 received the olive oil as in group 1 but were injected with acetaminophen (400mg/kg) ip. after the last dose of olive oil; those of group 4 received the scoparone as in group 2 but were injected with acetaminophen as group 3. Mice were fasted overnight prior to decapitation.

Enzymatic determinations

Serum transaminase(ALT, AST) activities were measured by the method of Reitman and Frankel(1957) using a commercial kit. Hepatic microsomal UDP-glucuronyltransferase activity was measured by the method of Reinke *et al*(1986) with p-nitrophenol and UDPGA as substrate. In brief, its enzyme activity was determined by measuring the amounts of decreased p-nitrophenol in n moles/mg protein/min (Morrison *et al.*, 1984). Under the assay conditions used, the initial rates of p-nitrophenol disappearance demonstrated linear function with time and protein concentration.

Chemical determinations

Lipid peroxidation of liver tissue was followed by measuring the formation of malondialdehyde with thiobarbituric acid according to the method of Ohkawa *et al*(1979). Acetaminophen in serum was determined after removal of protein using a 10% trichloroacetic acid by the method of Glynn and Kendal(1975). Acetaminophen level was expressed as mg/100m*l* of serum. Protein was determined by the method of Lowry *et al*(1951) using bovine serum albumin(BSA) as the standard. Student's t-test was used to establish significant differences of mean values between the control and treated groups.

RESULTS

Effect of scoparone on body and liver weights in the acetaminophen-treated mice.

Scoparone treatment at 5mg per kg per day for 5 days(ip.) did not influence mouse body weight, nor did it produce significant changes in liver weight and liver to body weight ratio when compared to the olive oil-treated controls(Table 1). However, when acetaminophen was injected at 400mg/kg, significant increases of liver weight and liver to body weight ratio were observed. Pretreatment of scoparone before the acetaminophen injection, however, maintained the liver weight and the liver to body weight ratio at control levels.

Effect of scoparone on serum transaminase activities in the acetaminophen-treated mice.

Blood samples for the determination of serum transaminase activities were collected 24hr after the administration of acetaminophen. The results are summarized in Table 2 and it showed that the increase of serum ALT activity was 10.5 fold, and the increase of serum AST was 2.5 fold after the acetaminophen treatment as compared to the control group. However, pretreatment of scoparone before the ace-

Table 1. Effect of scoparone on body and liver weights in acetaminophen -treated mice.

Treatment	Body weight	Liver weight	Liver/Body weight (g/100g body weight)
Control	23.66 ± 0.74	1.335 ± 0.079	5.636 ± 0.290
Scoparone	22.60 ± 1.01	1.301 ± 0.068	5.757 ± 0.261
Acetaminophen	22.69 ± 0.89	1.585 ± 0.065*	6.987 ± 0.245**
Scoparone + Acetaminophen	23.31 ± 1.00	1.347 ± 0.060	5.780 ± 0.217

a; Mice were killed 1 day after acetaminophen treatment(400mg/kg).
b; Values are means ± S.E. of 6 animals. *; $p<0.05$, **; $p<0.01$.

Table 2. Effect of scoparone on the serum alanine and aspartate transaminase (ALT, AST) activities in acetaminophen-treated mice.

Treatment	Transaminase activity(unit/ml of serum)	
	ALT	AST
Control	32.5 ± 8.9	66.5 ± 11.0
Scoparone	34.9 ± 7.0	59.0 ± 14.7
Acetaminophen	338.8 ± 19.7***	165.5 ± 14.7**
Scoparone + Acetaminophen	68.1 ± 7.8*	95.4 ± 12.3

a; The animals were sacrificed 1 day after the acetaminophen treatment (400mg/kg). b; Values are means ± S.E. of 6 animals. *; $p<0.05$, **; $p<0.01$, ***; $p<0.001$.

taminophen injection has markedly improved the status of serum transaminase activities when compared to the acetaminophen-treated group. Scoparone administration by itself had no effect the transaminase levels.

Effect of scoparone on the content of hepatic lipid peroxide in the acetaminophen-treated mice.

Fig. 1 shows the effect of scoparone on the formation of lipid peroxide in acetaminophen-treated mice. Scoparone treatment by itself had not changed the lipid peroxide content when compared to the control. On the other hand, the lipid peroxide level in the acetaminophen-treated group was increased to about 1.4 fold of the control. But the increase in lipid peroxide level in the scoparone-pretreated group was less than that of the group given acetaminophen alone.

Effect of scoparone on the hepatic microsomal UDP-glucuronyltransferase activity in the acetaminophen-treated mice.

When the UDP-glucuronyltransferase activity of liver microsomal fraction was measured using a p-nitrophenol as the acceptor substrate, scoparone treatment produced a significant induction of UDP-glucuronyltransferase (Fig. 2). When acetaminophen was injected to the control mice, the enzyme activity was powerfully decreased. However, in the scoparone-pretreated group, the decreasing effect caused by acetaminophen was markedly reduced.

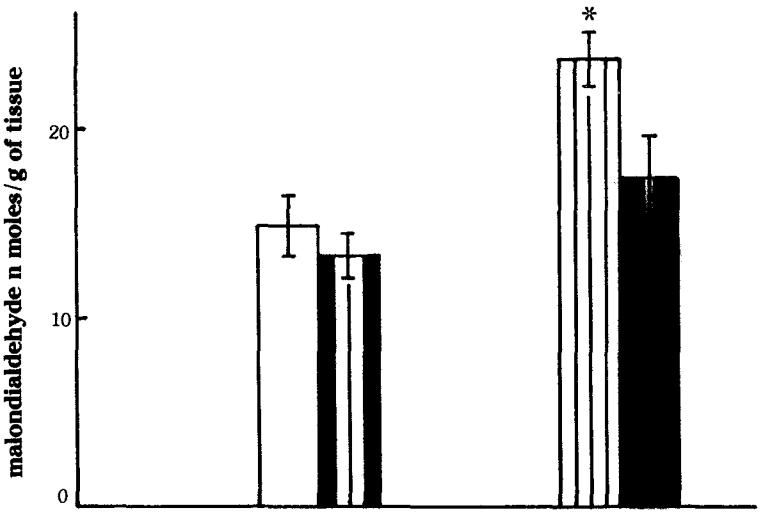

Fig. 1. Effect of scoparone on the formation of lipid peroxide in acetaminophen-treated mouse liver. Mice received scoparone(5mg/kg) ip. daily for 5 days. Mice were killed 1 day after the last dose of scoparone or after the single dose of acetaminophen(400mg/kg). Values are means ± S.E. of 6 animals. □; control, ▮▮; scoparone, ▯▯; acetaminophen, ■; scoparone + acetaminophen. *; $p<0.01$.

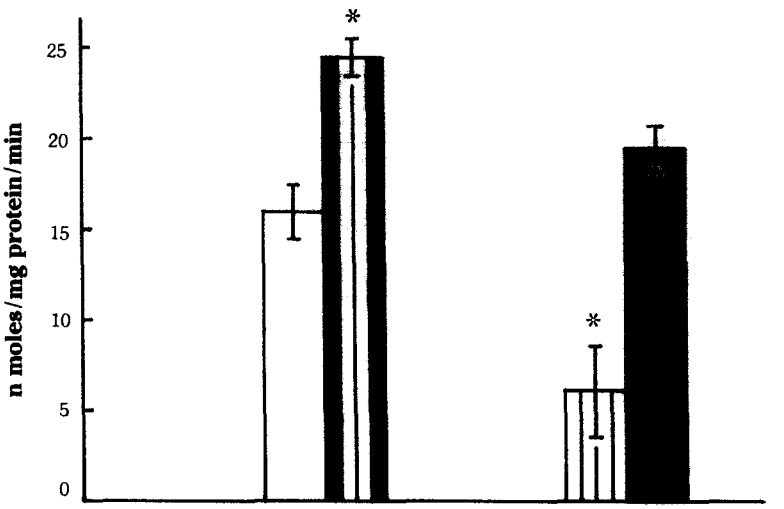

Fig. 2. Effect of scoparone on the hepatic microsomal p-nitrophenol UDP-glucuronyltransferase activity in mice.
The other conditions are the same as described in Fig. 1. *; $p<0.01$.

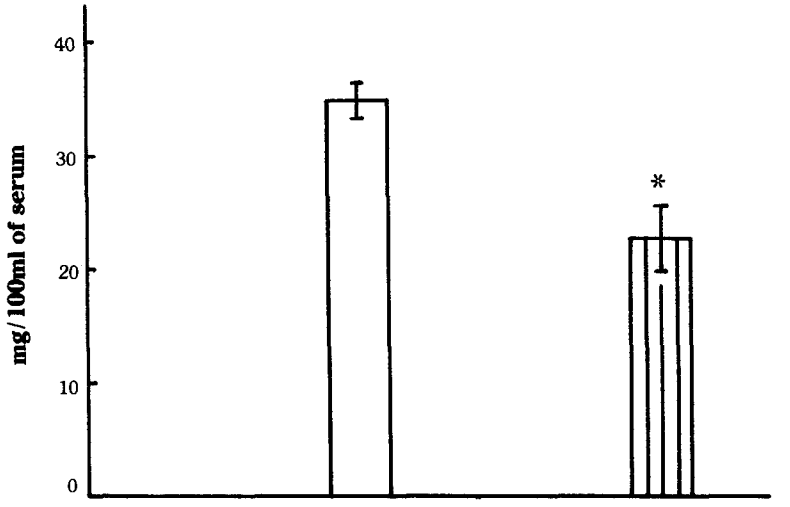

Fig. 3. Effect of scoparone on the serum acetaminophen level in acetaminophen-treated mice.
The animals were killed 60 min after administration of the acetaminophen. The other conditions are the same as described in Fig. 1. [ ]; acetaminophen, [|||]; scoparone + acetaminophen. *; $p<0.01$.

Effect of scoparone on the serum level of acetaminophen in mice.

Blood samples were collected for the determination of serum acetaminophen level 60min after the administration of acetaminophen. The effect of scoparone on the serum acetaminophen level is shown in Fig. 3. Scoparone pretreatment markedly decreased the serum level of acetaminophen(23.5 ± 2.8mg/100ml) when compared

to the acetaminophen-treated group(35.3 ± 1.4mg/100ml).

DISCUSSION

The results of the present study have demonstrated that scoparone, injected ip. into mice for 5 days at a dose of 5mg per kg per day, prevented the acetaminophen-induced hepatotoxicity. Scoparone pretreatment before the acetaminophen injection has blocked subsequent increases in liver weight, demonstrating that the dose of scoparone was optimal and effective for protection against the acetaminophen-induced hepatotoxicity. Furthermore, serum transaminase levels were increased many fold in the acetaminophen group while no significant rise was observed in the scoparone-pretreated group. These results indicated that biological changes caused by acetaminophen is prevented by the administration of scoparone. It was also observed that scoparone protects against acetaminophen-induced centrilobular congestion(data not shown).

The formation of lipid peroxide by acetaminophen was diminished by the pretreatment of scoparone given for 5 days. *In vivo*, as well as in the isolated perfused mouse liver, the acetaminophen was shown to increase lipid peroxidation in a dose-dependent manner(Reiter *et al.*, 1983). It is widely accepted that biological membrane damage was represented by lipid peroxide content(Tan *et al.*, 1984). The decrease in lipid peroxide found in the scoparone-pretreated mice is considered to have resulted from stimulation of acetaminophen conjugation. Our previous experiments with scoparone-treated mice show that hepatic microsomal UDP-glucuronyltransferase activity was gradually increased in correspondence with an increase of the dose, the characteristics of the increase in the enzyme activity may result from a change in the quantity of enzyme protein(Huh *et al.*, 1987). Decreasing effect of UDP-glucuronyltransferase activity caused by the acetaminophen administration was blocked by scoparone pretreatment. As a large percentage of the administered dose of acetaminophen is predominantly metabolized by conjugation with glucuronic acid(Price and Jollow, 1982), we also measured the acetaminophen level in serum. It was observed that scoparone pretreatment has significantly decreased the serum acetaminophen level. These results suggested that induction of UDP-glucuronyltransferase by scoparone pretreatment may have influenced the serum acetaminophen level as a result of an increased capacity to metabolize the acetaminophen to the nontoxic glucuronide. From our results, it is supposed that scoparone pretreatment shows a protective effect against the liver injuries which were induced by acetaminophen.

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