Androgen-mediated down-regulation of CYP1A subfamily genes in the pig liver

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Abstract

In Meishan and Landrace pigs, sex differences in the constitutive expression of hepatic cytochrome P4501A (CYP1A) subfamily enzymes were examined in terms of their mRNA, protein, and enzyme activity. All the results from the real-time RT-PCR, western blotting, and enzyme assays for CYP1A subfamily enzymes indicated that, in 5-month-old Meishan pigs, the expression levels of CYP1A1 and CYP1A2 in males were significantly low as compared with those in females. In contrast, there were no such significant sex differences in Landrace pigs. Castration of male Meishan pigs led to a female-type expression of the CYP1A subfamily enzymes, whereas no such effect was observed in male Landrace pigs after castration. In both breeds of pigs, the administration of testosterone propionate to the females and castrated males led to marked decreases in the expression levels of mRNAs and proteins in the CYP1A subfamily enzymes, and also in their enzyme activities. Furthermore, the correlation analyses between the serum testosterone level and the gene expression levels of CYP1A subfamily enzymes in different sex-matured (1–5-month-old) male pigs revealed that the clear decrease in expression levels of hepatic CYP1A subfamily enzymes occurred at concentrations of more than 33 ng/ml of serum testosterone. Incidentally, the mean concentrations of serum testosterone in 5-month-old Landrace and Meishan pigs were around 18 and 50 ng/ml respectively. In conclusion, we demonstrated for the first time that the serum testosterone level is one of the physiological factors which regulate constitutive expression of hepatic CYP1A1 and CYP1A2 in pigs.


Introduction

The metabolism of xenobiotics and steroid hormones is mainly catalyzed by hepatic cytochrome P450 (CYP), which consists of several subfamilies (Nelson et al. 1996). CYP enzymes have different substrate specificities (Omiecinski et al. 1999, Guengerich 2003), and their expression levels are influenced not only by physiological factors but also by exposure to xenobiotics (Parkinsin et al. 2004, Murray 2006, Graham & Lake 2008).

CYP1A subfamily enzymes are well known to play an important role in the metabolism of various carcinogens and drugs. For example, CYP1A1 and CYP1A2 catalyze the metabolic activations of the carcinogenic aryl hydrocarbons and aromatic amines respectively (Ishii et al. 1981, Conney 1982, Kamataki et al. 1983, Shimada 2006). Furthermore, the activities of CYP1A1 and CYP1A2 in target tissues are one of the host factors that determine the susceptibility of experimental animals toward carcinogenic aryl hydrocarbons (Conney 1982, Shimizu et al. 2000) and aromatic amines (Hashimoto et al. 1982, Degawa et al. 1985, Kleman et al. 1990) respectively.

Since many of the anatomical and physiological characteristics of pigs are similar to those of humans (Swindle & Smith 1998), pharmacological and toxicological studies using pigs can contribute to our understanding of human responses to xenobiotics, such as drugs and environmental chemicals. We have identified the cDNA sequences for several pig CYP isoforms including CYP1A1 subfamily enzymes (Kojima & Morozumi 2004). More recently, we have found a sex difference in the constitutive expression of hepatic CYP1A1 subfamily enzymes, CYP1A1 and CYP1A2, in Meishan pigs, and further suggested an androgen-dependent down-regulation of these genes (Kojima et al. 2008). Furthermore, it has been reported that the alteration in plasma testosterone levels in male pigs treated with human chorionic gonadotropin (Zamaratskaia et al. 2010) or immunized against GnRH (immunocastrated pigs) (Zamaratskaia et al. 2009) led to modulation of hepatic activities of CYP1A1 subfamily enzymes. Likewise, androgen is also considered to be one of the factors which determine sexual dimorphism in the constitutive and/or xenobiotic-induced expression of Cyp1a2 (Hashimoto et al. 1982, Degawa et al. 1985, 1988, 2006), Cyp2b (Honkakoski et al. 1992), and Cyp4b1.
(Degawa et al. 1990, Imaoka et al. 1995) in mice. However, the androgen-mediated down-regulation of hepatic CYP1A subfamily genes has not been completely elucidated.

In the present study we first examined, using two breeds of pigs, Meishan and Landrace pigs, whether there is a sex difference in the constitutive expression of hepatic CYP1A subfamily enzymes, CYP1A1 and CYP1A2, and further investigated the relationship between the physiological serum androgen level and the constitutive expression of the enzymes.

Materials and Methods

Animals

Meishan and Landrace pigs were bred and kept at the National Institute of Livestock and Grassland Science, Tsukuba, Japan. Pigs were fed a commercial grain diet and provided with water ad libitum. Pigs were killed at the age of 1, 2, 3, 4, or 5 months. Some of the male pigs were castrated at the age of 1 month and killed at 5 months of age. After each animal in the experimental groups was killed, a portion of the liver from the animal was quickly removed, frozen in liquid nitrogen, and kept at −80 °C for subsequent analysis.

All animals were handled humanely under the guidelines of the National Institute of Agrobiological Sciences and National Institute of Livestock and Grassland Science (Tsukuba, Japan).

Treatment with testosterone

Testosterone propionate (TP) was purchased from Sigma Chemical Co. TP (50 mg/ml) dissolved in corn oil was injected i.m., five times at a dose of 10 mg/kg body weight/injection, into the rear leg of each pig. Each injection was administered at 48-h intervals. The pigs were killed 24 h after the final injection.

Measurement of serum testosterone level

Blood samples were collected from individual pigs. After clotting at room temperature, the serum was separated from each blood sample by centrifugation at 1500 g for 15 min at 4 °C and stored at −80 °C until use. Serum testosterone levels were measured using the Correlate-EIA Testosterone Enzyme Immunoassay Kit (Assay Designs, Inc., Ann Arbor, MI, USA) according to the manufacturer’s instructions.

Gene expression of hepatic CYP1A subfamily enzymes

Total RNA was prepared from individual livers using TRIzol reagent (Invitrogen Corp.) and used to determine the CYP1A1 and CYP1A2 mRNA levels. The amounts of these mRNAs were measured according to the method described previously (Kojima et al. 2008). Briefly, a portion (4 µg) of total RNA was converted to cDNA in 20 µl of reverse transcription (RT)-reaction mixture using the Super-Script First-Strand Synthesis System for RT-PCR (Invitrogen) and oligo d(T)12–18 according to the manufacturer’s instructions. Semi quantitative RT-PCR was performed in a reaction mixture (25 µl) containing 0.5 µl of the RT-reaction mixture, 200 µM of dNTP, 1U of AmpliTaq Gold, and 200 or 400 nM of each primer (forward and reverse; Table 1). The internal standard used was 18S rRNA (18S). The amplification protocol was as follows: preactivation of AmpliTaq Gold for 10 min at 95 °C and then 17 or 23 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 55 °C, and extension for 30 s at 72 °C for 18S or CYP1A1/CYP1A2, respectively. The PCR products were electrophoresed in 2% agarose gel and visualized using ethidium bromide.

Real-time RT-PCR was performed with an ABI PRISM 7700 Sequence Detection System with SYBR Green Master Mix (PE Applied Systems, Tokyo, Japan) in a total reaction mixture (25 µl) containing 0.5 µl of the RT-reaction mixture and 100 or 200 nM of each primer set (forward and reverse; Table 1). The internal standard used was 18S.

Table 1 Primer pairs used in the present study

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer</th>
<th>Concentration of each primer (nM)</th>
<th>Reference or Accession no.</th>
</tr>
</thead>
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<tr>
<td><strong>For semi-quantitative RT-PCR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A1</td>
<td>atcctggagctcttcgac (forward)</td>
<td>400</td>
<td>Kojima et al. 2008</td>
</tr>
<tr>
<td></td>
<td>ggtatgatccctcagcgctt (reverse)</td>
<td>400</td>
<td>Kojima et al. 2008</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>tggaggagatgttcagcttgag (forward)</td>
<td>400</td>
<td>Kojima et al. 2008</td>
</tr>
<tr>
<td></td>
<td>cttctgtatctcaggatatg (reverse)</td>
<td>400</td>
<td>Kojima et al. 2008</td>
</tr>
<tr>
<td>18S</td>
<td>cgctaccaactcacaagggaag (forward)</td>
<td>200</td>
<td>AY265350</td>
</tr>
<tr>
<td></td>
<td>gctgaatacgccgcgg (reverse)</td>
<td>200</td>
<td>AY265350</td>
</tr>
<tr>
<td><strong>For real time RT-PCR</strong></td>
<td></td>
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<tr>
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<td>AB052254</td>
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<td>NM_001159614</td>
</tr>
</tbody>
</table>

*The primer set was also used for real time RT-PCR at 100 nM of each primer.
the relative standard curve method, as described in PE

The amplification protocol consisted of preactivation of AmpLiTaq Gold for 10 min at 95 °C and then 40 cycles of

denaturation for 15 s at 95 °C, followed by reaction with an HRP-conjugated rabbit anti-goat IgG (Sigma) as a secondary antibody. The bands responsible for CYP1A1 and CYP1A2 were detected with 0.05% 3,3'-diaminobenzidine tetrachloride (Wako Pure Chemical Industries Ltd, Osaka, Japan) as a substrate for HRP.

**CYP1A subfamily enzyme activities**

Hepatic microsomal activities for the ethoxyresorufin O-deethylation (EROD) and methoxyresorufin O-demethylation (MROD), which are mainly catalyzed by CYP1A1 and CYP1A2 respectively (Burke et al. 1985, 1994), were determined as described previously (Kojima et al. 2008). Briefly, a reaction medium (250 μl of 0·1 M sodium phosphate buffer at pH 7·4) containing hepatic microsomes (150 μg) and an NADPH-generating system was preincubated at 37 °C for 10 min. The reaction was started by the addition of 5 μl methoxyresorufin or ethoxyresorufin

**Preparation of hepatic microsomes**

Microsomal fractions were prepared from liver homogenates by differential centrifugations as described previously (Degawa et al. 1985). In brief, livers from individual pigs were homogenized with three volumes (v/v) of 1·15% KCl, and each homogenate was centrifuged at 9000 g for 20 min at 4 °C. Then each supernatant was further centrifuged at 105 000 g for 1 h at 4 °C, and the resultant pellet was homogenized with 1·15% KCl and used as microsomal fractions. The amount of microsomal protein was measured by the method of Lowry et al. (1951).

**Western blot analysis of CYP1A proteins**

Western blot analysis was performed as described previously (Kojima et al. 2008). In brief, each hepatic microsomal preparation (50 μg protein/lane) was separated by 9% SDS-PAGE. The separated proteins were transferred from the gel onto a nitrocellulose membrane (Hybond-ECL; GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) and then immunostained by reaction with a goat anti-rat CYP1A2 serum (Antiserum for human CYP1A1/1A2; Daiichi Pure Chemicals, Tokyo, Japan) as a primary antibody followed by reaction with a HRP-conjugated rabbit anti-goat IgG (Sigma) as a secondary antibody. The bands responsible for CYP1A1 and CYP1A2 were detected with 0·05% 3,3'-diaminobenzidine tetrachloride (Wako Pure Chemical Industries Ltd, Osaka, Japan) as a substrate for HRP.

**Figure 1** Hepatic gene expression of CYP1A1 and CYP1A2 in 5-month-old pigs. Total RNAs were prepared from individual livers of three pigs in each experimental group; intact male (M), castrated male (CM), and intact female (F). (A) Expression patterns for the CYP1A1 and CYP1A2 mRNAs in individual pigs. Equal volumes of RT-PCR products were subjected to electrophoresis on a 2% agarose gel, and the separated bands were visualized by u.v. fluorescence of ethidium bromide. (B) Expression levels of CYP1A1 and CYP1A2 mRNAs were determined by real-time RT-PCR. The levels were normalized to that of 18S, an internal standard. Each column represents the mean in each experimental group, and each bar represents the s.d. of the mean (n = 3). **a,b**Significant differences from the breed-matched intact male pigs assessed by Tukey’s post hoc test: *P*<0·05 and *P*<0·01.

**Figure 2** Effect of testosterone propionate (TP) treatment on the expression levels of hepatic CYP1A1 and CYP1A2 mRNAs in 5-month-old castrated male and female pigs. TP was injected i.m. to 5-month-old castrated male and intact female Meishan and Landrace pigs, as described in ‘Materials and Methods’. (A) Expression patterns for the CYP1A1 and CYP1A2 mRNAs in individual pigs. Equal volumes of RT-PCR products were subjected to electrophoresis on a 2% agarose gel, and the separated bands were visualized by u.v. fluorescence of ethidium bromide. In addition, electrophoresis pattern of the RT-PCR product from intact male Meishan pigs was also shown as a reference. (B) Expression levels of CYP1A1 and CYP1A2 mRNAs were determined by real-time RT-PCR. The levels were normalized to that of 18S, an internal standard. Each column represents the mean in each experimental group, and each bar represents the s.d. of the mean (n = 3). **a,b**Significant differences from the corresponding TP untreated pigs assessed by Student’s t-test: *P*<0·05 and *P*<0·01.

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(250 μM) dissolved in dimethylsulfoxide. After incubation at 37°C for 10 min, 250 μl cold ethanol was added to stop the reaction. The reaction mixture was centrifuged for 10 min at 2500 g, and the amount of resorufin formed in the resulting supernatant was measured using a Wallac 1420 AR VOSx Multilabel Counter (Perkin Elmer Life Sciences, Wellesley, MA, USA) at an excitation wavelength of 550 nm and an emission wavelength of 590 nm. In addition, the production of resorufin from methoxyresorufin or ethoxyresorufin increased in a reaction time-dependent manner at least for the first 10 min.

Statistical analysis

Significant differences were evaluated using Student’s t-test or Tukey’s post hoc test after ANOVA.

Results

Gene expression levels of CYP1A subfamily enzymes

Sex differences in the constitutive gene expression levels of hepatic CYP1A subfamily enzymes were first examined by RT-PCR in two breeds of pigs, Meishan and Landrace, at 5 months of age. In Meishan pigs, the CYP1A1 and CYP1A2 mRNAs were clearly detected in females but faintly in males, and castration of the males led to a female-type gene expression pattern (Fig. 1A). These results were identified with our previous report (Kojima et al. 2008). On the other hand, in Landrace pigs, these mRNAs were clearly detected even in males, and there were no sex differences.

The relative levels of hepatic CYP1A1 and CYP1A2 mRNAs were measured by real-time RT-PCR (Fig. 1B). In male Meishan pigs, these CYP1A mRNA levels were <10% of those in the castrated male and female pigs. On the other hand, in Landrace pigs, no such significant differences were observed among males, castrated males, and females.

Effects of testosterone treatment on the gene expression of hepatic CYP1A subfamily enzymes

To clarify whether or not androgen suppresses the gene expression of hepatic CYP1A subfamily enzymes, we administered TP to the castrated males and intact females of both 5-month-old Meishan and Landrace pigs. Treatments with TP led to marked decreases in the levels of CYP1A1 and CYP1A2 mRNAs in both breeds of pigs (Fig. 2A), and consequently, these mRNA levels in both breeds of pigs became <10% of the corresponding control (TP untreated) pigs (Fig. 2B). Incidentally, the CYP1A mRNA levels in the TP-treated pigs were almost the same as those of 5-month-old male Meishan pigs.

Activities of hepatic CYP1A subfamily enzymes

In both Meishan and Landrace pigs, CYP1A-mediated enzyme activities were comparatively examined in males, castrated males, and females. The enzyme assays were performed using ethoxyresorufin and methoxyresorufin as
substrates for CYP1A1 and CYP1A2 respectively (Burke et al. 1985, 1994). In female Meishan pigs, the activities of EROD and MROD were about 15- and 9-fold higher, respectively, than those in males (Fig. 3). In the castrated male Meishan pigs, these enzyme activities were similar to those in females. On the other hand, in Landrace pigs, no such significant differences were observed among males, castrated males, and females. Furthermore, in the castrated males and intact females of each breed of pig, treatments with TP led to an extreme reduction in the EROD and MROD activities.

Western blot analyses of hepatic CYP1A apoproteins

In both Meishan and Landrace pigs, the levels of hepatic CYP1A1 and CYP1A2 apoproteins were compared among males, castrated males, and females. Western blot analyses of CYP1A apoproteins were performed using a goat anti-rat CYP1A2 serum, which is cross-reactive with human CYP1A1 and CYP1A2. The bands corresponding to CYP1A1 and CYP1A2 were identified on the basis of Myers’ report (Myers et al. 2001). In 5-month-old Meishan pigs, the CYP1A apoproteins were clearly detected in the castrated males and females, but not in males (Fig. 4). On the other hand, in 5-month-old Landrace pigs, only the one band responsible for CYP1A2 apoprotein was detected in males, castrated males, and females, and their amounts were similar. Furthermore, treatment with TP almost completely diminished the CYP1A apoprotein(s) in all the pigs examined.

Correlation between the serum testosterone level and the hepatic gene expression of CYP1A subfamily enzymes

To clarify the relationship between the serum testosterone level and the levels of hepatic CYP1A subfamily enzymes, we first examined the serum testosterone level in 5-month-old Meishan and Landrace pigs. Serum testosterone levels in male Meishan pigs were about threefold higher than those in male Landrace pigs (Fig. 5). In addition, in both Meishan and Landrace pigs, serum testosterone levels in the castrated males and females were significantly lower than those in the corresponding male pigs.

Figure 5 Serum testosterone levels in 5-month-old pigs. Blood samples were collected from individual intact male (M), castrated male (CM), and intact female (F) pigs. Each column represents the mean in each experimental group, and each bar represents the s.d. of the mean (n = 3). *Significant difference between intact male Meishan and Landrace pigs assessed by Student’s t-test: *P < 0.01. **Significant differences from the breed-matched intact male pigs assessed by Tukey’s post hoc test: **P < 0.01.

Figure 6 Age-dependent changes in serum testosterone levels (A) and levels of hepatic CYP1A1 (B) and CYP1A2 (C) mRNAs in pigs. Blood samples and livers were collected from individual male Meishan (n = 3, 4, 5, 5, and 9 for 1-, 2-, 3-, 4-, and 5-month-old pigs respectively) and Landrace pigs (n = 3, 4, and 5 for 1-, 3-, and 5-month-old pigs respectively). Gene expression levels of CYP1A1 and CYP1A2 were measured by real-time RT-PCR and normalized to that of 18S. Closed and open circles represent the means for each experimental group in Meishan and Landrace pigs respectively. Each bar represents the s.d. of the mean. *Significant differences from the age-matched male Landrace pigs assessed by Student’s t-test: *P < 0.01.
We next examined the age-dependent changes in the levels of serum testosterone and hepatic CYP1A1 and CYP1A2 mRNAs in male Meishan and Landrace pigs. In Meishan pigs, the serum testosterone level increased in an age-dependent fashion up to 4 months of age (Fig. 6A), and this increased level remained at least up to 5 months of age. The testosterone levels in 4- and 5-month-old Meishan pigs were about 50 ng/ml. The levels in 3- and 5-month-old Landrace pigs were significantly lower than those in the age-matched Meishan pigs, although no breed difference in the level of serum testosterone was observed in 1-month-old pigs. The level of testosterone in 5-month-old Landrace pigs was about 18 ng/ml and almost the same as that in 2-month-old Meishan pigs.

There were no significant differences in the levels of hepatic CYP1A1 and CYP1A2 mRNAs between 1-month-old Meishan and Landrace pigs (Fig. 6B and C). These CYP1A mRNA levels in Meishan pigs decreased in an age-dependent fashion from 3 months of age, and the levels at 4 and 5 months of age were below 10% of the level at 1 month of age. On the other hand, such age-dependent decreases were considerably less pronounced in Landrace pigs. In addition, when serum testosterone levels in 1-month-old Landrace pigs (Fig. 7A) were increased by treatment with TP to almost the same as those in 4–5-month-old male Meishan pigs (Fig. 6A), levels of CYP1A1 and CYP1A2 mRNAs (Fig. 7B and C) decreased to almost the same as those in male 5-month-old Meishan pigs (Figs 1B and 2B).

On the basis of the data shown in Fig. 6, we examined the relationship between the serum testosterone level and the levels of hepatic CYP1A1 and CYP1A2 mRNAs. Negative correlations between these parameters were shown (Fig. 8A). A threshold of serum testosterone for androgen-mediated down-regulation of hepatic CYP1A subfamily genes was in the range of 30–40 ng/ml. Particularly, when serum testosterone level exceeded about 33 ng/ml, clear decreases in the levels of CYP1A mRNAs were observed. In addition, there was a positive correlation between the levels of CYP1A1 and CYP1A2 mRNAs (Fig. 8B).

Discussion

We have previously suggested that androgen suppresses the expression of hepatic CYP1A subfamily enzymes (Kojima et al. 2008). In the present study, we demonstrated for the first time that physiological level of serum testosterone regulates the constitutive gene expression of hepatic CYP1A subfamily enzymes in pigs.

Interestingly, sex differences in the constitutive gene expression of hepatic CYP1A subfamily enzymes were found in 5-month-old Meishan pigs, but not in Landrace pigs. Furthermore, in contrast to male Meishan pigs, no significant effects of castration on the expression of the CYP1A enzymes were observed in male Landrace pigs. On the other hand, in both 5-month-old Landrace and Meishan pigs, TP treatments of either castrated male or intact female pigs resulted in drastic decreases in expression levels of hepatic CYP1A1 and CYP1A2. These results suggest that a high level of serum testosterone suppresses the expression of CYP1A subfamily enzymes in pigs.
To clarify a relationship between the physiological serum androgen level and the constitutive expression levels of hepatic CYP1A1 and CYP1A2, we examined the age-dependent changes in these parameters in male pigs. Between 1-month-old Meishan and Landrace pigs, there were no significant differences in serum testosterone level and hepatic levels of the CYP1A mRNAs. A clear age-dependent increase in serum testosterone level was observed in Meishan pigs, and at 3 and 5 months of age, the levels were about 30 and 50 ng/ml respectively. On the contrary, decreases in the levels of hepatic CYP1A1 and CYP1A2 mRNAs occurred from 3 months of age in an age-dependent fashion. On the other hand, in Landrace pigs, no age-dependent changes in the levels of the CYP1A mRNAs occurred, although serum testosterone level slightly increased in an age-dependent manner. Serum testosterone level (about 18 ng/ml) in 5-month-old Landrace pigs was only about 36% of that in 5-month-old Meishan pigs. In addition, even in 1-month-old Landrace pigs, the administration of TP, which increases serum testosterone level to almost the same as those in 4–5-month-old male Meishan pigs, resulted in remarked decreases in the levels of hepatic CYP1A1 and CYP1A2 mRNAs. Such inhibitory effects of TP in 1-month-old Meishan pigs have been previously reported (Kojima et al. 2008).

The correlation analyses between serum testosterone level and hepatic gene expression levels of CYP1A1 and CYP1A2 revealed that there was a threshold for androgen-mediated down-regulation of hepatic CYP1A genes, and the clear down-regulation occurred at concentrations of more than 33 ng/ml of serum testosterone. These findings propose a hypothesis that the serum androgen level is one of the critical factors, which lead to the sex difference in the constitutive expression of hepatic CYP1A subfamily enzymes in pigs, although the secretion profile of GH is considered to be a main factor for determining the sex difference in the constitutive expression level of several CYPs (Agrawal & Shapiro 2001, Mode & Gustafsson 2006, Waxman & Holloway 2009). This hypothesis is further supported by the present findings that in spite of sex difference in the secretion profile of GH in Landrace pigs (Arbona et al. 1988), there was no sex difference in the constitutive expression of hepatic CYP1A subfamily enzymes. On the other hand, the modification of the secretion profile of GH by androgen administration had been reported (Davis et al. 1977, Somana et al. 1978, Link et al. 1986, Dubreuil et al. 1989). Accordingly, drastic decreases in the levels of CYP1A subfamily enzymes in androgen-treated Landrace pigs might occur, at least in part, through the change in secretion profile of GH, especially the emphasis of a male-type secretion profile.

The transcriptional activation of the CYP1A subfamily genes is well known to occur through binding of a ligand-activated aryl hydrocarbon receptor (AhR) to xenobiotic-responsive elements (XREs; Hankinson 1995, Denison & Nagy 2003). Our analyses of GenBank database revealed that CYP1A1 and CYP1A2 genes in pigs, as well as in other mammals (Corchero et al. 2001, Nukaya & Bradfield 2009), were located on the same chromosome and orientated head to head. An intergenic spacer region between these genes contains several XREs. On the other hand, we could not find
androgen receptor (AR)-binding element in the spacer region. Recently, it has been suggested that complex formation between activated AR and AhR plays an important role in suppression of the AhR-mediated transcription of CYP1 family genes (Sanada et al. 2009). Therefore, the androgen-mediated down-regulation observed in this study is thought to occur, at least in part, through a complex formation between AR and AhR. However, since no sex differences in the constitutive expression of CYP1A subfamily enzymes were observed in Landrace pigs, further studies are needed for understanding an exact mechanism underlying the androgen-mediated down-regulation of the CYP1A subfamily genes.

The levels of hepatic CYP1A1 and CYP1A2 apoproteins were not necessarily correlated with those of their mRNAs and enzyme activities. Particularly, in Landrace pigs, the band responsible for CYP1A1 apoprotein was not detected, despite its mRNA being clearly detectable. These findings suggest a disorder in the post-transcriptional process in the pig. The EROD activity in Landrace pigs might be exclusively dependent on the expression level of CYP1A2 because it shows not only a high activity of MROD but also a moderate activity of EROD (Burke et al. 1985, 1994, Messina et al. 2008). In Meishan pigs, the EROD and MROD activities were positively correlated with the expression amounts of CYP1A1 and CYP1A2 respectively, at both the levels of mRNA and apoprotein. CYP1A enzymes play an important role in the metabolism of xenobiotics including carcinogens and drugs (Guengerich 2003, Androutsopoulos et al. 2009), suggesting that androgen-mediated suppression of hepatic CYP1A genes would influence the chemical-induced toxicities and therapeutic effects of drugs among individual animals including human.

In conclusion, we demonstrated for the first time that the serum androgen level is one of the physiological factors regulating the constitutive expression of the CYP1A subfamily enzymes, CYP1A1 and CYP1A2, in pigs. Furthermore, we suggested that the androgen-mediated down-regulation of hepatic CYP1A subfamily enzymes occurs at concentrations of more than 33 ng/ml of serum testosterone in pigs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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