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Responses of the Hepatic Enzymes of a Teleost Fish to *trans*-Stilbene Oxide Treatment

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The microsomal mixed function oxidase (MFO) system and epoxide hydrolase are important enzymes in the metabolism of carcinogenic polynuclear aromatic hydrocarbons (JERINA & DALY 1974, WIEBEL et al. 1974, HEIDELBERGER 1975, NEBERT et al. 1975). The epoxide hydrolase may act in a detoxifying system as in the hydrolysis of benzo(a)pyrene 4,5,-oxide, or in an activating system, as in the metabolism of benzo(a)pyrene 7,8-oxide to benzo(a)pyrene 7,8-dihydrodiol, the precursor for the ultimate carcinogen, benzo(a)pyrene 7,8-dihydrodiol-9,10-epoxide (SIMS et al. 1974, THAKKER et al. 1976). Recently, trans-stilbene oxide (TSO) was found to be a selective inducer of epoxide hydrolase activity in the rat liver with no effect on five investigated monooxygenase parameters (SCHMASSMANN et al. 1978, SCHMASSMANN & OESCH 1978). TSO is involved in an important detoxification-activation role of the epoxide hydrolase but not directly in the MFO system. In this study, the effect of TSO on the hepatic electron transport components of the MFO system in a teleost fish, Tilapia aurea was investigated.

MATERIALS AND METHODS

Animals. Fish were transported from ponds to aquaria (100 L capacity) filled with dechlorinated water and were acclimated for 7 days before treatment. Aquaria were aerated and the water was maintained at 18± 1°C. One-year-old fish weighing between 150-200 g were fasted for various days prior to the treatment (by intraperitoneal injection) period, and were not sex differentiated.

Microsome preparations. Livers were removed from animals in each set of experiments and rinsed in 0.15 M KCl solution, and mitochondrial supernatants were prepared as previously described (MELIUS et al. 1980). Microsomal protein preparations were prepared by the calcium aggregation method (PHILLIPS & KHAN 1979).

Enzyme Assays. Protein concentrations were determined by the method of LOWRY et al. (1951) using bovine serum albumin as the standard. NADPH-cytochrome C reductase (YONETANI 1965) and NADH-cytochrome b_5 reductase (SCHELLENBERG & HELLERMAN 1958) were assayed spectrophotometrically at 25°C and pH 7.4. Aminopyrine N-demethylase activity was assayed at 25°C and pH 7.5 (NASH 1953) while the cholinesterase activity was measured colorimetrically at 25°C and pH 8.0 (ELLMAN et al. 1961). Both the hepatic glutamic oxaloacetic transaminase (HGOT) and hepatic glutamic pyruvic transaminase (HGPT) assays were conducted using the Sigma Kitset No. 505 (REITMAN & FRANKEL 1957), measuring the activities at 37°C and pH 7.5. A modified atomic

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absorption method was used for the epoxide hydrolase assay. The product of the enzyme catalyzed reaction contains a 1,2-diol moiety whic reacts with an excess of potassium periodate is preferentially precipated as the lead periodate (TAN et al. 1980). The precipitate was digested in nitric acid followed by atomic absorption analysis of the dissolved lead.

RESULTS AND DISCUSSION

It is well known that cytochrome P_{450} mediates the MFO system in the metabolism of xenobiotics. Perliminary experiments were conducted to determine the cytochrome P_{450} concentrations of TSO-treated fish. The carbon monoxide-bound dithionite-reduced spectra (OMURA & SATO 1964) did not yield any peak at 450 or 420 nm. A single peak at 438 nm was observed and the spectra was similar to that observed in the carp with peak at 432 nm (FUKAMI et al. 1969, CHAMBERS & YARBROUGH 1976). TSO-induced alteration in the conformation the lipoprotein during the treatment period may be responsible for these findings. Instability of the cytochrome P_{450} in the Tilapia or binding alteration of the protein during preparation for analysis could not be ruled out (GUINEY et al. 1980). In Figure 1, the concentration of the cytochrome P_{438} was estimated using the extinction

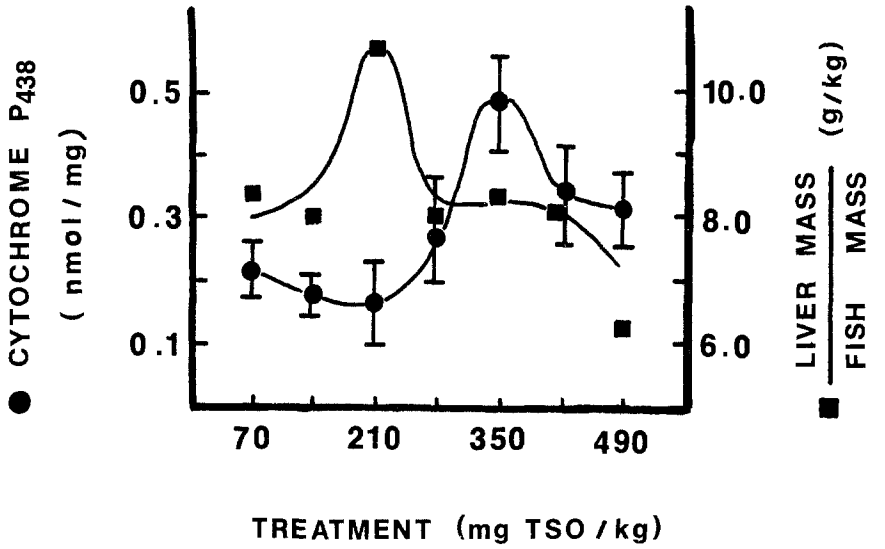

Figure 1. Dose-response curves of hepatic cytochrome P_{438} and liver to body mass ratio. Two fish were used for each treatment and kept in separate aquaria. Livers were taken for assays after a 2-day induction period. Error bars indicated the difference between 2 determinations.

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coefficient (91 cm^{-1} mM^{-1}) for the cytochrome P_{450} (OMURA & SATO 1964). Various dose levels were used for a 2-day period. The

cytochrome P_{438} reached maximum at the level of 350 mg TSO/kg fish mass. Although the cytochrome P_{438} was relatively low and the increase was moderate, it does suggest that the electron transport component is affected by the TSO treatment. The liver to body mass ratio reached a maximum at the 210 mg TSO/kg fish mass. This may not be significant because at the other treatment levels the ratio remained relatively constant. There were also no duplicated data for these ratios.

In Figure 2, a dose of 490 mg TSO/kg fish mass was used in all 12 fish and their livers were taken at various time periods. The

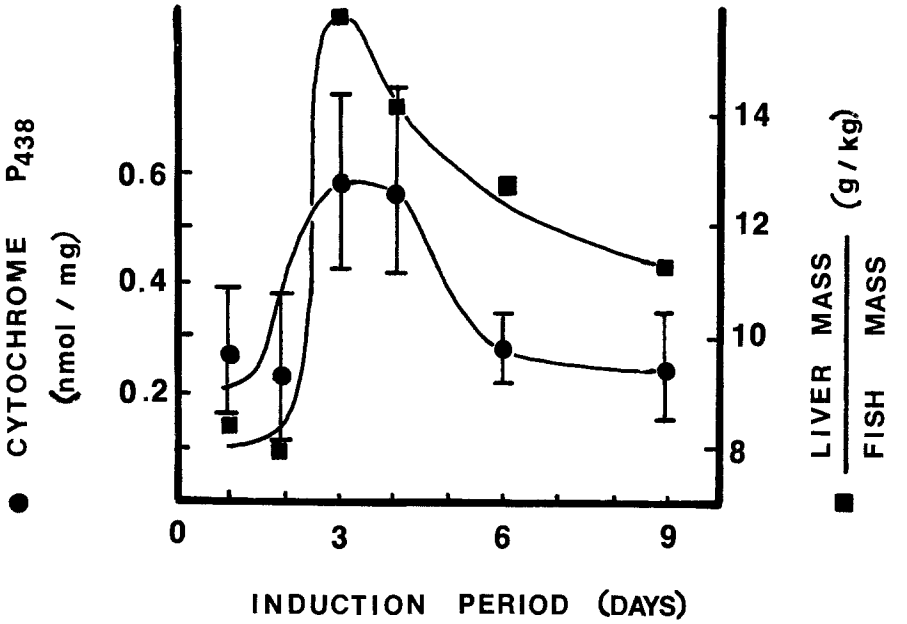

Figure 2. Time response curves of hepatic cytochrome P_{438} and liver to body mass ratio. All the fish were kept together in the same aquarium, and treated with 490 mg TSO/kg fish mass. Livers from two fish were taken at various time intervals from day 0. Error bars indicate the difference between 2 determinations.

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cytochrome P_{438} and the liver mass to fish mass ratio increased rapidly reaching a maximum after 3 days, and then declining gradually. Contrastingly, the treatment of 490 mg/kg body mass in the rat did not produce significant increase in the cytochrome P_{450} content (SCHMASSMANN & OESCH 1978). It is interesting that the increase of liver size appeared to be significant only after the 3rd day, irrespective of whether it was a constant dose of 490 mg TSO/kg or at various dose levels (Figure 2). Increase in fish liver size has been reported to be related to fish exposure to certain chemicals. For example, channel catfish exposed to Aroclor 1254 (HILL et al. 1976), Dover sole exposed to DDT (SHERWOOD &

MEARNS 1977), and black bullheads living in chlorinated sewage ponds (TAN et al. 1981).

Groups of 10 fish treated for 4 days with 200 mg TSO/kg fish mass were studied for their microsomal hepatic enzymes and the electron transport components. In Table 1, the TSO-treated fish showed a 240% induction of the epoxide hydrolase activity when compared to untreated control. At the 490 mg TSO/kg fish mass treatment the fish showed a 300% induction of the enzyme activity (results not shown on Table). This dramatic and almost linear induction of epoxide hydrolase activity in the Tilapia is similar to that reported for the rat (SCHMASSMANN & OESCH 1978). The NADPH cytochrome C reductase was unaffected by the TSO at 200 mg TSO/kg treatment level. The NADH cytochrome b_5 reductase decreased slightly but the aminopyrine N-demethylase increased slightly. These latter 3 electron transport-related hepatic enzymes did not change significantly.

The cholinesterase activity decreased by one half, which is suggestive of impairment of protein synthesis or denaturation of the enzyme. If this is so, then the induction of the liver with TSO treatment (Figures 1 and 2) may not be concomitant with protein synthesis. This observation was also reported in channel catfish exposed to Aroclor 1254 (HILL et al. 1976). The HGPT and HGOT showed increase in activities. The elevated transaminase activities indicated that there was hepatic cellular damage or tissue injury (TIETZ 1976) after 4 days at the 200 mg TSO/kg treatment. Elevated enzyme activities of these two enzymes had been noted in rainbow trout near a sewage plant outflow (WIESER & HINTERLEITNER 1980). The conclusion was that toxic effects had occurred from the sewage outflow.

TSO selectively induced epoxide hydrolase up to 300% at the 490 mg TSO/kg treatment. It did not affect significantly the NADPH cytochrome C reductase, NADH cytochrome b_5 reductase, and the aminopyrine N-demethylase activities but did increase the electron transport component, cytochrome P_{438}. SCHMASSMANN & OESCH (1978) had found that TSO is a selective inducer of epoxide hydrolase activity in the rat liver with no effect on five investigated monooxygenase parameter as we have found also in the Tilapia. It may be concluded that the Tialpia hepatic MFO responded in similar ways to the rat hepatic MFO, and therefore Tialpia have similar metabolic pathways for xenobiotic detoxification as the rat.

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Table 1. Microsomal Hepatic Enzymes and Electron Transport Components of TSO-Treated Tilapia

Hepatic Enzymes	Control	Treated (200 mg TSO/kg Fish Mass
Epoxide hydrolase[1*] (n mole/min/mg)	3.0 ± 0.1	7.3 ± 0.3
NADPH cyt c reductase[1] (nmole/min/mg)	8.26 ± 0.04	8.10 ± 0.08
NADH cyt b_5 reductase[1] (μmole/min/mg)	1.71 ± 0.03	1.43 ± 0.03
Aminopyrine N-demethylase[2] (μmole/min/mg)	6.05 ± 0.19	7.1 ± 0.2
Cholinesterase[2] (μmole/min/mg)	1.04 ± 0.01	0.54 ± 0.01
HGPT (SF units/ml)[3]	360 ± 1	421 ± 4
HGOT (SF units/ml)[3]	174 ± 1	544 ± 4

[1,2,3] are respectively average of 2,4 and 9 replicate experiments of same preparation (10 fish/preparation); mean ± standard error of mean. Treatment period was 4 days. Transaminase activities were recorded as Sigma-Frankel units/ml. *The epoxide hdyrolase activity was 9.2 ± 0.2 nmole/min/mg at the 490 mg TSO/kg treatment.

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