Maternal Exposure to Low Doses of DES Altered mRNA Expression of Hepatic Microsomal Enzymes in Male Rat Offspring

Osamu NISHIKAWA1, Kazuyoshi ARISHIMA1,*, Tetsuo KOBAYASHI1, Mitsuyuki SHIRAI2, Masaru MURAKAMI3, Motoharu SAKAUE1 and Masako YAMAMOTO3

1)Department of Anatomy II, School of Veterinary Medicine, Azabu University, 1–17–7 Fuchinobe, Chuo, Sagamihara, Kanagawa 252–5201, Japan
2)Department of Veterinary Pharmacology, School of Veterinary Medicine, Azabu University, 1–17–7 Fuchinobe, Chuo, Sagamihara, Kanagawa 252–5201, Japan
3)Department of Molecular Biology, School of Veterinary Medicine, Azabu University, 1–17–7 Fuchinobe, Chuo, Sagamihara, Kanagawa 252–5201, Japan

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ABSTRACT. Our previous studies demonstrated that prenatal diethylstilbestrol (DES) treatment disrupts steroidogenesis but induces high-level expression of androgen receptor (AR) mRNA to inhibit the disruption of spermatogenesis. This study examined which prenatal DES treatment influenced hepatic microsomal enzymes, CYP3A1, CYP2B1/2, CYP2C11, UGT2B1 (UDP-glucuronosyltransferase 2B1), and IGF-1 (insulin-like growth factor-1), in male rat offspring. DES treatment decreased the mRNA expression levels of CYP3A1 and CYP2B1/2, but did not alter the expression of CYP2C11. At 6 weeks, DES treatment increased the mRNA expression levels of UGT2B1 and IGF-1. These results suggest that prenatal DES treatment alters two hepatic enzymes (CYP3A1 and CYP2B1/2) and IGF-1 mRNA expression levels to counteract the low level of testosterone, but this disrupted UGT2B1 mRNA expression reduces the testosterone level.

KEY WORDS: CYP, DES, IGF-1, liver, rat.


Diethylstilbestrol (DES), a synthetic non-steroidal estrogen, was widely used as one of the best medications for preventing threatened abortion in the late 1940s and 1950s. However, Kaplan [13] first reported that DES may have affected the normal development of the reproductive system in male offspring. Since then, many reports have appeared on the undesirable effects of DES on the reproductive systems of men and women [12] as well as experimental animals [1, 19]. Numerous studies have used prenatal or postnatal exposure to DES, mostly in high-dose ranges from 10 to 300 mg/kg, to induce gross adverse changes in the developing male reproductive system, e.g., testicular cancer, reduced testicular size and sperm production [10, 19, 20, 24].

We administered doses of DES much lower (1.5 μg/kg) than those previously applied [10, 11, 29] to pregnant rats at days 7–21 of gestation (in the second and third trimesters), and demonstrated that DES induced not only suppression of plasma testosterone levels in adolescent male offspring (6 weeks after birth), but also promoted follicular maturation in female offspring [36, 37]; in addition, maternal DES treatment disrupted steroidogenesis but increased the expression level of androgen receptor (AR) mRNA to inhibit the disruption of spermatogenesis to thereby counteract the low level of testosterone in testis [15].

*CORRESPONDENCE TO: ARISHIMA, K., Department of Anatomy II, Azabu University, School of Veterinary Medicine, 1–17–71 Fuchinobe, Chuo, Sagamihara, Kanagawa 252–5201, Japan. e-mail: arishima@azabu-u.ac.jp

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includes multiple isoforms that catalyze the glucuronidation of exogenous and endogenous compounds. The two families of UGT, UGT1 and UGT2, consist of more than 35 enzymes found in various species [14, 18]. Glucuronidation of steroids leads to termination of biological activity and elimination from the body. Among the isozymes, the UGT2B subfamily is more specific to steroid hormones [38].

We have investigated the effects of maternally administered DES on offspring, but have yet to evaluate the reaction of the offspring’s liver to DES. In this study, we aimed to elucidate the responsiveness of steroid-metabolizing enzymes in the liver of mature and immature male offspring of mothers who received low doses of DES during pregnancy.

Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were given a commercial diet (CE-2, CLEA, Tokyo, Japan) and water, both ad libitum. Females were mated with males overnight and were examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was detected was counted as day 0 of gestation. Pregnant rats were housed individually and maintained in a 12/12 hr light-dark cycle at room temperature of 21 ± 2°C and humidity of 55 ± 5%. Pregnant rats were divided into three groups, respectively (DES 0.5 and DES 1.5 µg/kg/day and Control group) dissolved in corn oil (tocopherol-stripped, ICN Biomedicals In c., Aurora, OH, U.S.A.) or corn oil alone (Control group) on days 7–21 of gestation.

Levels of significance were analyzed by Fisher’s one-way analysis of variance. All values are expressed as the mean ± standard error of the mean (SEM). Differences are considered significant at P<0.05.

Table 1. PCR primers for the detection of various gene expressions used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction</th>
<th>Sequence (5’–3’)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A1</td>
<td>Forward</td>
<td>ATCCGATATGGAGATCAC</td>
<td>580</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GAAGAAGTCTTGTTGTCG</td>
<td></td>
</tr>
<tr>
<td>CYP2B1</td>
<td>Forward</td>
<td>GAGTTCTTCTCTGGTGTTCTG</td>
<td>549</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>ACTGTTGGTCTATGGGAGTCG</td>
<td></td>
</tr>
<tr>
<td>CYP2C11</td>
<td>Forward</td>
<td>TGCCCCTTTTTTACGAGGCT</td>
<td>367</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GGAACAGATGACTCTGAATCTCT</td>
<td></td>
</tr>
<tr>
<td>UGT2B1</td>
<td>Forward</td>
<td>AATCACATGGTAGCCAAAGGA</td>
<td>288</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GACCAATAGGAAACCAATGACA</td>
<td></td>
</tr>
<tr>
<td>IGF-1</td>
<td>Forward</td>
<td>GTGCTCCGCTGAAAGGCTAC</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AGGTCCTGTTCTCTGACATC</td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>Forward</td>
<td>CACGCTTCTTCTCTGGGTATG</td>
<td>246</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TAGAGCCACCAATCCACACAG</td>
<td></td>
</tr>
</tbody>
</table>
groups (Fig. 2D–F). At 6 weeks of age, CYP2B1/2 mRNA expression levels were significantly lower in the DES 1.5 than in the other two groups (Fig. 3). At 1 and 3 weeks of age, CYP2C11 mRNA expression was not detected. At 6 and 15 weeks, CYP2C11 mRNA expression levels remained unchanged after DES administration (Fig. 3). At 6 weeks of age, UGT2B1 mRNA expression levels were significantly higher in the DES 1.5 than in the Control group (Fig. 4). At 15 weeks of age, UGT2B1 mRNA expression levels were significantly lower in the DES 1.5 and 0.5 groups than in the Control group (Fig. 4). At 1 week of age, IGF-1 mRNA expression was not detected. At 6 weeks of age, IGF-1 mRNA expression levels were significantly higher in the DES 1.5 and 0.5 groups than in the Control group (Fig. 5).

In our previous study [15], DES, administered during the fetal stage, reduced plasma LH level for three weeks after birth, the plasma testosterone levels of the DES1.5 (6 weeks old) and DES0.5 (15 weeks old) groups decreased, but the release of gonadotropins from the pituitary gland did not increase in response to this change. To compensate for the decreased testicular testosterone level due to DES, the AR (androgen receptor) mRNA expression level in the testicles increased, and the spermatogenesis in the testicles was rescued from the inhibitory effects of DES. However, the altered mRNA expression levels of enzymes associated with testicular steroidogenesis were found to be not directly correlated with the decreased plasma testosterone levels [15]. Therefore, we considered that the steroid metabolism in the liver, responsible for the maintenance and excretion of circulating hormones, might be altered by DES and contribute to the decreased plasma testosterone level. In this experiment, we investigated CYP3A1, CYP2B1/2, and CYP2C11 mRNA expression levels closely associated with steroid metabolism, the normal developmental changes in the mRNA expressions of these three hepatic cytochrome P450s were very similar to those in the previous report [40].

DDE induces hepatic CYP enzymes (CYP2B1, 3A1, and 2C11), which inactivate circulating androgens in the liver by their hydroxylating actions [39]. DDE could modulate the expression and activity levels of liver CYP enzymes in developing rats at early postnatal stages when DDE was given to pregnant dams during late gestation. For male rats, CYP2B1 and 3A1 increased in response to the DDE treatment at both infantile and adult stages, whereas CYP2A1, as a testosterone 7α-hydroxylase, increased only in the neonatal period and CYP2C11 decreased in the adult stage [39]. However, maternal treatment with DDE did not affect a newborn’s testosterone production, no direct evidence is available to show whether the circulating androgen levels are indeed affected by the DDE-induced alterations of testosterone hydroxylase activities. DES administered to a pregnant mother in our experiment, tended to decrease CYP3A1 and CYP2B1/2 mRNA expression levels and plasma testosterone levels at 6 weeks of age. These observations suggest that CYP3A1 and CYP2B-2/1 in the DES group were downregulated by circulating hormones to maintain the normal level of testosterone.

CYP2C11 is an enzyme specifically expressed only in male rats, a major isoform of the CYP family in the liver [30, 39]. In our study, CYP2C11 was not expressed for three weeks after birth as previously reported [30] and CYP2C11 expression was not altered in response to the decreased testosterone due to DES. Previous studies report gender-divergent mRNA expression patterns for both rat and mouse UGTs [4, 25]. Gonadectomy decreased mRNA expression of UGT2B1 in male mouse liver; testosterone treatment of gonadectomized mice increased this expression [5]. This report suggests that UGT2B1 mRNA expression in liver was regulated by testosterone. In our experiment, UGT2B1 mRNA increased in the liver of the DES1.5 group at 6 weeks after birth, but decreased in the liver of DES groups at 15 weeks; these changes did not parallel those of testosterone levels in our previous study [15]. These results suggest that maternal DES treatment might disrupt metabolic enzyme, UGT2B1, expressions in the liver of offspring to decrease the plasma testosterone level at 6 weeks of age.

Absence of IGF-1 leads to poor prenatal growth [31], and intrauterine growth restriction decreases serum IGF-1 [8]. Some studies demonstrated that GH plays a role in gonadal...
steroidogenesis and gametogenesis [2], exerting endocrine activity either directly at gonadal sites or indirectly via IGF-1 [9]. IGF-1 and its receptor mRNAs are highly expressed in testicular Leydig cells [16]. IGF-1-deficient mouse model confirmed that liver is indeed the major source of circulating IGF-1 [6, 17, 22]. Deficiency of liver-derived IGF-1 reduces the histological compartments of the prostate and decreases AR expression in prostate [27]. Because maternal DES treatment did not impair spermatogenesis despite the resulting low level of testosterone [15], we determined the level of liver-derived IGF-1. As a result, the IGF-1 level of

Fig. 2. Histomicrographs of the livers at 15 weeks. A-C: Sections of the livers stained with Hematoxylin and Eosin. There are no histological changes in livers of the DES 0.5 and DES 1.5 groups (B and C) to compared to the liver of Control group (A). Scale bar, 50 μm. D-F: Sections of the livers stained with anti-CYP3A1 serum. Many CYP3A1-positive hepatocytes are observed in surrounding the central veins (*) in Control group (D). In DES 0.5 and DES 1.5 groups (E and F), CYP3A1-positive hepatocytes are observed in small numbers. Scale bar, 200 μm.
DES ALTERS HEPATIC MICROSOMAL ENZYMES

the DES group increased at 6 and 15 weeks of age, suggesting that the spermatogenesis in the DES group may have been maintained in part by IGF-1 from liver to compensate for the decreased plasma testosterone level due to DES.

In conclusion, maternal DES treatment decreased plasma testosterone, followed by the decreased expression levels of CYP3A1 and CYP2B1/2 to maintain the normal plasma testosterone level and the increased expression of IGF-1 to maintain the normal spermatogenesis, but DES treatment disrupted the expression level of UGT2B1, reducing the testosterone level at 6 weeks of age. We demonstrated the pos-
sibility that prenatal DES treatment may disrupt the expression of the microsomal enzyme, UGT2B1, and this change induced in part the low levels of plasma testosterone. However, at 15 weeks of age, some changes in mRNA expression levels of metabolic enzymes and the growth factor do not explain the decreased levels of testosterone in the DES 0.5 group.

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