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SHORT COMMUNICATION

Characterization of the Induction of Rat Microsomal Cytochrome P450 by Tacrine

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ABSTRACT. The effect of multiple-dose tacrine (THA) administration at 2 and 20 mg/kg (single oral doses for 2 weeks) on cytochrome P450 (CYP) was examined in male and female Wistar rats. Changes in CYP were determined by measuring total spectral CYP, the rates of ethoxy- and pentoxyresorufin dealkylations, and the protein expression of several CYPs by western blot analysis of liver microsomes. Animals treated with β-naphthoflavone or phenobarbital were employed as positive controls. No physiological or metabolic changes were observed in male or female rats treated with 2 mg/kg THA for 2 weeks. Male and female animals treated with 20 mg/kg THA for 2 weeks demonstrated increased CYP1A activity (increased ethoxyresorufin deethylase activity) and increased expression of CYP1A1 with only minor increases in CYP1A2 expression. Compared with the effects of β-naphthoflavone induction of CYP1A, the induction observed with THA at 20 mg/kg was considered minor. BIOCHEM PHARMACOL 54;3:425–427, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. tacrine; rat; cytochrome P450; liver microsomes; induction; Alzheimer's disease

THA† (9-amino-1,2,3,4-tetrahydroacridine, Cognex®) is a centrally active acetylcholinesterase inhibitor approved for use in treating Alzheimer's disease [1, 2]. A relatively common side-effect associated with THA administration to Alzheimer's patients has been asymptomatic elevations in liver marker enzymes, in particular ALT [3]. THA is known to undergo extensive oxidative metabolism *in vivo* in rats and humans to mono- and dihydroxylated metabolites [4, 5]. In addition, THA has been shown to be biotransformed into chemically reactive cytotoxic metabolites *in vitro* using rat and human liver microsomes [6–8]. The primary enzyme involved in the formation of stable and cytotoxic metabolites is CYP1A2 [6–8]. The CYP1A subfamily comprises two isoforms, CYP1A1 and CYP1A2, that are inducible by cigarette smoking, charcoal-broiled beef, and polycyclic aromatic hydrocarbons [9]. As part of our efforts to better understand the mechanism(s) underlying THA-induced hepatotoxicity, we choose to assess the potential for THA to induce CYP, especially CYP1A1/2. We evaluated the potential of THA to induce CYP in male and female Wistar rats by examining the changes in total spectral CYP content, degree of protein expression, and enzymatic activities of CYP1A and CYP2B. These changes were compared with those of BNF and PB, two known potent inducers of CYP1A and CYP2B.

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† *Abbreviations:* THA, tacrine; ALT, alanine aminotransferase; CYP, cytochrome P450; BNF, β-naphthoflavone; PB, phenobarbital; ER, ethoxyresorufin; and PR, pentoxyresorufin.

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MATERIALS AND METHODS

Chemicals

THA monohydrochloride monohydrate (78.5% parent) was synthesized at Parke–Davis Pharmaceutical Research Laboratories (Ann Arbor, MI). Antibodies to CYPs 1A2, 2B1, 2C6, 2C7, 2E1, and 3A1/2 and glucuronyltransferase (testosterone as substrate) were obtained from C. R. Wolf (University of Dundee, Scotland). Antibody to CYP1A1 was obtained from Oxygene Dallas (Dallas, TX). All other reagents were of the highest quality commercially available.

Animals and Treatment

Male (236–267 g) and female (174–199 g) Wistar rats (7/group) were administered single oral daily doses of either 2 or 20 mg/kg THA (free base equivalents, in saline), or saline vehicle for 2 weeks. Male rats (3/group) were induced with BNF (80 mg/kg, i.p.) or PB (80 mg/kg, i.p.) once daily for 3 days. Twenty-four hours after the last dose of THA or inducer, rats were weighed and killed, and each liver was removed, weighed and stored at −80°.

Tissue Preparation and Assay

Microsomes were prepared from pooled male and female livers by standard procedures and stored at −80° in 2.5 nM sucrose/10 mM potassium phosphate buffer, pH 7.4. CYP concentrations were determined by the method of Omura and Sato [10] and microsomal protein concentrations by the method of Bradford [11]. The enzymatic activities of CYP1A and 2B were determined by measuring the rate of

TABLE 1. Comparison of oral administration of THA (2 or 20 mg/kg) on male and female rats and PB- or BNF-treated male rats on total spectral cytochrome P450 content, ER deethylase activity, and PR depentylase activity from pooled liver microsomes

Animal treatment	Total CYP (nmol/mg protein)	Rate of ER metabolism (pmol/min/mg protein)	Rate of PR metabolism (pmol/min/mg protein)
Male			
THA, 0 mg/kg	1.08 ± 0.10	53.7	13.6
THA, 2 mg/kg	1.16 ± 0.02	51.0	14.7
THA, 20 mg/kg	1.06 ± 0.16	103	17.7
BNF	1.80 ± 0.12	980	ND*
PB	2.30 ± 0.21	ND	803
Female			
THA, 0 mg/kg	1.09 ± 0.10	67.0	6.5
THA, 2 mg/kg	0.94 ± 0.04	74.3	8.2
THA, 20 mg/kg	0.84 ± 0.05†	112	7.8

Data are means ± SD for total CYP (N = 3) and mean values determinations for ER and PR.
* Not determined.
† Significantly different from control (0 mg/kg, female), $P < 0.005$.

ER deethylation and PR depentylation, respectively, using pooled liver microsomes [12].

For immunoblotting analysis, 2.5 mg of microsomal protein was solubilized in SDS reducing buffer (62 mM Tris–HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol) and heated at 95° for 4 min. The samples were loaded on a 4% polyacrylamide stacking gel with a 12% resolving gel. After SDS–PAGE, the proteins were transferred to a nitrocellulose membrane. The membrane was blocked with a 2% BSA solution, incubated with the primary CYP antibody, washed with a solution of PBS containing 0.2% Tween 20, incubated with a secondary antibody (donkey anti-rabbit immunoglobulin conjugated to horseradish peroxidase), and subsequently washed. Protein band intensities were quantitated by scanning densitometry subsequent to visualization by chemiluminescence (Amersham ECL western blotting detection system).

RESULTS

Minor decreases were observed in body and liver weights of male and female animals when treated for 2 weeks at 20 mg/kg THA (data not shown). No induction of total spectral CYP was observed in male animals at either dose level, while a significant decrease ($P < 0.005$) was observed in total CYP content between control and 20 mg/kg THA-treated female animals (Table 1). The ER deethylase activity increased approximately 2-fold in both male and female rats at the 20 mg/kg dose level. In comparison to the ER activity in BNF-induced rat liver microsomes (*ca.* 18-fold increase), our findings indicate that THA at 20 mg/kg resulted in minor induction of CYP1A activity in male and female rats. No apparent changes were observed with the PR depentylase activity in either gender or dose group, although PB-induced mircosomes did show an increase in depentylase activity (57-fold) (Table 1). SDS–PAGE immunoblots of liver microsomes from all three dose levels indicated no significant changes in the amounts of expressed CYP proteins 2B1, 2C6, 2C7, 2E1, and 3A1/2 or glucuronyltransferase (data not shown). However, CYP1A1 microsomal protein expression was increased in male and female rats treated with 20 mg/kg THA, as compared with control and 2 mg/kg treated animals (Fig. 1). In addition, minor increases were observed in the expression of CYP1A2 in liver microsomes from male and female animals, 2.4- and 1.2-fold (mean of duplicate immunoblots), respectively (Fig. 1).

DISCUSSION

These cumulative results indicate that no changes in CYP activity and content occurred in male and female animals after 2 weeks of THA treatment at 2 mg/kg. However, in animals dosed for 2 weeks at 20 mg/kg THA, body weight gain suppression and increases in CYP1A1 enzyme activity and protein expression were found. The sensitive ER assay demonstrated a significant increase in CYP1A enzyme activity (*ca.* 2-fold) at 20 mg/kg. Due to the extrahepatic nature of CYP1A1 in control rat liver tissue, quantitation of the CYP1A1 immunoblot is more difficult. No CYP1A1 was detected in our immunoblots of control rat liver microsomes or microsomes obtained from animals treated

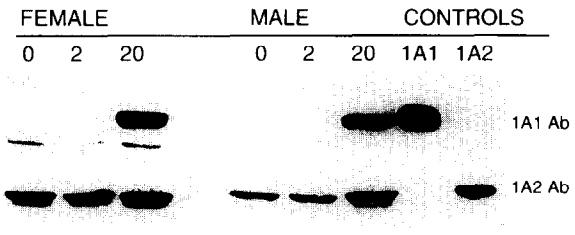

FIG. 1. Representative western blot of liver microsomes from male and female rats treated with single oral doses of 0, 2, or 20 mg/kg THA for 2 weeks. Controls were isolated preparations of CYP1A1 and 1A2. Rows labeled 1A1 Ab and 1A2 Ab were probed with anti-rat CYP1A1 and 1A2 antibodies, respectively.

with 2 mg/kg THA, albeit CYP1A1 was detectable after treatment at 20 mg/kg THA. Although no absolute increase in CYP1A1 protein expression can be determined, clearly CYP1A1 expression was increased at a 20 mg/kg dose of THA but not at 2 mg/kg.

While THA is metabolized by CYP1A1/2 in rats [13], there was little effect on the expression and activity of CYP1A1/2 at pharmacological doses (2 mg/kg THA) and only minor effects at higher doses (20 mg/kg THA). Interestingly, the increase in CYP1A1/2 activity was not paralleled by an increase in total CYP content. In fact, a reduction in CYP content was found in female animals when dosed at 20 mg/kg THA. These results suggest that examining total spectral CYP content may not always be an adequate indication of enzyme induction, especially when the extent of induction is minor, although total CYP measurements are certainly adequate for potent enzyme inducers, such as BNF and PB.

These results suggest that the induction of CYP1A1/2 by THA is not involved in the hepatotoxic mechanism(s) of THA, although other known inducers of CYP1A1/2 may affect the hepatotoxicity of THA [13]. The human doses of THA (40–160 mg/day) are similar to the 2 mg/kg dose used in these studies where no induction was observed. In addition, there is no evidence that multiple dosing of THA in humans has any significant effect on the pharmacokinetics of THA [14]. However, cigarette smoking, which is known to induce CYP1A1/2, has been shown to reduce substantially the plasma concentrations of THA in smokers versus nonsmokers [15]. Therefore, in light of our results, the therapeutic doses of THA in humans should not be high enough to elicit an induction response; however, at higher toxicological doses, THA has the potential to elicit an inductive response in rats.

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