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trans-STILBENE OXIDE: A NEW INDUCER OF RAT LIVER MICROSOMAL UDP-GLUCURONYLTRANSFERASE

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INTRODUCTION

trans-Stilbene oxide has been shown to induce hepatic microsomal mixed-function oxidase and epoxide hydrase activities in the rat [1]. Since many of the products of the above enzyme systems undergo glucuronidation before they can be effectively excreted, and since the enzyme(s) catalyzing this conjugation reaction (UDP-glucuronyltransferase) has been shown to respond to pretreatment with inducers, such as phenobarbital and 3-methylcholanthrene [2-6], 2,3,7,8-tetrachlorodibenzo-p-dioxin [7], some polychlorinated biphenyls [8] as well as natural inducers, e.g. glucocorticoids [9], it was of interest to investigate the effects of this new inducer on hepatic microsomal UDP-glucuronyltransferase activity. The results of these studies are reported herein.

MATERIALS AND METHODS

Male Sprague-Dawley rats (95-100g) were injected i.p. with the desired amount of trans-stilbene oxide (TSO) in 0.5 ml corn oil or with phenobarbital (PB) (100 mg/kg in 0.5 ml saline for four successive days) or with 3-methylcholanthrene (3-MC) (40 mg/kg in 0.5 ml corn oil for four consecutive days). Control animals received the vehicle alone. Rats were sacrificed 24 hr after the last injection. The livers were homogenized in 0.25 M sucrose and microsomes were prepared as described previously [10].

The activity of UDP-glucuronyltransferase (EC 2.4.1.17) was measured using p-nitrophenol (Sigma) as an aglycone. The incubation mixture was essentially that described in reference 11, consisting of 10 mM UDP-D-[U-^{14}C] glucuronic acid (New England Nuclear, radiochemical purity > 99.4%), cold uridine-5'-diphosphoglucuronic acid, ammonium salt, (Sigma), 5 mM p-nitrophenol, 25 mM Tris-HCl buffer, pH 7.4, and 0.3 mg microsomal protein to give a final volume of 0.1 ml. Maximal activation of the microsomal enzyme was

achieved by the addition of digitonin (Sigma) to give a concentration of 0.3% (w/v) in the final assay medium. Incubations were carried out for 5 min at 37° in a Dubnoff metabolic shaker. Controls were prepared as above but lacked p-nitrophenol. Reactions were stopped by the addition of 0.3 ml absolute ethanol and vortexing. The mixture was immediately frozen on solid CO_2, thawed and centrifuged, and the clear supernatant was chromatographed on LQD thin-layer plates (Quantum Laboratories, Kontes, N.J.). Authentic p-nitrophenyl-glucuronide (Sigma) was cochromatographed on the same plates and developed in n-butanol:acetone:acetic acid:5% aqueous ammonia (7:5:3:3). The conjugate was localized under ultraviolet light, the silica gel region was scraped, and the radioactivity was determined in a Beckman LS 250 liquid scintillation counter. Protein was determined by the procedure of Lowry et al. [12].

RESULTS AND DISCUSSION

The effect of treatment of male rats with TSO on hepatic microsomal UDP-glucuronyltransferase activity was studied using p-nitrophenol as the aglycone (Table 1). A dose-dependent increase in the enzyme activity was observed over the control values when TSO was administered to male rats at dosages of 100, 200 and 500 mg/kg body weight on four consecutive days. However, maximal activity was obtained at the 200 mg/kg dose. Pretreatment with TSO at a dosage of 200 mg/kg for six consecutive days gave lower values for UDP-glucuronyltransferase activity than treatment with the same dose for four consecutive days (Table 1). This could be due to toxic side effects of the compound since the increase in liver weight and microsomal protein yield was also less (Table 1).

Data shown in Table 2 indicate that treatment of rats with TSO increased hepatic microsomal UDP-glucuronyltransferase activity in detergent-activated microsomes to a level close to that obtained upon treatment with PB (2.0- and 2.3-fold, respectively). 3-MC treatment, on the other hand, markedly increased the glucuronidation of p-nitrophenol (4.4-fold) as compared to treatment with PB which is in agreement with previously published reports [13].

The possibility that the observed stimulation of p-nitrophenol glucuronidation following administration of TSO to rats was a result of activation of the enzyme in the microsomal membrane was eliminated in this study since the enzyme activity was determined in maximally detergent-activated microsomes [13-15]. In addition, the increase in microsomal protein yield is suggestive of an induction process; however, studies using protein synthesis inhibitors would be of more significance in this respect.

We have previously reported [1] that epoxide hydrase and mixed-function oxidase activities were increased upon treatment with TSO. Maximum increases, however (70% for epoxide hydrase, 58% for aminopyrine N-demethylase, and 66% for aryl hydrocarbon hydroxylase), were observed when rats were pretreated with a 200 mg/kg dose twice for 2 days.

Table 1. Effect of trans-stilbene oxide administration on hepatic microsomal UDP-glucuronyltransferase activity in male rats

Treatment	Liver Weight (g)	Microsomal Protein (mg/kg wet livers)	UDP-glucuronyl-transferase activity[a]
CO	6.54 ± 0.68	20.54 ± 2.11	34.51 ± 3.49
TSO:			
(200 mg/kg x 2)	7.16 ± 0.70 (9)	21.53 ± 2.03 (5)	39.89 ± 4.86 (16)
(100 mg/kg x 4)	7.23 ± 0.64 (11)	21.66 ± 1.94 (5)	45.29 ± 7.42 (31)[b]
(500 mg/kg x 4)	7.83 ± 0.36 (20)	24.68 ± 1.19 (20)	52.78 ± 6.82 (53)[b]
CO	6.25 ± 0.19		29.04 ± 4.70
TSO:			
(200 mg/kg x 4)	7.52 ± 0.27 (20.3)		58.05 ± 8.86 (100)[c]
CO	6.32 ± 0.54	20.88 ± 1.41	36.41 ± 3.12
TSO:			
(200 mg/kg x 6)	7.71 ± 0.42 (22)	24.43 ± 1.14 (17)	53.98 ± 4.89 (48)[c]

Percent increase over control is indicated between parentheses. Values represent mean ± S.D. of 3-5 individual animals. Male rats (95-110g) were injected i.p. daily with TSO as indicated in parentheses for 2, 4 or 6 days and were sacrificed 24 hr after the last injection. Results shown are those of digitonin-activated liver microsomes as described in the text.

CO: corn oil controls; TSO: trans-stilbene oxide.

[a] nmoles/min/mg protein.

[b] $p < 0.05$.

[c] $p < 0.01$.

Table 2. Effect of trans-stilbene oxide pretreatment on male rat hepatic microsomal UDP-glucuronyltransferase activity as compared to phenobarbital and 3-methylcholanthrene pretreatment

Treatment	UDP-glucuronyltransferase activity (nmoles/min/mg protein)
Control	29.04 ± 4.70
trans-stilbene oxide: (200 mg/kg x 4)	58.05 ± 8.86 (100)[a]
Phenobarbital: (100 mg/kg x 4)	66.82 ± 13.20 (130)[a]
3-Methylcholanthrene: (40 mg/kg x 4)	129.02 ± 12.18 (344)[a]

Saline and corn oil values did not differ significantly and were grouped together accordingly. For details refer to Table 1.

[a] $p < 0.01$.

Our results in this study show that induction of UDP-glucuronyltransferase activity follows a time course of response different from that of epoxide hydrase and mixed-function oxidase activities (Table 1). We have also observed an increase in cytochrome P-450 content upon prolonged administration of trans-stilbene oxide (data not shown) that was not evident in the previous pretreatment schedule [1]. The dissociated observed effects of TSO pretreatment on microsomal mixed-function oxidase activities and cytochrome P-450 content are interesting since type I (e.g. PB) and type II (e.g. 3-MC) inducers show their effects on these two parameters concomitantly [16].

The effects of TSO administration to rats on hepatic microsomal UDP-glucuronyltransferase could be utilized in studies concerning multiplicity of this enzyme. The use of different inducers and different substrates in different species and organs has been a valuable tool to study the properties of various enzymes and their relationship to other enzymic pathways.

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