Original

The Effects of Inducers of Different Types of Cytochrome P-450 Isozymes on Serum Dimethadione/Trimethadione Ratio in Rats

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Abstract: Trimethadione (TMO) is regarded as a model drug for monitoring hepatic drug N-demethylation capacity *in vivo*. However, the isozyme of cytochrome P-450 that is responsible for N-demethylation of TMO has not been identified. Considering these facts, this study was designed to determine the cytochrome P-450 species that participate in the TMO N-demethylation by employing several typical inducers of cytochrome P-450 I, II, III and IV isozymes. Male Sprague Dawley (SD) rats were pretreated with typical cytochrome P-450 inducers, such as 3-methylcholanthrene (3MC), isosafrole, ethanol, phenobarbital (PB), pregnenolone-16 α -carbonitrile (PCN), dexamethasone (DEX), triacetyloleandomycin (TAO) and clofibrate, and the serum dimethadione (DMO)/TMO ratio was determined for evaluation of the effects of these inducers on hepatic TMO metabolism. In clofibrate or PB pretreated rats, the serum DMO/TMO ratio was increased to approximately 2 or 4 times those in the control. In rats pretreated with other inducers however, the ratio did not change significantly compared to the controls. These results suggest that the cytochrome P-450 gene family(ies) induced by PB or clofibrate may be responsible for TMO metabolism in rats. On the other hand, it has also been suggested that the cytochrome P-450 I family that is induced by 3MC and isosafrole is not responsible for TMO metabolism in rats.

Key words: trimethadione N-demethylase, cytochrome P-450 inducer, rat liver

Introduction

Trimethadione (TMO) is a compound that is almost completely absorbed from the gastrointestinal tract and is distributed uniformly into the total body fluids when given orally¹⁾. Furthermore, TMO is extensively metabolized to dimethadione (DMO), its single metabolite, by hepatic microsomal cytochrome P-450²⁾. Neither TMO nor DMO is bound to plasma protein³⁾. From these properties, TMO is regarded as a useful model drug for monitoring hepatic drug N-demethylation capacity in rats and humans⁴⁻⁸⁾. However, the isozyme of cytochrome P-450 that is responsible for N-demethylation of TMO has not been identified. Recently, cytochrome P-450 gene families (I, II, III, IV) in rats and their typical inducers and inhibitors have been reported by Loft⁹⁾ (Table 1). If TMO is metabolized to DMO by some specific cytochrome P-450 isozyme(s), the serum DMO/TMO ratio might be increased due to the enhancement of cytochrome P-450 by its inducers. In the present study we investigated whether or not these inducers for cytochrome P-450 isozymes enhance the N-

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P-450 gene	Trivial names	Prototype inducers	Prototype inhibitor	Reference
I	P448I/ rat c/P1 P448h/ rat d/P3	3-MC, TCDD, β -NF isosafole β -NF, 3-MC	α -NF	Pelkonen et al. 1986 ¹⁴⁾ Grant et al. 1987 ¹⁵⁾ Sesardic et al. 1988 ¹⁶⁾ Testa & Jenner 1987 ¹⁷⁾
II	rat a hum(1) rat b rat e hum mp hum TB db1 rat j hum j	 PB PB EtOH EtOH	SKF525A quinidine imidazole	Testa & Jenner 1987 ¹⁷⁾ Wilson et al. 1987 ¹⁸⁾ Wilson et al. 1987 ¹⁸⁾ Knodell et al. 1987 ¹⁹⁾ Otton et al. 1988 ²⁰⁾ Jacqz et al. 1986 ²¹⁾ Raucy et al. 1987 ²²⁾ Gadeholt et al. 1984 ²³⁾
III	rat pcn1 hum HLP	PCN, DEX RIF TAO	TAO	Wrighton et al. 1985 ²⁴⁾ Kronbach et al. 1988 ²⁵⁾
IV	P450LAW	clofibrate		

3-MC, 3-methylcholanthrene; TCDD, 2,3,7,8-dioxin;

α -NF, α -naphthoflavone; β -NF, β -naphthoflavone; PB, phenobarbital;

PCN, pregnenolone-16 α -carbonitrile; DEX, dexamethasone;

RIF, rifampicin; TAO, triacetyloleandomycin

Adapted from Nebert et al. (1987²⁶⁾ and 1989²⁷⁾, Beaune & Guengerich (1988)²⁸⁾, Gonzales (1988)²⁹⁾ and Guengerich (1989)³⁰⁾

Table 1. Summary of prototype inducers and inhibitors of rat and human cytochrome P-450s.

demethylation of TMO and we determined the selectivity of these inducers of the cytochrome P-450 isozyme(s) which is responsible for N-demethylation of TMO in SD rats.

Materials and Methods

Materials

A commercial preparation of TMO powder (66.7%) was used (Mino-Aleviation: Dainippon Pharmaceutical Co. Ltd., Osaka, Japan). DMO and maleinimide were purchased from Tokyo Kasai (Tokyo, Japan). Isosafole was purchased from Aldrich Chemical Co. (Milwaukee, WI). 3-Methylcholanthrene (3MC), clofibrate, dexamethasone (DEX), and triacetyloleandomycin (TAO) were purchased from Sigma Chemical Co. (St. Louis, MO), and phenobarbital (PB) from Sankyo Pharmaceutical Co. Ltd. (Tokyo, Japan). Pregnenolone-16 α -carbonitrile (PCN) was kindly donated by Upjohn Co. (Kalamazoo, MI). All other chemicals were of analytical reagent grade. 3MC, isosafole and clofibrate were dissolved in corn oil. PB was dissolved in 0.9% NaCl containing 1.1 equivalent mol of NaOH. Ethanol was diluted to 10% (v/v) in the drinking water, TAO was mixed into powder chow as 1.5 g/100 g chow. PCN and DEX were suspended in water containing 2% Tween 80 for administration to rats.

Animal treatment

Male SD rats, weighing 200–300 g were pretreated with 3MC (20 mg/kg i.p.), isosafrole (150 mg/kg i.p.), clofibrate (400 mg/kg i.p.), and PB (80 mg/kg i.p.) for 4 days, ethanol (with free access) for 3 weeks, TAO (with free access) for 10 days, and PCN, DEX (300 mg/kg p.o.) for 4 days. The control rats were given an equal volume of corn oil, and 2% Tween 80. The control rats, pretreated with ethanol or TAO, were given drug-free drinking water and powder chow. TMO (100 mg/kg p.o.) was administered 24 hours after the last dose and blood samples were collected from the jugular vein 2 hours after the TMO administration.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Analysis of rat liver microsomal protein using polyacrylamide (15%) slab gel electrophoresis was carried out using SDS, according to the method of Laemmli¹⁰⁾. Liver microsomal protein (20 µg) was prepared from P-450 inducer-treated rats. Bovine serum albumin (66 kda), hen-egg white ovalbumin (45 kda), carbonic anhydrase (29 kda) and hen-egg white lysozyme (14.5 kda) were used as standards.

Extraction procedure

Serum concentrations of TMO and DMO were determined as described by Tanaka et al.¹¹⁾ Serum (100 µl) was added to 200 µl of 5 M monobasic sodium phosphate with 100 mg of sodium sulphate and magnesium sulphate, and with 100 µl of ethyl acetate containing maleinimide as the internal standard. The tube was shaken for 2 min and centrifuged at 3000 rpm for 30 min. A 0.5 µl portion of the organic phase was then analyzed by GC.

Analysis

Analysis of serum TMO and DMO was carried out with a Shimadzu-GC14A equipped with FTD. Data was computed with a Shimadzu CR4A integrator. Tenax GC (60–80 mesh) was used as the packing material in a glass column (2.6 mm I.D. × 50 cm). For the GC conditions, the column temperature was increased from 130°C to 230°C at a rate of 15°C per min. The injection port and detector temperatures were 230°C. Helium, hydrogen and air flow rates were 50, 30 and 145 ml/min, respectively.

Results

The pattern of microsomal proteins after treatment of cytochrome P-450 inducers separated by SDS-PAGE is shown in Fig. 1. The band of cytochrome P-450 proteins representing molecular weights of about 45,000 to 55,000 were increased compared to the controls by pretreatment with inducers.

As shown in Fig. 2, in the rats pretreated with ethanol and TAO 2 hours after TMO administration, the serum DMO/TMO ratio was changed to 80% and 145%, respectively, of that of the control. However, in rats pretreated with PB, this ratio was increased to approximately 4 times that of the control.

Fig. 3 shows that in rats pretreated with 3MC and isosafrole, the ratio changed to 76% and 114%, respectively, of that of the control. In the rats pretreated with clofibrate the ratio was increased about 2 times that of the controls.

In the rats pretreated with PCN and DEX the serum DMO/TMO ratio (Fig. 4) was changed slightly to 133% and 143% of the control, respectively.

Discussion

In this study, we investigated the effects of typical cytochrome P-450 inducers on the

Fig. 1. SDS-PAGE of microsomal protein after treatment with typical cytochrome P-450 inducers in SD rats.
3MC, 3-methylcholanthrene; EtOH, ethanol; TAO, triacetyloleandomycin; PB, phenobarbital; PCN, pregnenolone-16 α -carbonitrile; DEX, dexamethasone.

Fig. 2. Effects of pretreatment with EtOH, TAO and PB on TMO N-demethylation in SD rats. Values are mean $\pm$ S.E. of 6 rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 3. Effects of pretreatment with 3MC, isosafrole and clofibrate on TMO N-demethylation in SD rats.

Values are mean $\pm$ S.E. of 6 rats. ** $P < 0.01$.

Fig. 4. Effects of pretreatment with PCN and DEX on TMO N-demethylation in SD rats.

Values are mean $\pm$ S.E. of 6 rats. * $P < 0.05$, ** $P < 0.01$.

isozyme of cytochrome P-450 that is responsible for N-demethylation of TMO in SD rats.

Tanaka et al. reported that TMO metabolism in rat liver is not significantly affected by 3MC treatment¹²⁾. Also in our study, the serum DMO/TMO ratio was not increased by 3MC. However, the band of cytochrome P-450 in microsomal proteins was increased on SDS-PAGE by 3MC treatment. Similar results were obtained by isosafrole. Among the

cytochrome P-450 gene families, isozymes of cytochrome P-450 that are induced by 3MC or isosafrole have been indicated to be in the cytochrome P-450 I gene family¹³⁾. These results suggest that the cytochrome P-450 I gene family that is induced by 3MC or isosafrole is not responsible for TMO metabolism.

Tanaka *et al.* also reported that the serum DMO/TMO ratio is induced by PB treatment *in vivo* in rats¹²⁾. Furthermore, they indicated that the cytochrome P-450 IIB₁ subfamily and IIC₁₁ subfamily are related to TMO metabolism according to a recent study using purified P-450 and the antibody for each P-450 gene family (personal communication). Among the cytochrome P-450 gene families, the cytochrome P-450 IIB and IIC subfamily are reported to be the most PB-inducible¹³⁾. In the present study, the serum DMO/TMO ratio was also increased significantly by PB. Therefore, the cytochrome P-450 IIB and IIC gene families were probably partly responsible for TMO metabolism.

On the other hand, it has been suggested that the cytochrome P-450 IIE subfamily is induced by ethanol¹³⁾. In the present study, the serum DMO/TMO ratio was not elevated by ethanol treatment. However, the band of cytochrome P-450 in microsomal protein pre-treated by ethanol was increased on SDS-PAGE. These results suggest that the P-450 IIE subfamily was induced by ethanol but may not be related to the TMO metabolism.

The cytochrome P-450 III gene family is reported to be primarily induced by PCN, DEX or TAO¹³⁾. In the present study, the serum DMO/TMO ratio was only slightly affected by PCN, DEX or TAO. These results also show that the cytochrome P-450 gene family induced by PCN, DEX or TAO was not primarily responsible for TMO metabolism. The cytochrome P-450 IV gene family has been reported to be induced by clofibrate¹³⁾. The serum DMO/TMO ratio by clofibrate was also increased significantly in the present study.

These results may indicate that in SD rats, cytochrome P-450 isozymes responsible for TMO metabolism are primarily induced by PB or clofibrate, so that in selectivity for inducers, they are considered to be cytochrome P-450 IIB, IIC and IV gene families. Further studies will be necessary to determine the isozyme of cytochrome P-450 which is responsible for TMO metabolism.

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