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Effect of Scoparone on the Hepatic Microsomal UDPglucuronyltransferase Activity in Mice

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Abstract□The effect of scoparone on UDPglucuronyltransferase in mouse hepatic microsomes was studied. After treatment with scoparone, hepatic microsomal UDPglucuronyltransferase activity was increased with dose-dependent manner as compared to control. The V_{max} value (control = 23.2 n moles/mg protein/min, scoparone = 31.2 n moles/mg protein/min) without affecting the K_m value (414 μM) for p-nitrophenol was increased by the scoparone treatment, and the pattern of kinetic studies for UDP-glucuronic acid was also similar to those of p-nitrophenol. Whereas, the hepatic microsomal UDPglucuronyltransferase was not changed by the addition of scoparone in vitro. The results obtained suggest that the characteristics of increase in the enzyme activity may include induction of enzyme proteins.

Keywords□Scoparone, UDPglucuronyltransferase, UDP-glucuronic acid, *p*-Nitrophenol.

Detoxication by conjugative pathways is a known mechanism by which many exogenous and endogenous substances are efficiently removed from the body[1,2]. In mammals a large array of xenobiotics (drugs, carcinogens, mutagens and antibiotics) as well as endogenous compounds (steroids and bilirubin) are metabolized by conjugation with glucuronic acid which is the main pathway of the Phase II (elimination) of the detoxication process[3,4]. The enzyme that catalyzes this reaction is known as UDPglucuronyltransferase (UDPGT, EC 2.4.1.17)[5,6].

It is well known that scoparone is a derivative of coumarin which is biologically active component of Artemesiae capillaris flos[7,8]. Scoparone is known to have medicinal properities[9,10]. In the previous study in our laboratory, it was recognized that scoparone inhibited xanthine oxidase which catalyze the oxidation of many purine and pyrimidine derivatives[11]. However, the action mechanism of scoparone on the detoxication pathway has not been completely elucidated yet. In the present report, it was undertaken for further investigation of scoparone on the hepatic microsomal UDPGT activity in mice.

EXPERIMENTAL METHODS

Materials

Uridine 5'-diphosphoglucuronic acid (UDPGA) and bovine serum albumin were purchased from Sigma Chemical Co., p-nitrophenol from Nakarai Chemical Co., scoparone from Aldrich Chemical Co., and triton X-100 from Waco Pure Chemical Co. All other reagents were of reagent grade commericially available.

Treatment of animals

Male ICR-mice weighing 25g were used for all studies. They divided into 4 groups. 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. All the animals had free access to food and water but deprived of the 16hr prior to sacrifice.

Preparation of hepatic microsomal fraction

The animals were killed by exsanguination from inferior vena cava. The liver was exhaustively perfused with cold 0.15M sodium chloride solution through the portal vein until uniformly pale and

quickly excised. After mincing, the piece of liver was homogenized in 4 vol. of 0.25M sucrose. Each homogenate was centrifuged at 10,000 × g for 20 min. The resulting supernatant was centrifuged at 105,000 × g for 60min and the microsomal fraction (pellet) resuspended in 0.25M sucrose to a final concentration of 10 to 20mg of protein per m*l* and used immediately.

Assay for UDPGT

Hepatic microsomal UDPGT activity was measured by the method of Reinke et al[12]. with p-nitrophenol and UDPGA as substrate. In brief, incubation mixtures consisted of 0.05M potassium phosphate buffer (pH 7.0) containing 3mM UDPGA, 1mM $MgCl_2$, 0.02% bovine serum albumin, 0.05% triton X-100, microsomes and 1mM *p*-nitrophenol. Incubations were terminated by the addition of 0.6N perchloric acid. p-Nitrophenol remaining was determined by diluting 0.5m*l* of the supernatant fraction with 2.0m*l* of 1.6M glycine buffer (pH 10.3) and read absorbance at 436nm ($E_{436} = 7.11 m M^{-1}cm^{-1}$). Enzyme activity defined as decreased p-nitrophenol n moles/mg protein/min[13]. Under the assay conditions used, the initial rates of p-nitrophenol disappearance were linear function of time and protein concentration. Protein was determined by the method of Lowry et al. using bovine serum albumin as standard[14].

RESULTS

In vitro effect of scoparone on the hepatic microsomal UDPGT activity

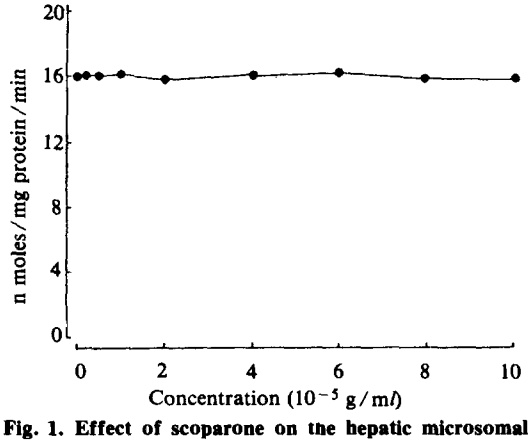

Fig. 1. Effect of scoparone on the hepatic microsomal UDPGT activity in vitro.
The assay procedure was described in the experimental methods. Values are mean of 5 experiments.

Table I. Dose response of scoparone on the hepatic microsomal UDPGT activity in mice

Treatment (mg/kg)	UDPGT activity (n moles/mg protein/min)
0	16.087 ± 1.180
2.5	19.204 ± 1.439
5.0	21.717 ± 1.121*
10.0	24.335 ± 1.867*

Mice received scoparone intraperitoneally once daily for 5 days. The assay procedure was described in the experimental methods. Values are mean ± SE of 5 animals in each group. *; $p<0.01$.

The UDPGT activities were measured at various concentration of scoparone in vitro. The results are shown in Fig. 1. The hepatic microsomal UD-PGT activities in the presence of scoparone (2.5 to 100 ug/m*l*) were not affected in this study.

Dose response of scoparone on the hepatic microsomal UDPGT activity

Hepatic microsomal UDPGT activities by the scoparone treatment are shown ine table I. The enzyme activity of control mice was 16.087 n moles/mg protein/min. In the hepatic microsomal fraction of scoparone-treated mice, the enzyme activities were 19.204, 21.717 and 24.335 n moles/mg protein/min with an increase of dose, respectively. The enzyme activity was significantly elevated as compared to the control group when scoparone (5.0 and 10.0mg/kg was injected to mice.

Effect of scoparone on the kinetic properties of the hepatic microsomal UDPGT

To study the effect of scoparone on the kinetic parameters, initial rates of UDPGT activity were measured as a function of variable concentrations of p-nitrophenol at a fixed concentration of UDPGA and as a function of varying concentrations of UDPGA at a fixed concentration of p-nitrophenol (Fig. 2. and Fig. 3). Apparent K_m value for p-nitrophenol in microsomes in olive oil-treated control mice was 414uM; respective K_m value in scoparone treated mice was also 414uM (Fig. 2). There was no change in the apparent K_m values for p-nitrophenol in the scoparone-treated mice, compared to controls. When plotted on double reciprocal form, the V_{max} value for p-nitrophenol was increased about 1.35 fold by the treatment of scoparone. Moreover the V_{max} value (control; 23.15 n moles/mg protein/min, scoparone; 31.20 n moles/mg protein/min) without affecting the K_m value (286uM

for UDPGA was also increased by the scoparone treatment (Fig. 3).

DISCUSSION

Since scoparone is known to have medicinal properties, the understanding of its interaction in detoxication mechanism is essential for a complete description. Moreover, glucuronidation is one of the major conjugation reaction involved in the metabolic conversion of xenobiotics to more water soluble products[15].

Serum transaminase (ALT, AST) activities were not changed by the treatment of various concentrations of scoparone (data not shown). UDPGT activity was not changed by the addition of scoparone in vitro. In addition, this result suggested that scoparone was unlikely to have arisen directly UDPGT activity (Fig. 1). Our present experiments with scoparone-treated mice show that UDPGT activity was gradually increased in correspondence with an increase of dose. As mentioned above, the hepatic microsomal UDPGT is intracellular membrane proteins which catalyze the transfer of glucuronic acid from UDPGA to a lipophilic acceptor[16]. Thus, process of glucuronidation is of major importance in the detoxication and elimination of numerous foreign chemicals. The increment of UDPGT activity can change either due to an alteration in the quantity of enzyme protein or due to catalytic

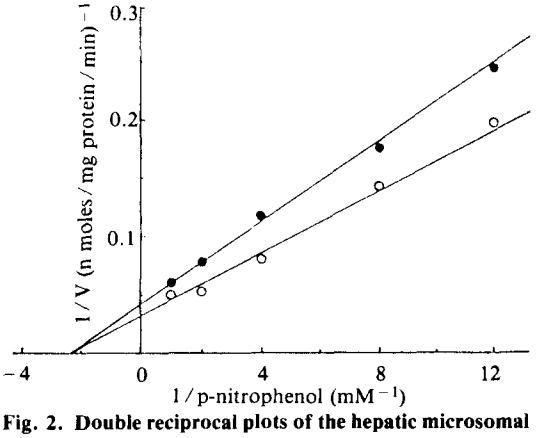

Fig. 2. Double reciprocal plots of the hepatic microsomal UDPGT activity as a function of varying concentrations of *p*-nitrophenol at fixed level of UDPGA (3mM).
Mice received scoparone intraperitoneally once daily for 5 days. The assay procedure was described in the experimental methods. Data points represent the mean of 3 experiments. Control: ●, Scoparone (5mg/kg): o.

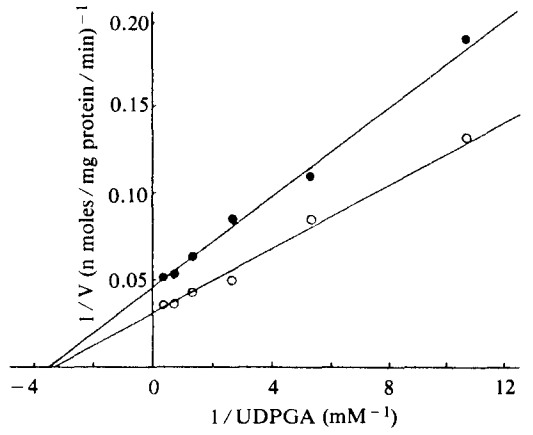

Fig. 3. Double reciprocal plots of the hepatic microsomal UDPGT activity as a function of UDPGA concentration at fixed level of *p*-nitrophenol (1mM).
The other conditions are the same as described in Fig. 2. Control: ●, Scoparone (5mg/kg): o.

activation of existing enzyme. To differentiate between these possibilities, we determined the kinetic properties of UDPGT. All the K_m values of the reaction (Fig. 2, Fig. 3) were not significant change for p-nitrophenol, and UDPGA, respectively. Whereas, all the V_{max} values were increased with scoparone treatment compared to control groups. Thus, as 3-methylcholanthrene[17], the characteristics of the increase in the enzyme activity may result from a change in the quantity of enzyme proteins, rather than activation of enzyme activities due to changes in the lipid environment or other factors. From the above discussion it may be concluded that scoparone would regulate the hepatic microsomal UDPGT activity to prevent the toxic effect of xenobiotics. Further experiments to confirm this postulate are under development.

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