Arch. Pharm. Res. **13**(1), 51-54 (1990)

Effect of Scoparone on the Hepatic Sulfotransferase Activity in Mice

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(Received December 15, 1989)

Abstract□Effect of scoparone (6,7-dimethoxycoumarin) on the hepatic cytosolic sulfotransferase activity was investigated. After treatment with scoparone, hepatic cytosolic sulfotransferase activity was increased with dose and time-dependent manner as compared to control. The V_{max} value (control = 1.33 n moles/mg protein/min, scoparone = 2.39 n moles/mg protein/min) without affecting the K_m value for p-nitrophenol was increased by the scoparone treatment. Whereas, the hepatic cytosolic sulfotransferase was not changed by the addition of scoparone *in vitro*, and was strongly inhibited by the addition of metabolites of scoparone. The results obtained suggest that the characteristics of increase in the enzyme activity may include induction of enzyme proteins, and may be due to the metabolites of scoparone.

Keywords□Sulfotransferase, p-nitrophenol, scoparone, coumarin.

Sulfotransferase (EC 2.8.2.1) is found in a wide variety of organisms and tissue[1,2]. In mammals, a wide range of xenobiotics (drugs, carcinogens and antibiotics) as well as endogenous compounds (steroids and catecholamines) are metabolized by conjugation with sulfate which is the pathway of phase II of the detoxication process[3-5]. The conjugation of a compound with sulfate renders it more water soluble and hence readily excretable in urine or bile[6]. However, effect of scoparone on the hepatic sulfotransferase activity has not been elucidated yet. Scoparone is a coumarin derivative and is biologically active component of *Artemesiae capillaris flos.*[7,8]. We have previously reported that scoparone induced the activity of hepatic microsomal UDP-glucuronyltransferase[9] which plays an important role in the conjugative elimination of toxic substances.

In the present report, it was undertaken for further investigation of scoparone on the hepatic cytosolic sulfotransferase activity in mice.

MATERIALS AND METHODS

Materials

ATP and bovine serum albumin were purchased from Sigma Chemical Co., p-nitrophenol from Nakarai Chemical Co., scoparone from Aldrich Chemical Co.. All other reagents were of reagent grade and obtained from commercial sources.

Treatment of animals

Male ICR-mice weighing 20 to 25g were used for these experiments. They were divided into 4 groups of 6 mice each. One group, the control, received olive oil intraperitoneally. The other groups received scoparone (2.5, 5.0 and 10 mg/kg in olive oil) intraperitoneally once daily for 5 days. Mice were allowed free access to food and water but deprived of food for 24 hr prior to sacrifice.

Preparation of cytosolic fractions

Animals were sacrificed by exsanguination from inferior vena cava. After perfused with ice-cold 0.15 M NaCl solution, livers were rapidly removed and homogenized in 4 volumes of 0.25 M Tris/HCl buffer (pH 7.4). The homogenates were centrifuged at 10,000xg for 20 min and the pellets discarded. The supernatants were recentrifuged at 105,000xg for 60 min and resultant supernatants were used as the cytosolic fractions.

Enzyme assay

Hepatic cytosolic sulfotransferase activity was measured by the method of Dawson *et al.*,[10]. In brief, the reaction mixture consisted of 0.25 M Tris/HCl buffer (pH 7.4) containing 2 mM K_2SO_4, 0.01 mM $MgCl_2$, 5 mM ATP, 0.25 mM p-

nitrophenol and cytosolic fraction. The reaction was terminated by heating in a boiling-water bath for 2 min, followed by cooling in ice-water. In assay for the sulfotransferase activity towards p-nitrophenol, 2.0 m*l* of 0.2 M glycine buffer (pH 10.4) was subsequently added to the reaction mixture, centrifuged and substrate disappearance was measured at 400 nm. Under the assay conditions used, the initial rates of p-nitrophenol disappearance demonstrated linear function with time and protein concentration. Protein was determined by the method of Lowry *et al.*, using bovine serum albumin as standard[11].

RESULTS

Dose response of scoparone on the hepatic cytosolic sulfotransferase activity

Hepatic cytosolic sulfotransferase activity by the scoparone treatment is shown in Fig. 1. In control mouse liver, cytosolic sulfotransferase activity was 1.20 n moles/mg protein/min. Treatment of mouse with scoparone caused about a 1.5 to 2.0 fold increment of cytosolic sulfotransferase activity.

Time course of scoparone on the hepatic cytosolic sulfotransferase activity

Table I. shows the time course of scoparone on the hepatic cytosolic sulfotransferase activity. In the hepatic cytosolic fraction of scoparone-treated mice, the enzyme activity was increased with time-dependent manner as compared to the control. The enzyme activity was significantly elevated as compared to the control group when scoparone (5 and 7 day) was injected to mice.

Effect of scoparone on the kinetic properties of the hepatic cytosolic sulfotransferase activity

Fig. 2. shows the double reciprocal plots of sulfotransferase activity versus p-nitrophenol concentration. The V_{max} value (control = 1.33 n moles/mg protein/min, scoparone = 2.39 n moles/mg protein /min) was increased about 1.9 fold by the treatment of scoparone. Whereas, the K_m value was not changed in the scoparone-treated mice, compared to

Table I. Change of the hepatic cytosolic p-nitrophenol sulfotransferase activity in mouse after scheduled-administration of scoparone.

Day	Sulfotransferase activity (n moles/mg protein/min)
0	1.201 ± 0.079
1	1.254 ± 0.132
3	1.530 ± 0.174
5	2.183 ± 0.216*
7	2.483 ± 0.297*

Mice were injected i.p. daily with scoparone (5 mg/kg) for 1, 3, 5 or 7 days, and killed 24 hr after the last injection. Values are Mean ± S.E. for 6 animals. Significantly different from control.
*$p < 0.01$

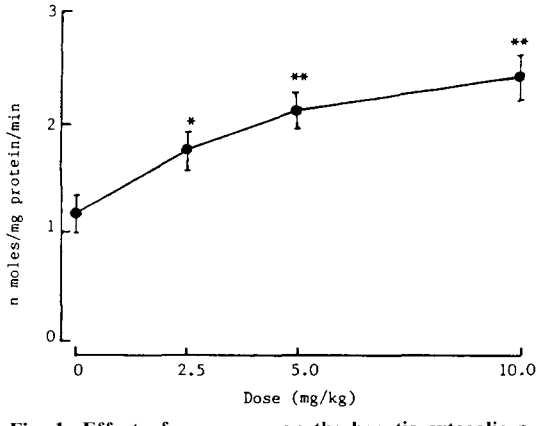

Fig. 1. Effect of scoparone on the hepatic cytosolic p-nitrophenol sulfotransferase activity in mice.
Mice were injected scoparone i.p. daily for 5 days, and killed 24 hr after the last dose. Values are Means ± S.E. for 6 animals. Significantly different from control. *; $p < 0.05$, **; $p < 0.01$.

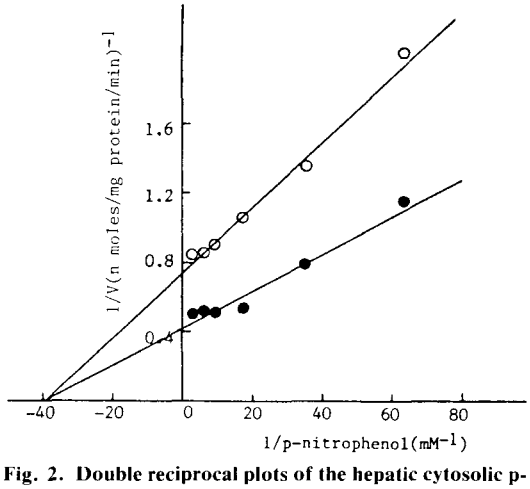

Fig. 2. Double reciprocal plots of the hepatic cytosolic p-nitrophenol sulfotransferase activity as a function of p-nitrophenol at fixed level of ATP (5 mM).
Mice received scoparone (5 mg/kg) i.p. daily for 5 days. Data points represent means of 3 experiments. Control; -○-, Scoparone; -●-

control group.

Changes on the hepatic cytosolic sulfotransferase activity in various concentration of scoparone in vitro.

The sulfotransferase activity was measured at various concentration of scoparone *in vitro* (Fig. 3.). The hepatic cytosolic sulfotransferase activity in the presence of scoparone (1.0 to 100 mM) was affected in this study.

Effect of coumarin derivatives on the hepatic cytosolic sulfotransferase activity in vitro.

Table II shows effect of coumarin derivatives on the sulfotransferase activity *in vitro*. As shown in Table II, 4-hydroxycoumarin and coumarin were not changed the hepatic cytosolic sulfotransferase activity. On the other hand, addition of the other coumarin derivatives strongly inhibited the enzyme activity. In particular, scopoletin (7-hydroxy-6-methoxy coumarin) strikingly inhibited the enzyme activity.

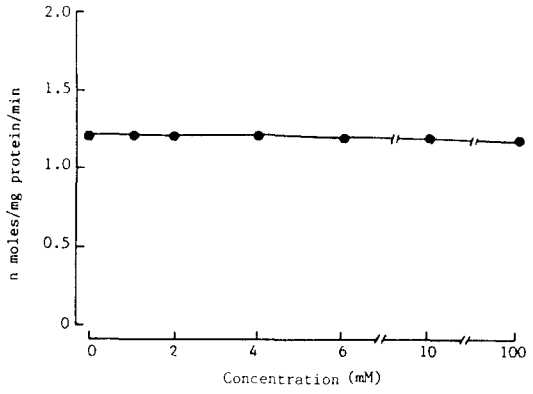

Fig. 3. Changes on the hepatic cytosolic p-nitrophenol sulfotransferase activity in various concentration of scoparone *in vitro*.

Values are means for 4 separate experiments.

Table II. Effect of coumarin derivatives on the hepatic cytosolic sulfotransferase activity *in vitro*.

Coumarin derivatives (concentration)	Sulfotransferase activity (n moles/mg protein/min)
None (control)	1.201
Scopoletin (3 mM)	0.336
4-Methylumbelliferone (3 mM)	0.497
Umbelliferone (3 mM)	0.526
Esculetin (3 mM)	0.734
Dicumarol (3 mM)	0.746
Warfarin (3 mM)	0.872
4-Hydroxycoumarin (3 mM)	1.173
Coumarin (10 mM)	1.218
Scoparone (10 mM)	1.230

Values are means for 3 separate experiments.

DISCUSSION

In general, sulfation of a xenobiotic will render it less toxic, more water soluble and thus more readily excreted[12, 13]. The present study was investigated the effect of scoparone on the sulfotransferase activity in mouse liver. When scoparone was given for 5 days, liver cytosolic sulfotransferase activity was significantly enhanced. The increment of sulfotransferase activity can change either due to catalytic activation of existing enzyme. To differenciate between these possibilities, we determined the kinetic properties of sulfotransferase. V_{max} value without afffecting the K_m value for p-nitrophenol was increased with scoparone treatment compared to control group. These increasing effects indicated that scoparone treatment might result from a change in the quantity of enzyme proteins, rather than activation of enzyme activities. Whereas, sulfotransferase activity was not changed by the addition of scoparone *in vitro*. In addition, this result suggested that scoparone was unlikely to have arisen directly sulfotransferase activity as well as to affect as substrate. As mentioned above, it is well known that scoparone is a coumarin derivative, and is transformed by demethylation in body[14]. Therefore, we also examined the effect of coumarin derivatives on the sulfotransferase activity. The metabolites (scopoletin, esculetin and 6,7-dihydroxycoumarin) of scoparone[14] was strongly inhibited the enzyme activity *in vitro*. This result indicated that metabolites rather than scoparone may affect the induction of sulfotransferase activity in body, as phenobarbital[15]. From the above discussion, it may be concluded that scoparone would regulate the hepatic cytosolic sulfotransferase activity to prevent the toxic effect of xenobiotics. But more active researches in this field are needed.

ACKNOWLEDGEMENT

This research was supported by a grant from the Korean Science and Engineering Foundation in 1989 (*891-0408-056-1).

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