Dose-dependent difference of nuclear receptors involved in murine liver hypertrophy by piperonyl butoxide

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ABSTRACT — Nuclear receptors play important roles in chemically induced liver hypertrophy in rodents. To clarify the involvement of constitutive androstane receptor (CAR) and other nuclear receptors in mouse liver hypertrophy induced by different doses of piperonyl butoxide (PBO), wild-type and CAR-knockout mice were administered PBO (200, 1,000, or 5,000 ppm) in the basal diet for 1 week. Increased liver weight and diffuse hepatocellular hypertrophy were observed at 5,000 ppm for both genotypes, accompanied by increased \( \text{Cyp3a11} \) mRNA and CYP3A protein expression, suggesting that CAR-independent pathway, possibly pregnane X receptor (PXR), plays a major role in the induction of hypertrophy. Moreover, wild-type mice at 5,000 ppm showed enhanced hepatocellular hypertrophy and strong positive staining for CYP2B in the centrilobular area, suggesting the localized contribution of CAR. At 1,000 ppm, only wild-type mice showed liver weight increase and centrilobular hepatocellular hypertrophy concurrent with elevated \( \text{Cyp2b10} \) mRNA expression and strong CYP2B staining, indicating that CAR was essential at 1,000 ppm. We concluded that high-dose PBO induced hypertrophy via CAR and another pathway, while lower dose of PBO induced a pathway mediated predominantly by CAR. The dose-responsive path on liver hypertrophy is important for understanding the involvement of nuclear receptors.

Key words: Constitutive androstane receptor, Pregnane X receptor, Liver hypertrophy, Mouse model, Piperonyl butoxide

INTRODUCTION

Many agricultural chemicals and pharmaceutical agents induce liver hypertrophy in rodent livers with alterations in metabolic enzyme expression. Xenosensing nuclear receptors, such as constitutive androstane receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator-activated receptor (PPAR), play important roles in this pathology. For example, phenobarbital induces liver hypertrophy accompanied by induction of metabolizing enzymes, such as CYP2B, in rodents and humans, and long-term exposure to phenobarbital induces liver tumors in rodents (Whysner et al., 1996); however, these effects disappeared completely in CAR-knockout (CARKO) mice, indicating that murine liver hypertrophy and tumor induction by phenobarbital are mediated by a CAR-dependent pathway (Yamamoto et al., 2004). In contrast, many chemicals are known to induce liver hypertrophy mediated by 2 or more nuclear receptors. A recent detailed analysis of dose-responsiveness was shown to be useful and important for clarifying the involvement of CAR and other pathways in liver hypertrophy (Boobis et al., 2009; Bercu et al., 2010; Goetz et al., 2011). Tamura et al. (2013) revealed that high-dose tebuconazole (a triazole fungicide, 1,500 ppm) induces severe liver hypertrophy in the presence or absence of CAR (i.e., independent of CAR), while CAR is the principal trigger to induce liver hypertrophy at lower doses. Thus, their study indicated that the involvement of nuclear receptors in liver hypertrophy changes according to the chemical exposure level.

Piperonyl butoxide (PBO, a pesticide synergist), is known to induce liver hypertrophy and to promote liv-
er tumor development in rodents (Okamiya et al., 1998; Takahashi et al., 1994, 1997). In the induction of liver hypertrophy, PBO increases expression of liver metabolizing enzymes such as CYP1A1, 1A2, 2B1/2, 3A and 4A in rats (Okamiya et al., 1998; Watanabe et al., 1998; Tasaki et al., 2010; Morita et al., 2013) and CYP1A1, 1A2 and 2B10 in mice. Among them, CYP1A1 induction in mice was reported to be mediated by the aryl hydrocarbon receptor activation (Adams et al., 1993; Ryu et al., 1996; Muguruma et al., 2006). However, the involvement of other nuclear receptors in induction of CYPs and liver hypertrophy have not been fully understood. We previously reported that PBO induced liver tumors via CAR-dependent pathway, while another mechanism may contribute to induction of liver hypertrophy (Sakamoto et al., 2013). In this previous study, since we employed only one high dose (5,000 ppm), we could not determine whether PBO had dose-dependent effects on various pathways for liver hypertrophy induction in terms of nuclear receptor involvement.

Therefore, in the present study, we investigated the specific involvement of CAR and other nuclear receptors in PBO-inducible liver hypertrophy using three different doses of PBO in wild-type and CARKO mice.

MATERIALS AND METHODS

Chemicals
PBO (CAS No. 51-03-6, purity: > 90%) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Animals
CARKO mice in the C3H/HeNCrl background were generously provided by Dr. M. Negishi, National Institute of Environmental Health Sciences (Research Triangle Park, NC, USA) (Kodama et al., 2004; Yamamoto et al., 2004). C3H/HeNCrlCrlj mice were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan) and used as wild-type mice in the present study. All protocols were approved by the Animal Care and Utilization Committee of the National Institute of Health Sciences, and all studies followed the guidelines for the use of laboratory animals of the National Institute of Health Sciences.

Experimental procedures
Seven-week-old male wild-type and CARKO mice were divided into 4 groups consisting of 5 mice per group for each genotype. The animals were kept in plastic cages according to group and genotype, under a 12-hr light/dark cycle. The wild-type and CARKO mice in the control group were fed a basal diet (powdered CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan). The other 3 groups were given PBO at concentrations of 200, 1,000, or 5,000 ppm in the basal diet for 7 days. The highest dose was determined from previous reports in which the dose was sufficient to induce liver hypertrophy and liver tumors in wild-type mice (Fujitani et al., 1993; Takahashi et al., 1997; Sakamoto et al., 2013).

All mice were examined daily to check for changes in clinical condition. Body weights were measured on days 0 (the first day of dosing), 1, 5, and 7 (the day of termination). Food intake per cage was measured on days 0 to 1 and days 5 to 6, and the values were divided by the number of animals in each cage. At termination of the experiment, all mice were anesthetized by isoflurane inhalation and euthanized by exsanguination. The livers were removed and weighed, then processed for use in subsequent analyses.

Real-time quantitative polymerase chain reaction (PCR) analysis of genes related to drug metabolism
The left lobes of the liver were immediately frozen in liquid nitrogen for later RNA isolation and preparation. Total RNA was extracted from the frozen liver tissue samples using ISOGEN (Nippon Gene Co. Ltd., Tokyo, Japan), following the manufacturer’s instructions. Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using High Capacity Reverse Transcription kits (Applied Biosystems, Foster City, CA, USA). After RNA quantity and quality were checked, real-time PCR was performed using a TaqMan Fast Universal PCR Master Mix (Applied Biosystems). For primer-probe sets, we used TaqMan Gene Expression Assays from Applied Biosystems for the quantitative analysis of specific mRNA expression levels of the following genes: Cyp1a1 (Mm00487218_m1), Cyp2b10 (Mm01972453_s1), Cyp3a11 (Mm00731567_m1), Cyp4a10 (Mm02601690_g1), and cytochrome P450 reductase (Mm00435876_m1). Real-time PCR was performed using a 7900HT Fast Real-time PCR System (Applied Biosystems). The relative changes in gene expression levels were calculated by the relative standard curve method, normalized to endogenous GAPDH (TaqMan Rodent GAPDH Control Reagent, Applied Biosystems).

Pathological and immunohistochemical examination of the liver
The median lobes of the liver were immediately fixed in 10% buffered formaldehyde solution, embedded in paraffin wax, sliced in 4-μm sections, and stained with hematoxylin-
Liver hypertrophy by PBO and involved nuclear receptors

Clinical signs, food intake, body weights, and liver weights

No treatment-related clinical signs or deaths were detected during the experimental period. The food intake was lower in the middle- and high-dose groups for both genotypes compared to the corresponding control groups from day 0 to day 1 and from day 5 to day 6 (Table 1). The calculated mean intakes of PBO were 44-56, 186-220, and 714-1122 mg/kg/day in the low-, middle-, and high-dose groups, respectively (Table 1). CARKO mice in the middle- and high-dose groups showed significantly lower body weights than CARKO mice in the control group (Table 2). Absolute and relative liver weights were largely increased in the middle-dose group of wild-type mice and in the high-dose groups of both genotypes compared to the control group (Table 2).

mRNA expressions in the liver

mRNA expression levels of genes encoding liver metabolic enzymes are shown in Fig. 1. The expression levels of Cyp2b10 were significantly increased in the middle-dose group and dramatically increased in the high-dose group of wild-type mice. In CARKO mice, the middle- and high-dose groups showed increases in Cyp2b10 expression compared to the CARKO control group; however, the expression levels were much lower than those in the corresponding groups of wild-type mice. The expression levels of Cyp3a11 were significantly increased in the low-, middle-, and high-dose group of CARKO mice, while only the high-dose group showed a significant increase in wild-type mice. With regard to the expression level of Cyp1a1, a statistically significant increase was detected only in the high-dose group of CARKO mice. The expression patterns of Cyp4a10 were similar to those of Cyp1a1, for which only the high-dose group of CARKO mice showed a substantial increase. The expression levels of cytochrome P450 reductase mRNA showed statistically significant increases in the middle-dose group of wild-type mice and in the high-dose group of both genotypes.

Histopathological findings in the liver

The results of histopathological examination with H&E stain sections are shown in Table 3, and representative photomicrographs are shown in Fig. 2. Hepatocellular hypertrophy was observed in the high-dose group of both genotypes and in the middle-dose group of wild-type mice. In wild-type mice, mildly (at 1,000 ppm) or moderately to severely (at 5,000 ppm) hepatocellular hypertrophies were observed mainly at the centrilobular area. Some animals in the high-dose group showed diffuse hypertrophy throughout the lobule with severely hypertrophied hepatocytes in centrilobular area. In CARKO mice, moderately hypertrophied hepatocytes are diffusely observed throughout lobule in the high-dose group only.

Representative photomicrographs of CYP2B and CYP3A immunohistochemistry are shown in Fig. 2, and those of CYP1A and CYP4A immunohistochemistry are shown in Fig. 3. Furthermore, the schematic results of immunohistochemistry with anti-CYP2B, anti-CYP3A, anti-CYP1A, and anti-CYP4A antibodies are shown in Fig. 4. In wild-type mice, positive reactions to the anti-
CYP2B antibody became stronger in a dose-dependent manner. The 5,000 ppm group showed more than mildly positive reactions throughout the lobules, but the centrilobular area showed a stronger reaction than the midzonal and periportal areas. In CARKO mice, the reactions to the anti-CYP2B antibody were weaker than those in wild-type mice in each dose group. For the other antibodies, there were no apparent differences in the intensities and areas of positive staining between wild-type and CARKO mice. Positive reactions to anti-CYP3A antibodies were observed in the centrilobular and midzonal areas in the control groups of both genotypes, and the positive area expanded to the periportal area in the high-dose group. Slightly positive reactions to anti-CYP1A and anti-CYP4A antibodies were observed at the centrilobular area in the control, low-dose, and middle-dose groups of both genotypes. In the high-dose group, positive reactions to anti-CYP1A and anti-CYP4A antibodies were expanded to the midzonal or periportal area; however, the staining intensity was still weak in each area.

**DISCUSSION**

In this study, we examined the dose-dependent effects

| Table 1. Food intake, calculated PBO intake, and final body weight. |

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (ppm)</th>
<th>Day 0-1</th>
<th></th>
<th>Day 5-6</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Food intake (g/animal/day)</td>
<td>PBO intake (mg/kg/day)</td>
<td></td>
<td>Food intake (g/animal/day)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>Control</td>
<td>0</td>
<td>6.10</td>
<td>0</td>
<td>5.28</td>
</tr>
<tr>
<td></td>
<td>PBO</td>
<td>200</td>
<td>6.02</td>
<td>52</td>
<td>5.38</td>
</tr>
<tr>
<td></td>
<td>PBO</td>
<td>1000</td>
<td>4.46</td>
<td>193</td>
<td>5.40</td>
</tr>
<tr>
<td></td>
<td>PBO</td>
<td>5000</td>
<td>3.30</td>
<td>714</td>
<td>4.74</td>
</tr>
<tr>
<td>CARKO</td>
<td>Control</td>
<td>0</td>
<td>6.58</td>
<td>0</td>
<td>6.52</td>
</tr>
<tr>
<td></td>
<td>PBO</td>
<td>200</td>
<td>7.04</td>
<td>56</td>
<td>6.02</td>
</tr>
<tr>
<td></td>
<td>PBO</td>
<td>1000</td>
<td>5.16</td>
<td>207</td>
<td>4.70</td>
</tr>
<tr>
<td></td>
<td>PBO</td>
<td>5000</td>
<td>4.34</td>
<td>865</td>
<td>5.54</td>
</tr>
</tbody>
</table>

All values are the mean values of each group for each genotype.

| Table 2. Final body weight, absolute and relative liver weight. |

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (ppm)</th>
<th>Final body weight (g)</th>
<th>Absolute liver weight (g)</th>
<th>Relative liver weight (% of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>Control</td>
<td>24.9 ± 1.3</td>
<td>1.2 ± 0.1</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>PBO (low)</td>
<td>26.0 ± 0.7</td>
<td>1.2 ± 0.1</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>PBO (middle)</td>
<td>25.0 ± 1.0</td>
<td>1.4 ± 0.1*</td>
<td>5.7 ± 0.4**</td>
</tr>
<tr>
<td></td>
<td>PBO (high)</td>
<td>23.4 ± 1.1</td>
<td>1.9 ± 0.1**</td>
<td>8.1 ± 0.3**</td>
</tr>
<tr>
<td>CARKO</td>
<td>Control</td>
<td>27.8 ± 1.3</td>
<td>1.5 ± 0.1</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>PBO (low)</td>
<td>27.7 ± 1.1</td>
<td>1.4 ± 0.1</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>PBO (middle)</td>
<td>25.4 ± 1.2*</td>
<td>1.3 ± 0.1</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>PBO (high)</td>
<td>25.3 ± 1.0**</td>
<td>1.8 ± 0.1**</td>
<td>7.2 ± 0.3**</td>
</tr>
</tbody>
</table>

All values are mean ± S.D. (n = 5 for each group for each genotype)

*, **: Significantly different from the corresponding controls at p < 0.05 and p < 0.01, respectively.
Liver hypertrophy by PBO and involved nuclear receptors

of PBO on CAR-mediated liver hypertrophy in mice. Our results suggested that the involvement of nuclear receptors in liver hypertrophy differed depending on the dosage of PBO. The differences are summarized in Table 4.

At 5,000 ppm PBO, the highest dose tested, both wild-type and CARKO mice showed remarkable increases in liver weights and diffuse hepatocellular hypertrophy despite strong induction of CYP2B immunohistochemical reaction and Cyp2b10 mRNA expression only in wild-type mice. In this dose level, diffuse positive reactions to anti-CYP3A antibodies and increased expression levels of Cyp3a11 mRNA were observed both in wild-type and CARKO mice. These result was consistent with our previous report conducted at the same dose of PBO as in the

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Table 3. Histopathological findings of livers from wild-type and CARKO mice treated with PBO for 1 week.

<table>
<thead>
<tr>
<th>Findings of livers</th>
<th>Genotype:</th>
<th>Wild-type</th>
<th></th>
<th></th>
<th>CARKO</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group:</td>
<td>200</td>
<td>1000</td>
<td>5000</td>
<td>200</td>
<td>1000</td>
<td>5000</td>
</tr>
<tr>
<td>No. of mice examined:</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Grade</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Hepatocellular hypertrophy

- Centrilobular:
  - mild: 0 0 3 0 0 0 0 0
  - moderate: 0 0 0 2 0 0 0 0

- Diffuse:
  - moderate: 0 0 0 0 0 0 0 5

- Diffuse and severer in the centrilobular area: severe 0 0 0 3 0 0 0 0

Fig. 2. H&E sections or immunohistochemistry for CYP2B and CYP3A in the livers of wild-type and CARKO mice in the control, middle-dose, and high-dose groups. Upper row represents H&E. Middle row and lower row represent CYP2B and CYP3A, respectively. The blood vessel on the right side in each photo is the central vein. Bar = 100 μm.
Liver hypertrophy by PBO and involved nuclear receptors

**Fig. 3.** Immunohistochemistry for CYP1A and CYP4A in the liver of wild-type and CARKO mice in the control, middle-dose, and high-dose groups. Upper row and lower row represent CYP1A and CYP4A, respectively. The blood vessel on the right side in each photo is the central vein. Bar = 100 μm.

**Fig. 4.** Histological localization of positive reactions for CYP2B, CYP3A, CYP1A, and CYP4A in the liver. Concentric circles represent hepatic lobules. Upper and lower hemispheres represent hepatic lobules from wild-type and CARKO mice, respectively.
present study (Sakamoto et al., 2013), and clearly indicated the CAR-independent pathway was involved in the induction of liver hypertrophy. We assume that PXR plays an important role in liver hypertrophy by 1 week treatment of PBO at 5,000 ppm, since CYP3A induction is mainly regulated by PXR in rodents and other species including human (Quattrochi and Guzelian, 2001; LeCluyse, 2001), though there is no clear evidence for a direct effect of PBO on PXR. The comprehensive morphological analysis in the present study revealed that the wild-type mice receiving 5,000 ppm demonstrated more advanced liver hypertrophy and remarkable CYP2B protein expression in the centrilobular area than those in the periportal area, while CARKO mice showed diffuse hypertrophy at this dose. These results suggested that CAR also contributed to liver hypertrophy at 5,000 ppm, particularly in the centrilobular area. On the other hand, at 1,000 ppm PBO, the middle-dose tested in this study, CAR was a predominant mediator of liver hypertrophy because only wild-type mice showed increases in liver weight and centrilobular hepatocellular hypertrophy with increased positive reactions to anti-CYP2B antibodies, along with the increased expression of Cyp2b10 mRNA. Cyp3a11 mRNA was slightly induced in CARKO mice at 1,000 ppm and also at 200 ppm but not in wild-type mice, although there was no liver weight increase, hepatocellular hypertrophy and induction of CYP3A in protein level at these dose levels. There is no obvious evidence but PXR might be weakly activated in these lower doses in compensation for absence of CAR, given the fact that CAR and PXR regulate overlapping sets of genes involved in detoxication of xenobiotics including Cyp2b10 and Cyp3a11 (Maglich et al., 2002; Xie et al., 2000), and that these nuclear receptors share retinoid X receptor as a partner of heterodimer. On the contrary to these two lower doses, there were no differences in Cyp3a11 mRNA expression levels and CYP3A immunohistochemistry between wild-type and CARKO at 5,000 ppm, possibly because the high-dose of PBO had a sufficient inducibility of CYP3A regardless of the presence or absence of CAR. Taken together, the present results revealed that CAR and another pathway such as PXR were involved in liver hypertrophy in mice induced by PBO and that involvement of these nuclear receptors changed with the dosage of PBO.

The expression levels of Cyp1a1 and Cyp4a10 mRNAs were significantly increased only in the high-dose group of CARKO mice, suggesting that PBO may activate the transcription of Cyp1a1 and Cyp4a10 mRNAs. Although it was not clear why only CARKO mice showed increased levels of these mRNAs, phenobarbital has also been reported to induce PPAR alpha and Cyp4a10/4a14 mRNA only in mice lacking CAR (Tamasi et al., 2009; Ueda et al., 2002), suggesting that CAR suppresses the activation of PPAR following exposure to extremely high doses of PBO. Unexpectedly, immunohistochemical examination with anti-CYP1A and anti-CYP4A antibodies showed inconsistent results, with no obvious differences between wild-type and CARKO mice. The expression level of CYP1A1 in untreated mouse liver is generally very low; therefore a slight induction of mRNA expression level could make remarkable increase in fold expression. In addition, the anti-CYP1A antibody reacts with CYP1A1 and CYP1A2, the latter has more abundant expression in the normal liver. Unfortunately we did not examine the expression level of Cyp1a2 mRNA, but if the high dose of PBO could affect on CYP1A2 expression level both in wild-type and CARKO mice to the same extent, the result of CYP1A immunohistochemistry could be reasonable. Also, since anti-CYP4A antibody may react with CYP4A10, 4A12, and 4A14, so more detailed investigation for members of CYP4A subfamily mRNA expression would explain the discrepancy between the results of CYP4A immunohistochemistry and Cyp4a10 mRNA expression.

The expression level of cytochrome P450 reductase, an enzyme which gives electron to CYPs, significant-

<table>
<thead>
<tr>
<th>Dose</th>
<th>Genotype</th>
<th>Hypertrophy</th>
<th>CYP2B</th>
<th>CYP3A</th>
<th>Nuclear receptors involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle</td>
<td>Wild-type</td>
<td>Centrilobular</td>
<td>Centrilobular to midzonal</td>
<td>NI</td>
<td>CAR</td>
</tr>
<tr>
<td></td>
<td>CARKO</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>Wild-type</td>
<td>Diffuse and severer in centrilobular area</td>
<td>Diffuse but stronger in centrilobular area</td>
<td>Diffuse</td>
<td>CAR and Others (possibly PXR)</td>
</tr>
<tr>
<td></td>
<td>CARKO</td>
<td>Diffuse</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, Not detected; NI, Not increased compared to the control group of each genotype.

Table 4. Summary of effects of middle- and high-dose PBO on liver hypertrophy and CYP2B/CYP3A immunohistochemistry in wild-type and CARKO mice.
Liver hypertrophy by PBO and involved nuclear receptors

Liver hypertrophy in rodents is frequently found in toxicity studies of chemicals; however, toxicological concerns of liver hypertrophy vary depending on the mode of action of the drug or chemical (Holsapple et al., 2006). Therefore, it is very important to know which nuclear receptor(s) are involved when addressing safety evaluations of liver hypertrophy-inducing chemicals. As shown in the present study and in a previous report using triazole fungicides (Tamura et al., 2013), the types of nuclear receptors involved in liver hypertrophy could be changed with different dose levels of chemicals. When comparing the effects of several doses of chemicals on the liver pathology between wild-type and CARKO mice, we can elucidate the involvement of CAR and possible involvements of other nuclear receptors in induction of liver hypertrophy. To investigate the evidence of the direct involvement of nuclear receptors in the induction of liver hypertrophy, a reporter gene assay is a well-known research tool. However, although it is difficult to clarify whether PBO is a direct ligand of CAR or not, morphological analyses including immunohistochemistry is also a useful tool to show activated CAR through the CYP2B induction in combination with CARKO mice. In the present study, CYP2B induction was observed CAR-dependently and we have already clarified that PBO induces eosinophilic foci/adenoma by its promotion effects independently and we have already clarified that PBO induces eosinophilic foci/adenoma by its promotion effects. The increases in cytochrome P450 reductase mRNA are basically coincident with the induction of liver weight increase and hepatocellular hypertrophy in the present study as well as our previous report (Sakamoto et al., 2013), suggesting the increased demands of electron by activated CYP enzymes caused the induction of cytochrome P450 reductase.

In conclusion, high-dose PBO induced liver hypertrophy via CAR and another pathway, possibly PXR, while lower dose of PBO induced it CAR-dependently. The dose-responsiveness on liver hypertrophy is important for understanding the involvement of nuclear receptors.

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Conflict of interest—- The authors declare that there is no conflict of interest.

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