Increased Activity of Hepatic UDP-Glucose Dehydrogenase after Treatment of Rats with *trans*-Stilbene Oxide

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Stilbene and its derivatives occur naturally and are also used in industrial chemical processes. It has been reported that stilbene is metabolized to a number of different products in mammalian systems[1] (see Fig. 1).

Our laboratory[2] and others[3,4] have shown that *trans*-stilbene oxide is a new type of inducer of drug-metabolizing systems. Treatment of rats with this xenobiotic results in a 620 % increase in hepatic epoxide hydratase activity, a 200–300 % increase in cytosolic hepatic glutathione *S*-transferase activity, and a 120 % increase in the cytochrome P-450 content of rat liver microsomes.[2] Thus, *trans*-stilbene oxide induces "phase II" enzymes to a much greater extent than the "phase I" cytochrome P-450 system; whereas the pattern of response to the classical inducers phenobarbital[5] and methylcholanthrene[6] is exactly the opposite in this respect.

It is also of interest to characterize the effect of treatment with *trans*-stilbene oxide on enzyme pathways which are indirectly involved in the metabolism of xenobiotics. The pentose phosphate pathway, which provides NADPH to the NADPH-cytochrome P-450 reductase, is one example of such a pathway. We have found that *trans*-stilbene oxide increases the activity of glucose-6-phosphate dehydrogenase, the first enzyme in this shunt, to a level 3.4-fold higher than that of controls; while the third enzyme, 6-phosphogluconate dehydrogenase, is increased only about 70 % and enzymes further along in this pathway are not affected et all.[7]

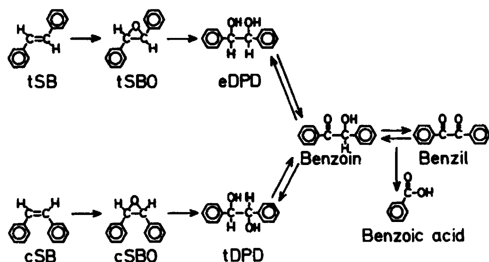

Fig. 1. Metabolic pathway of stilbene. For an explanation of the abbreviations used, see Table 1.

Another enzyme indirectly involved in the metabolism of xenobiotics is the cytosolic UDP-glucose dehydrogenase, which provides UDP-glucuronic acid for the glucuronidation of certain functional groups, including hydroxyl groups. Under certain circumstances the activity of this enzyme may be rate-limiting for glucuronidation and it was therefore of interest to determine the level of this enzyme after induction with *trans*-stilbene oxide.

Male Sprague-Dawley rats weighing 180–200 g were used in these experiments. The animals were injected intraperitoneally once daily with stilbene or a metabolite in 1 ml sunflower oil. The number of injections and the dose are given in the figure and table legends. After starvation overnight the rats were decapitated and the total microsomal and supernatant fractions from liver were prepared in the usual manner.[8] UDP-Glucuronosyl transferase[9] was assayed in the microsomes and UDP-glucose dehydrogenase[10] was assayed in the supernatant using published procedures. In addition, epoxide hydratase and glutathione *S*-transferase activities were determined routinely in order to be certain that induction had been achieved. In every rat which we have treated with *trans*-stilbene oxide (about 100 animals to date) the expected induction of these two enzymes has been observed.

We found that treatment with *trans*-stilbene oxide (400 mg/kg once daily for 5 days) increases microsomal UDP-glucuronosyl transferase activity from 20.3 ± 2.6 to 32.9 ± 7.8 nmol *p*-nitrophenol conjugated/min(mg protein) (means ± standard deviations of 7 rats). Thus, the inducer increases this activity no more than about 60 %, a finding in agreement with an earlier report.[11]

Since there may be several different forms of UDP-glucuronosyl transferase with different substrate specificities, it is possible that measurement with other subtrates might yield other results, a possibility which we are presently investigating.

On the other hand, it is clear from Fig. 2 that treatment of rats with *trans*-stilbene oxide has a more pronounced effect on hepatic UDP-glucose dehydrogenase activity. After 5 days of treatment with the maximal dose (400 mg/kg body weight), a plateau of activity about 2.9-fold higher than the control activity is reached. This time course closely resembles the induction of both epoxide hydratase and glutathione *S*-transferase by *trans*-stilbene oxide.[2] After cessation of treatment hepatic UDP-glucose dehydrogenase activity returns to about 140 % of the control level within 3 days. This return to normal is somewhat faster than that seen with epoxide hydratase and glutathione *S*-transferase.[2]

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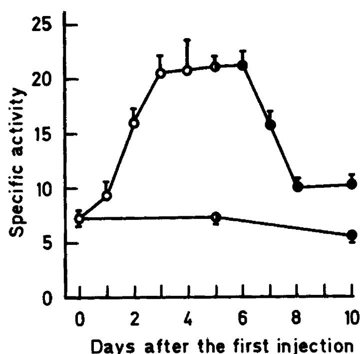

Fig. 2. Time course of the increase in UDP-glucose dehydrogenase activity caused by treatment with *trans*-stilbene oxide (○) and return to control level after cessation of treatment (●). The animals were treated with 400 mg/kg once daily on days 0–4. C = Control, I = Induced.

For the induction 6 animals were used for each time point, while 3 animals were used each day during the return to control levels. The error limits shown are the standard deviations.

Fig. 3 illustrates the response of UDP-glucose dehydrogenase activity to different doses of *trans*-stilbene oxide. This response is approximately linear up to about 300 mg/kg body weight, after which it seems to level off. The only ill-effect of treating rats with the maximal dose (400 mg/kg) is a slight loss of appetite. The dose-response curve for UDP-glucose dehydrogenase resembles those seen for epoxide

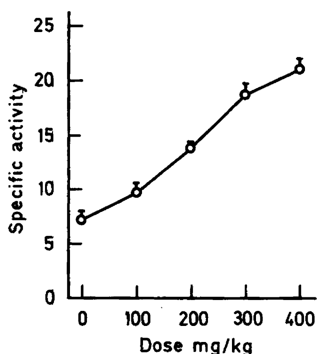

Fig. 3. Response of UDP-glucose dehydrogenase to different doses of *trans*-stilbene oxide. The animals were treated once daily for 5 days before sacrifice. The number of animals used was 16 for 0, 3 for 100, 11 for 200, 6 for 300 and 6 for 400/(mg/kg). The error limits shown are the standard deviations.

Table 1. UDP-Glucose dehydrogenase activity after treatment of rats with metabolites from stilbene.

Derivatives [a]	% of control activity [b]
trans-Stilbene (tSB)	132 ± 36 (6) *
trans-Stilbene oxide (tSBO)	193 ± 37 (11) ***
erythro-1,2-Diphenylethane-1,2-diol (eDPD)	97 ± 23 (3)
cis-Stilbene (cSB)	156 ± 27 (6) ***
cis-Stilbene oxide (cSBO)	143 ± 28 (6) **
threo-1,2-Diphenylethane-1,2-diol (tDPD)	120 ± 15 (3)
Benzoin	182 ± 37 (6) ***
Benzil	191 ± 35 (7) ***
Benzoic acid	81 ± 18 (3)

[a] The structures of the metabolites are shown in Fig. 1. 1 mmol/kg body-weight of each compound was injected *i.p.* once daily for 5 days. [b] The control activity was 7.20 ± 0.88 (16) nmol NAD^+ reduced/min per mg supernatant protein. The values presented are the means and standard deviations for the number of animals given in parenthesis. Statistical differences between means of control and treated animals were determined using Student's *t* test. (* $P<0.05$; ** $P<0.01$; *** $P<0.001$).

hydratase and glutathione *S*-transferase, but cytochrome P-450 appears to be maximally induced with considerably lower doses of *trans*-stilbene oxide.[2]

Table 1 illustrates the effect of treatment of rats with different metabolites of stilbene on liver UDP-glucose dehydrogenase activity. The most effective inducers are *trans*-stilbene oxide, benzoin, and benzil. This is also the case for induction of epoxide hydratase and glutathione *S*-transferase activities by stilbene derivatives.[1,2]

As a control for the possibility that metabolites of stilbene stimulate UDP-glucose dehydrogenase by direct interaction, this activity was measured in the supernatant fraction from the livers of untreated rats in the presence of different metabolites. The metabolites used were those which increase UDP-glucose dehydrogenase actvity upon injection intraperitoneally and the concentration used (*i.e.* 2 mM) was calculated assuming that the total amount of drug injected accumulated in the liver. None of the compounds stimulated UDP-glucose dehydrogenase activity. The maximal effect seen was a 16 % inhibition by *trans*-stilbene oxide and benzil.

It remains to be seen whether the increase in UDP-glucose dehydrogenase activity caused by *trans*-stilbene oxide is a true induction, *i.e.*, reflects

an increase in the amount of enzyme protein due to increased synthesis. Because of the nature of the assay for cytochrome P-450, increases in this component reflect increases in the total amount of holoenzyme. In addition, SDS disc gel electrophoretic patterns suggest that *trans*-stilbene oxide dramatically increases the amount of epoxide hydratase protein.[12] Finally, we have shown with immunological techniques and by purification that *trans*-stilbene oxide increases the amount of glutathione *S*-transferase protein 3–4-fold.[13] Thus, the effect on UDP-glucose dehydrogenase is also expected to be an induction.

The effect of *trans*-stilbene oxide on hepatic UDP-glucose dehydrogenase activity also supports the interesting suggestion that this enzyme may be rate-limiting for glucuronidation under certain circumstances. This possibility is one line of further investigation in our laboratory.

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