Biochemical Pharmacology, Vol. 36, No. 24, pp. 4355–4359, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

DIFFERENTIAL INDUCTION OF CYTOCHROME P-450 BY THE ENANTIOMERS OF *TRANS*-STILBENE OXIDE

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(*Received* 9 *February* 1987; *accepted* 26 *May* 1987)

Abstract—Optically pure (+)- and (−)-*trans*-stilbene oxide (TSO) enantiomers were administered to immature male Sprague–Dawley rats. (+)-TSO was the more potent inducer of liver microsomal cytochrome P-450-dependent monooxygenases. The greater potency of (+)-TSO may be explained on the basis of stereoselective metabolism since a far greater concentration of TSO was found in liver microsomes of (+)-TSO-treated rats. Furthermore, of the enzymes known to metabolize TSO, cytosolic epoxide hydrolase turned over the (−)-TSO enantiomer at a faster rate, consistent with the greater persistence of the (+)-enantiomer. Although this report is of chiral effects in potency of enzyme induction, stereoselective metabolism (i.e. disposition) rather than inherent structural characteristics (recognition) may be responsible for these effects.

The administration of drugs or other xenobiotics may lead to increases in the activities of various liver enzymes, responsible for the metabolism of foreign compounds. If this increase in activity of a xenobiotic-metabolizing enzyme is due to an increased level of the enzyme, then the process is known as induction and the xenobiotic is called an inducer. Inducers have been traditionally classified either as like PB§ or like MC, based on the cytochrome P-450 isozymes (phase I enzymes) that are increased [1].

Although TSO induces the same cytochrome P-450 isozymes as PB [2, 3], TSO does not fit very well into the above classification since it is somewhat weaker than PB as a cytochrome P-450 inducer while being much more potent than PB as an inducer of microsomal epoxide hydrolase (EC 3.3.2.3) and several conjugating (phase II) enzymes [4–9]. Compounds like TSO are of great interest to the toxicologist since they are able to shift the spectrum of xenobiotic-metabolizing enzyme activities such that conjugating, potentially detoxifying, activities are enhanced. Administration of TSO, for example, leads to a profound shift in the metabolism of benzo[*a*]pyrene which is accompanied by a marked lowering of mutagenic metabolites [10].

The induction process itself is poorly understood, as is the regulation of xenobiotic-metabolizing enzymes in general. A single receptor, responsible for the expression of several xenobiotic-metabolizing enzymes, has been described in the literature. Known as the *Ah* receptor, this receptor avidly binds coplanar xenobiotics like MC or TCDD. PB [11] and TSO,‖ however, are not ligands of the *Ah* receptor, and all attempts to find a receptor for PB and related compounds have been unsuccessful. The mechanism by which PB and TSO induce xenobiotic-metabolizing enzymes is unknown. In the present study we describe the effects of the optically-active TSO enantiomers, the use of which allows us to determine chiral effects in recognition or disposition, both of which can directly influence the induction of xenobiotic-metabolizing enzymes.

MATERIALS AND METHODS

Chemicals. 4-Dimethylaminoantipyrine, MC, and TSO (further purified by ethanol recrystallization) were obtained from the Aldrich Chemical Co. (Milwaukee, WI), while aldrin (Serva GmbH, Heidelberg, FRG), cytochrome *c* (type III from horse heart, Sigma Chemie GmbH, Deisenhofen, FRG), PB (Merck-Schuchardt, Hohenbrunn/München, FRG) and [^{3}H]benzo[*a*]pyrene (New England Nuclear, Dreieich, FRG) were purchased from the sources indicated. [^{3}H]Benzo[*a*]pyrene-4,5-oxide [12] and [^{3}H]TSO [13] were synthesized as described.

Separation and purification of TSO enantiomers. Racemic TSO (Aldrich) was completely resolved into (+)-(1*R*,2*R*)- and (−)-(1*S*,2*S*)-enantiomers by HPLC on a semipreparative chiral column (30 × 2 (i.d.) cm) packed with cellulose triphenylcarbamate

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§ Abbreviations: PB, phenobarbital; MC, 3-methylcholanthrene; TSO, *trans*-stilbene oxide; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

‖ Dr. A. B. Okey, personal communication, cited with permission.

[14]. The separation was accomplished on a Jasco Trirotar-II chromatograph equipped with ultraviolet (Uvidec-100-III) and polarimeter (DIP-181C) detectors using hexane–2-propanol (90:10, 10 ml/min) as an eluant at 25°. After recrystallization from ethanol–water (7:3), each enantiomer showed a melting point of 69.5–70.5° and was optically pure as determined by analytical HPLC with the chiral column described below.

The enantiomers of [^{3}H]TSO were separated by HPLC using an analytical Chiralpak OT column at 10° and methanol as eluent, exactly as described [15]. The specific activity of each [^{3}H]TSO enantiomer was determined by liquid scintillation counting and by u.v. absorbance at 228 nm ($\varepsilon = 20{,}800\ M^{-1}\ cm^{-1}$ [16]).

Animal treatment. Male weanling Sprague–Dawley rats, obtained from Süddeutsche Versuchstierfarm, Tuttlingen, were acclimatized in a temperature-controlled room (20–22°) equipped with a 12-hr light–dark cycle and were housed in plastic cages and provided with a standard rat chow (Altromin 1324, Altromin GmbH, Lage, FRG). At 4 weeks of age (approximately 100 g body weight), rats received daily i.p. injections of either PB (400 μmol/kg) in saline, MC (100 μmol/kg) in corn oil, (±)-, (+)- or (−)-TSO (400 mg/kg or 200 mg/kg) in corn oil (10 ml/kg) or corn oil alone for 3 consecutive days. At the time of the last injection, the rats were taken off feed and were fasted until they were killed 24 hr later.

Preparation of liver cytosolic and microsomal fractions and measurement of drug-metabolizing enzyme activities. After each rat was killed by cervical dislocation, the peritoneal cavity was immediately opened and the liver was perfused *in situ* with ice-cold 0.25 M sucrose containing 0.1 mM EDTA, pH 7.4. The liver was removed, washed, minced and homogenized in the sucrose solution described above, and 100,000 *g* microsomal and cytosolic fractions were obtained by differential centrifugation as described [17]. Microsomal suspensions and cytosols were adjusted to 6 and 10 mg protein/ml respectively. Samples were then frozen in liquid nitrogen and stored at −70° until used for the determination of enzyme activities.

Our methods for measuring cytochrome P-450 content, aminopyrine *N*-demethylase, benzo[*a*]pyrene hydroxylase, and NADPH–cytochrome *c* reductase were carried out as previously described [18]. The rate of aldrin epoxidation was measured essentially as published [19], while microsomal epoxide hydrolase activity was determined using [^{3}H]benzo[*a*]pyrene-4,5-oxide [20] with the modifications that the final incubation volume was 200 μl and the reaction was started by the addition of 10 μl of an acetonitrile solution containing approximately 1 nmol benzo[*a*]pyrene 4,5-oxide/μl. Racemic, (+)- and (−)-[^{3}H]TSO were used as substrates for cytosolic epoxide hydrolase. The published method [21] was modified as described [22].

Statistics. Statistics were performed using Dunnett's test for multiple comparisons with a control [23].

Extraction and quantification of TSO. Cyclohexanone was found to be a useful solvent for the selective extraction of TSO. Liver microsomal suspensions (1.5 ml) were extracted with 2 × 1.0 ml cyclohexanone (Merck, Darmstadt, FRG). The cyclohexanone fractions were combined and evaporated to a volume of 500 μl. An aliquot of the latter was directly injected, without further cleanup, and the TSO concentration was determined by packed column (6 feet, 2 mm i.d., 3% OV-17 on 80–100 mesh chromosorb W-HP) gas chromatography with flame ionization detection. Extraction of microsomes spiked with racemic TSO yielded a recovery of 88.6 ± 0.7%.

RESULTS

A detailed examination of the inducing properties of (±)-TSO, (+)-TSO and (−)-TSO is presented in Table 1. Included for comparison are the effects of the classical inducers, PB and MC. Like PB, (±)-TSO, at a total dose of 1200 mg/kg, showed statistically significant increases in cytochrome P-450 content and in the cytochrome P-450-dependent monooxygenase activities, aminopyrine *N*-demethylase and aldrin epoxidase, and in NADPH–cytochrome *c* reductase and microsomal epoxide hydrolase activities. (+)-TSO and (−)-TSO at 1200 mg/kg also elicited statistically significant increases in these variables, except that the increase in aldrin epoxidase activity following (−)-TSO administration was not statistically significant. Generally, a dose-dependency was observed with the higher dose, in each case eliciting the greater effect. Surprisingly, however, (+)-TSO was a more potent inducer of each cytochrome P-450-dependent enzyme activity than was (−)-TSO when corresponding doses were compared. The activities of cytochrome *c* reductase and microsomal epoxide hydrolase were equally increased by the TSO enantiomers. None of the treatments resulted in a statistically significant increase in cytosolic epoxide hydrolase nor, with the exception of MC, in benzo[*a*]pyrene hydroxylase.

The differing potencies of the TSO enantiomers as inducers may be related to differing pharmacokinetic properties or alternatively to differences inherent in the structures of this enantiomer pair. The former possibility was checked by extracting and measuring the residual of TSO present in the prepared microsomes. The results, presented in Table 2, show that the concentration of TSO in microsomes purified from rats treated with (−)-TSO had fallen below the detection limit of 9.3 nmol TSO/ml microsomes, whereas the concentrations of TSO in microsomes from rats treated with (+)-TSO or (±)-TSO were easily measured. These results indicate that (−)-TSO is metabolized more rapidly than (+)-TSO or that (+)-TSO is preferentially bound to microsomal proteins in the liver. There is, however, no evidence for the latter. Recovery of about 90% of the racemate from spiked microsomes (cf. Materials and Methods) indicates that there are no remarkable differences in binding of the TSO enantiomers to liver microsomal protein.

One of several liver enzymes which turn over TSO is cytosolic epoxide hydrolase. Figure 1 shows that indeed this enzyme metabolized (−)-[^{3}H]TSO faster than (+)-[^{3}H]TSO.

Table 1. Effects of phenobarbital (PB), 3-methylcholanthrene (MC) and (±)-, (+)- and (−)-*trans*-stilbene oxide (TSO) on drug-metabolizing enzyme activities in the immature male Sprague–Dawley rat

Treatment group (total dose)	Cytochrome P-450 (nmol cyto. P-450/mg microsomal protein)	Aminopyrine *N*-demethylase (nmol/mg protein/min)	Aldrin epoxidase (nmol/mg protein/min)	Benzo[*a*]pyrene hydroxylase (pmol/mg protein/min)	NADPH–cytochrome *c* reductase (nmol/mg protein/min)	Microsomal epoxide hydrolase (nmol/mg protein/min)	Cytosolic epoxide hydrolase (pmol/mg protein/min)
Control (N = 7)	0.41 ± 0.08	3.95 ± 0.38	3.5 ± 0.8	101 ± 19	42.6 ± 3.9	5.8 ± 1.4	21 ± 9
PB, 1200 μmol/kg (N = 7)	1.34 ± 0.19*	12.4 ± 1.8*	39.4 ± 9.4*	157 ± 47	63.7 ± 7.8*	9.8 ± 2.4*	15 ± 6
MC, 300 μmol/kg (N = 7)	0.99 ± 0.21*	5.16 ± 0.45	2.8 ± 0.6	1780 ± 150*	60.6 ± 7.9*	6.3 ± 1.7	21 ± 7
(±)-TSO, 600 mg/kg (N = 3)	0.84 ± 0.03*	9.85 ± 0.79*	11.6 ± 2.3*	69 ± 13	73.2 ± 2.3*	8.8 ± 1.9	38 ± 10
(±)-TSO, 1200 mg/kg (N = 4)	1.04 ± 0.11*	9.13 ± 0.79*	20.0 ± 5.1*	110 ± 24	62.2 ± 6.6*	15.5 ± 2.6*	23 ± 9
(+)-TSO, 600 mg/kg (N = 3)	0.79 ± 0.02*	8.64 ± 0.64*	11.4 ± 0.65*	62 ± 3	44.9 ± 6.2	8.4 ± 0.9	36 ± 9
(+)-TSO, 1200 mg/kg (N = 4)	1.08 ± 0.17*	9.75 ± 0.53*	15.4 ± 2.0*	100 ± 15	57.5 ± 4.1†	17.8 ± 2.2*	15 ± 7
(−)-TSO, 600 mg/kg (N = 3)	0.48 ± 0.06	5.83 ± 0.54†	4.47 ± 0.65	84 ± 10	49.5 ± 1.7	7.4 ± 0.5	16 ± 4
(−)-TSO, 1200 mg/kg (N = 4)	0.74 ± 0.06*	6.31 ± 0.60*	8.33 ± 1.73	125 ± 34	64.9 ± 4.4*	16.7 ± 1.1*	11 ± 2

All values are means ± SD. N = the number of rats.
*,† Significantly different from control at *$P \leqq 0.01$ or †$P \leqq 0.05$ respectively.

Table 2. Concentration of *trans*-stilbene oxide (TSO) in liver microsomes purified from rats treated with either (±)-, (+)- or (−)-TSO

Treatment group (total dose)	Rat	TSO (nmol/ml liver microsomes)	TSO* (nmol/g liver) ($\bar{x}$)	TSO* (nmol/liver) ($\bar{x}$)	Calculated liver TSO content as a percent of administered dose* ($\bar{x}$)
(±)-TSO (1200 mg/kg)	A	50.2	243	1420	0.21
	B	51.0			
	C	52.6			
(+)-TSO (1200 mg/kg)	D	61.4	408	1810	0.28
	E	85.0			
	F	92.7			
(−)-TSO (1200 mg/kg)	G	NF†			
	H	NF			
	I	NF			

* Extrapolation based on the assumption that TSO was to be found exclusively in liver microsomes.
† None found; detection limit 9.3 nmol TSO/ml microsomes.

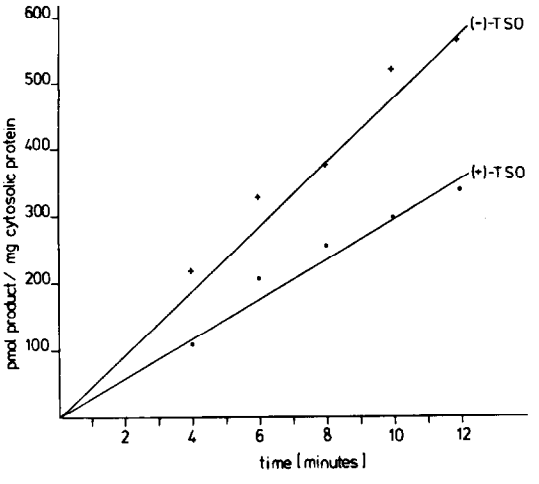

Fig. 1. Rates of metabolism of (+)-[^{3}H]- and (−)-[^{3}H]*trans*-stilbene oxide by 100,000 *g* cytosolic proteins isolated from livers of untreated rats.

DISCUSSION

The results presented in this paper show clearly that (+)-TSO was a more potent inducer of cytochrome P-450 than (−)-TSO. At this point, the likely explanation for these data is that (−)-TSO is metabolized more rapidly. Similar results were obtained with the PB-like inducer normephenytoin. Although both the (*R*)- and (*S*)-enantiomers of normephenytoin were potent inducers of drug-metabolizing enzymes, they were equipotent when stereospecific differences in rates of metabolism were taken into account [24]. In the context of PB-inducible enzymes, there is no evidence that chiral differences can be "recognized" by whatever begins the induction process, whereas chiral differences are clearly recognized by those enzymes so induced.

This lack of recognition of chiral differences may not, however, be universal among the drug-metabolizing enzymes. A recent report suggests that a form of cytochrome P-450 (corresponding to lauric acid 12-hydroxylase activity, inducible by hypolipidemic drugs) is more potently induced by the (*S*)-enantiomer of 2-phenylpropionic acid than by the (*R*)-enantiomer [25]. The interpretation of these results, however, is complicated by the observation that the (*R*)-enantiomer is inverted *in vivo* (enzymatically converted to its antipode) [26], rendering a measurement of its potency of induction all but impossible. Certainly additional studies will be required to elucidate these effects.

In conclusion, it should be noted that differential rates of metabolism may alter dramatically the induction response, masking, in the present study, possible chiral differences in recognition.

Acknowledgements—The authors thank Dr. Frans Setiabudi for the synthesis of [^{3}H]*trans*-stilbene oxide, Dr. Peter Wedlund for helpful discussions, Ms. Martha Butts for typing the manuscript, and the Alexander von Humboldt Foundation and the Deutsche Forschungsgemeinschaft (SFB 302) for financial support.

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