— Research Letter —

Strain Differences in the Induction of Cytochrome P450 3A1/3A2 and Nuclear Receptors in the Liver by Phenobarbital and Dexamethasone in Sprague-Dawley Rats and Dark Agouti Rats

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Strain differences in the induction of cytochrome P450 (CYP) affect drug actions and side effects. Strain differences in the induction of CYP are important to evaluate drug-drug interactions in CYPs. We clarified strain differences in the induction of CYP3A1/3A2 and nuclear receptors by evaluating mRNA levels and metabolic activities in Sprague-Dawley (SD) rats and Dark Agouti (DA) rats (models for extensive and poor metabolism of CYP2D6, respectively). To clarify strain differences in CYP levels, we examined nuclear receptors such as the constitutive androstane receptor (CAR) and pregnane X receptor, which regulate the transcription of CYPs and transporters. We investigated CYP3A inductions in the liver after repeated intraperitoneal injections of phenobarbital (PB) or dexamethasone (DEX) into SD rats and DA rats for 3 d. mRNA levels of CYP and nuclear receptors were determined by real-time reverse transcriptase-polymerase chain reaction. Metabolic activities of CYP3A were also determined. Increased CYP3A mRNA levels were observed in both rat strains after treatment with PB or DEX compared with the respective rat strains treated with vehicle alone. Induction of CYP3A mRNAs by DEX was higher in SD rats than in DA rats, suggesting that SD rats could be more susceptible to DEX than DA rats. Inductions of CAR by PB differed between strains. The increase in mRNA levels and activity of CYP3A by PB in SD rats and DA rats were similar. However, there were strain differences in CYP3A1/3A2 inductions after DEX treatment.

Key words —— cytochrome P450, induction, nuclear receptor

INTRODUCTION

Cytochrome P450 (CYP) has important roles in xenobiotic metabolism, including the first-pass effect in the liver. Strain differences in CYP-mediated drug metabolism among rats has been reported.[1–3] Dark Agouti (DA) rats exhibit quite low mRNA levels of CYP2D2[4] and low metabolic activities for the biotransformation of some typical CYP2D6 substrates (debrisoquine, bunitrolol, alprenolol, metoprolol) compared with Sprague-Dawley (SD) rats or Wistar rats.[5–9] Therefore, DA rats have been regarded as an animal model for poor metabolism of CYP2D6 in humans. In our previous report, we demonstrated that mRNA levels of CYP3A1/3A2 were significantly higher in DA rats than in SD rats, whereas the mRNA levels of CYP2D2 were remarkably lower in DA rats than in SD rats.[10] Therefore, SD rats and DA rats could be assumed to exhibit strain differences in CYP3A1/3A2 induction by CYP inducers. To take advantage of DA rats as a model animal for poor metabolism of CYP2D6 in humans, evaluation of CYP induction in SD rats and DA rats is required.

Several attempts have been made to clarify the induction or inhibition of CYP and drug interactions via CYP *in vitro* and *in vivo*.[11–13] The frequency and strength of drug interactions has been reported to differ between extensive metabolizers and poor metabolizers.[14–17]

Phenobarbital (PB) and dexamethasone (DEX) are CYP inducers via nuclear receptors, including the constitutive androstane receptor (CAR) and pregnane X receptor (PXR), respectively.[18–22] Nuclear receptors regulate the transcription of genes encoding enzymes involved in phase-I (functionalization reactions) and phase-II (conjugation reactions) metabolism and transporters.[23, 24] To clarify the strain differences in CYP induction between

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CYP2D-expressing SD rats and CYP2D-deficient DA rats after treatment with PB and DEX, we examined the mRNA levels and metabolic activities of CYP3A1/3A2 and their activities as the major CYP isoforms in the livers of these rats. Moreover, the mRNA levels of CAR and PXR were also determined to investigate the correlation between the mRNA levels of nuclear receptors and basal levels of CYP.

MATERIALS AND METHODS

Materials —— PB (10% Phenobal®) and DEX were purchased from Fujinaga Pharmacy (Tokyo, Japan) and Wako Pure Chemicals (Osaka, Japan), respectively. All other chemicals were reagent grade products obtained commercially.

Treatments —— Eight-week-old male SD and DA rats were purchased from CLEA Japan (Tokyo, Japan) and Japan SLC (Shizuoka, Japan), respectively. The rats were injected intraperitoneally with 50 mg/kg of PB in saline or 100 mg/kg DEX in corn oil for 3 d. Control rats for the PB and DEX treatments were treated with saline and corn oil for 3 d, respectively. At 24 hr after the final administration, the animals were anesthetized with diethyl ether. The liver was perfused with ice-cold saline and then removed. After flash freezing with liquid nitrogen, each sample was preserved at -80°C until analysis. The experiments were approved by the Committee for the Care and Use of Laboratory Animals at Kinki University School of Pharmacy.

Determination of mRNA Levels by Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) —— Determination of mRNA levels was performed using real-time reverse transcriptase polymerase chain reaction as described by Kawase *et al.*[10] The reverse-transcribed cDNA was used as a template for real-time PCR. Amplification was performed in 50 μl reaction mixtures containing 2 × SYBR Premix Ex Taq (TaKaRa, Shiga, Japan), 0.2 mM primer set of target gene or 18S ribosomal RNA (18S rRNA) as endogenous reference. In Table 1, the oligonucleotide sequences for each target are shown. The data were analyzed using the ABI Prism 7000 SDS Software (Applied Biosystems, Carlsbad, CA, U.S.A.) for the multiplex comparative method.

Measurement of CYP3A Activities —— Liver microsomes were prepared using the procedure described by Komura *et al.*[25] CYP3A activity was evaluated using a P450-Glo™ CYP3A4 Assay (Promega, Madison, WI, U.S.A.). An incubation mixture was prepared that contained 200 mM potassium phosphate buffer (pH 7.4), an NADPH regeneration system (Promega), 20 μg of rat liver microsomes and 50 μM luciferin 6′ benzyl ether (luciferin-BE) as a substrate for CYP3A1/3A2. Luciferin-BE is a relative specific substrate for CYP3A1/3A2 as well as CYP3A4 as per the manufacturer's instructions. The reaction was allowed to proceed for 30 min at 37°C with constant shaking. The production of luciferin by CYP3A1 and CYP3A2 was determined using luciferase assays. Luminescence was measured using a FLUOstar Optima (Moritex, Tokyo, Japan).

Table 1. Primers Used in PCR for mRNA Detection and Quantification

Genes		Primer sequences (5′–3′)
CYP3A1	Upper	GCCTTTTTTTGGCACTGTGCT
	Lower	GCATTTGACCATCAAACAACCC
CYP3A2	Upper	TTTGCCATCATGGACACAGAGA
	Lower	GCTTTCCCCATAATCCCCACT
CAR	Upper	GACGGCAGCATCTGGAACTAC
	Lower	TGATGACGCCCTTGAACATG
PXR	Upper	CCACGGGCTATCATTTCCAT
	Lower	CCCAGCAAACGGACAGATG
18S rRNA	Upper	CGCCGCTAGGTGAAATTC
	Lower	CCAGTCGGCATCGTTTATGG

Statistical Analysis —— The significance of differences between SD and DA rats was estimated by analysis of variance (ANOVA) followed by a Tukey's test. Values of $p < 0.05$ were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Figures 1 and 2 show the effects of treatment with PB or DEX on the mRNA levels and activities of CYP3A in SD rats and DA rats, respectively. The basal mRNA levels of CYP3A in control DA rats were higher than those in control SD rats.

PB treatment increased the mRNA levels of CYP3A1/3A2 by 6–8-fold compared with control levels in SD rats and DA rats. As a result, mRNA levels in DA rats were significantly greater than those in SD rats owing to the difference in the corresponding levels in untreated rats. CYP3A activities in DA rats were also significantly higher than those in SD rats after PB treatment. The strain differences in CYP3A activities between SD rats

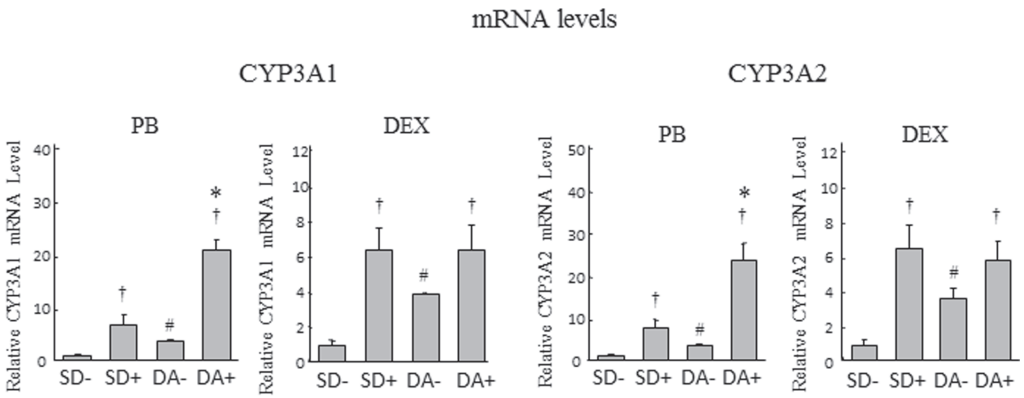

Fig. 1. CYP3A1 and CYP3A2 mRNA Levels in the Livers of SD and DA Rats Treated Intraperitoneally with PB or DEX for 3 d

Relative mRNA levels were represented for SD− group. The results are expressed as the means ± S.D. of 4 rats in each group. *, $p < 0.01$ between SD+ and DA+; #, $p < 0.01$ between SD− and DA−; †, $p < 0.01$ to corresponding control group (untreated group).

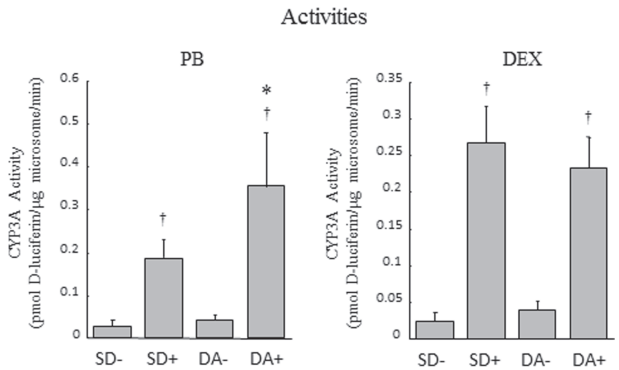

Fig. 2. CYP3A Activities in Liver Microsomes Derived from SD and DA Rats Treated Intraperitoneally with PB or DEX for 3 d

The results are expressed as the means ± S.D. of 4 rats in each group. *, $p < 0.01$ between SD+ and DA+; †, $p < 0.01$ to corresponding control group (untreated group).

and DA rats after PB treatment were more marked than those in untreated rats. The induced extents of CYP3A activities in DA rats were larger than those in SD rats after PB treatment. These results suggest that the drug interactions via CYP3A induction after PB treatment showed strain differences between SD rats and DA rats. Conversely, DEX treatment increased the CYP3A1/3A2 mRNA levels by only 1.5-fold in DA rats but by 7-fold in SD rats. DEX treatment of SD rats caused stronger effects on CYP3A1/3A2 mRNA expressions compared with DA rats, suggesting that SD rats could be more susceptible to DEX than DA rats. Consequently, almost identical mRNA and activity levels between the two strains after DEX treatment were observed. The induction profiles of CYP3A activities by PB (but not DEX) were parallel with those of the mRNA levels in SD rats and DA rats. Similar tendencies were observed for other doses of DEX (50 mg/kg) and PB (40 mg/kg) in preliminary experiments even though we chose typical doses, vehicles and injection routes. Mei *et al.* reported that the mRNA levels of CYP3A1 (but not mdr1a/1b) in the intestine, colon, kidney, and liver were significantly increased by DEX (20 mg/kg per day, per os) in rats for 3 d.[26] Strain differences in the induction of transporters could exist.

The transcription of CYP is regulated by nuclear receptors such as PXR and CAR. Qatanani *et al.* described the complexity of the overlap in the functions of the xenosensors CAR and PXR using CAR-null mice.[27] There could be strain differences in the induction of PXR and CAR between SD rats and DA rats after treatment with PB and DEX. Figure 3 shows the effects of PB or DEX treatments on the mRNA levels of CAR and PXR in SD rats and DA rats. The mRNA levels of CAR in DA rats treated with PB (a typical CAR ligand) and DEX (a typical PXR ligand) were significantly higher than the corresponding levels in SD rats after treatment. In PXR, slight differences in the mRNA induction by PB were observed between SD rats and DA rats, although DEX treatment increased PXR mRNA levels in SD rats and DA rats compared with each control. After DEX treatment, induction of PXR (but not CAR) was observed in SD rats and DA rats. Further studies are needed to clarify the cause of the differences between the levels of PXR and CAR after DEX treatment. These results suggested that the higher mRNA levels of CAR in DA rats could participate in strain differences in the basal levels of CYP.

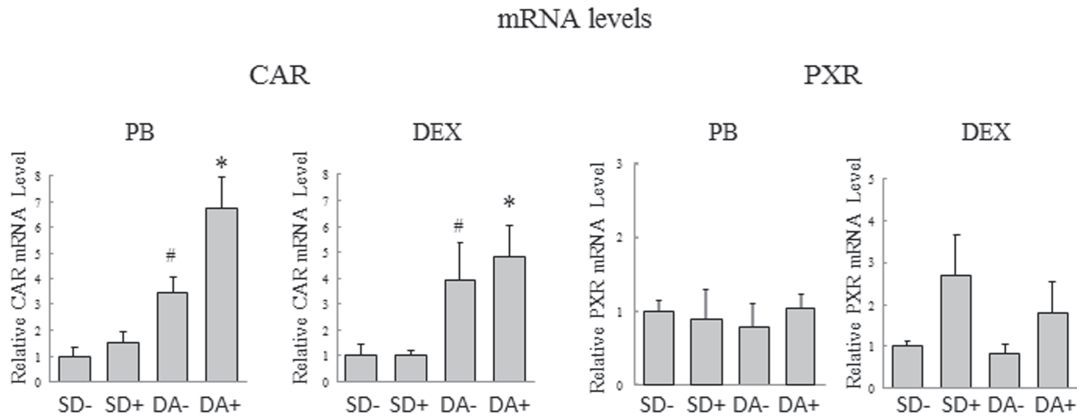

Fig. 3. CAR and PXR mRNA Levels in the Livers of SD and DA Rats Treated Intraperitoneally with PB or DEX for 3 d

Relative mRNA levels were represented for SD− group. The results are expressed as the means ± S.D. of 4 rats in each group. *, $p < 0.01$ between SD+ and DA+; #, $p < 0.01$ between SD− and DA−; †, $p < 0.01$ to corresponding control group (untreated group).

In DA rats, the increased mRNA levels of CAR and PXR could promote the induction of CYP3A1/3A2 after treatment with PB and DEX. Strain differences in the induction of CAR by typical CAR agonists such as PB could be involved in the development of strain differences of induced CYP3A levels between SD rats and DA rats. However, the induction of CAR and PXR mRNA was not entirely consistent with the induction and activity of CYP3A mRNA. Our previous report demonstrated that about 4-fold higher CAR and CYP3A1/3A2 mRNA levels were detected in the livers of DA rats compared with the livers of SD rats, and that the mRNA levels of PXR and hepatocyte nuclear factor 4α exhibited slight differences between SD rats and DA rats under normal conditions.[10] Few strain differences in the induction of PXR were observed, but strain differences in the induction of CYP3A were noted after DEX treatment. The glucocorticoid receptor also participates in the regulation of CYP3A. Differences in glucocorticoid receptor-mediated regulation of CYP3A may be involved in the higher responsivity to DEX in SD rats. To clarify the cause of strain differences in the induction of CYP in SD rats and DA rats, further studies are needed to examine the strain differences of ligand-binding domains in CAR and PXR in these rats. The results obtained in the present study indicate the need for consideration of strain differences in the induction of CYP3A to decide if test compounds are CYP inductions.

In conclusion, the mRNA level and activity of CYP3A inductions by PB in SD rats and DA rats were similar but the basal levels of CYP3A1/3A2 and CAR in DA rats were higher than those in SD rats. However, there were strain differences in the induction of CYP3A1/3A2 after DEX treatment. Therefore, it is important to consider the strain differences of CYP induction in animal experiments.

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