Histopathological Effect of Ketoconazole on Rat Placenta

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ABSTRACT. In order to investigate the morphological effects of ketoconazole on hypertrophied placentas, we examined the sequential histopathological changes in the placenta from rats exposed to ketoconazole. Ketoconazole was administered orally at 0 and 25 mg/kg/day during gestation days (GDs) 12 to 14, and the placentas were sampled on GDs 15, 17 and 21. All dams showed neither effect on body weight nor any abnormal clinical signs during the experimental period. In the treated group, the placentas appeared more hypertrophic with increases in the weight, diameter and thickness on GD 21. Histopathologically, increased thickness was noted in the labyrinth zone and basal zone on GDs 17 and 21, while on GD 15 the change had been already evident in the former zone. In the labyrinth zone, the mitotic figures of the trophoblasts were significantly elevated on GD 15. A multiple cystic dilatation of maternal sinusoids was observed in some placentas on GDs 15, 17 and 21. In the basal zone, an increase in spongiotrophoblasts and clusters of glycogen cells were detected on GDs 17 and 21. In the decidua basalis, there were no significant changes in either histology or thickness between the control and treated group during GDs 15 to 21. In conclusion, ketoconazole increased the population of composed cells in the labyrinth and basal zone, leading to placental hypertrophy in pregnant rats.

KEY WORDS: hypertrophy, ketoconazole, placenta, rat.

The placenta secures the embryo and fetus to endometrium and releases a variety of steroids, hormones and cytokines. It is also responsible for maternoembryonic exchange of O2/CO2 and nutrient/metabolite requirements during embryonic development, and serves as a protective barrier of xenobiotics. Although placenta is a transient organ, its growth and function play important roles in the maintenance of pregnancy and fetal growth. Therefore, even a subtle alteration or deviation of function in the placenta induced by chemicals may lead to miscarriage and/or fetal death. Whereas, overgrowth of placentas is induced with/without fetal effects under various conditions, such as hormonal incoordination [2, 8], unfavorable maternal environment, facilitating compensatory recovery of fetal-growth retardation [3, 4, 17, 18], and others. Placental hypertrophy is also detected in the intact uterus with less than six fetuses in rats [7]. Experimentally, placental hypertrophy is induced by a reduction in the number of corpora lutea [15], ovariectomy with estrogen and progesterone treatment [6], and exposure to some chemicals, such as ethanol [1, 10], indomethacin [27], methylhydrazine [13], etc.

Ketoconazole, an imidazole compound, has been used as an antifungal agent for candiduria, coccidioidomycosis, histoplasmosis, chromomycosis and paracoccidiomycosis [14]. It is known that ketoconazole interferes with the synthesis and permeability of fungal cell membranes [26]. The mechanism of action involves inhibition of the cytochrome P450 (CYP) enzyme responsible for conversion of lanosterol to ergosterol, the major sterol of most fungal cell membranes.

There have been evidences of adverse effects of ketoconazole on pregnancy in mammals. The administration of ketoconazole during gestation days (GDs) 6 to 21 in rats and during GDs 6 to 18 in mice results in a high incidence of resorption, increase in stillbirth, delayed parturition, reduction in birth weight, and delayed descensus testis and vaginal opening [5]. It is also reported that ketoconazole induced placental hypertrophy associated with reduced plasma estradiol level in pregnant rats [16]. However, there have been no reports on the histopathology of the placenta exposed to ketoconazole. In the present report, we examined the sequential histopathological changes in the placenta from rats exposed to ketoconazole during GDs 12 to 14.

MATERIALS AND METHODS

Animals: A total of 24 pregnant specific pathogen-free Crlj:CD(SD) rats (Charles River Laboratories Japan, Inc., Japan), at approximately 10–14 weeks of age, were purchased. The animals were single-housed in wire-mesh cages in an air-conditioned room (22 ± 2°C; humidity, 55 ± 10%; light cycle, 12 hr/day). Feed (CRF-1: Oriental Yeast Co., LTD., Japan) and water were available to the animals ad libitum.

Experimental design: GD 0 was designated as the day when the vaginal plug was identified. The pregnant rats were randomly allocated to 2 groups of 12 rats each. Ketoconazole (Sigma, U.S.A.) was suspended in olive oil and treated to the group at 0 (control) or 25 mg/kg/day with a volume of 1 ml/100g B.W. during GDs 12 to 14. The dose level and treated period in this study were previously reported to induce placental hypertrophy in rats [16]. All treatments were made between 10 and 11 a.m. Maternal body weight was recorded on GDs 0, 6, 11, 12, 13, 14, 15,
17, 18 and 21. Four dams each from the control and treated groups were euthanized by exsanguination under diethyl ether anesthesia, and necropsied on GDs 15, 17 and 21. All embryos/fetuses and half of the placentas were removed from the uterus and weighed. The placentas removed from each dam were fixed in 10% neutral buffered formalin and measured major and minor axis and thickness on the fixed samples.

Histopathological examination: Five placentas, which were not removed, were selected randomly from each dam. These tissues were embedded in paraffin, sectioned 4-µm in thickness, and stained routinely with hematoxylin and eosin (H&E) for histopathological examination. Immunohistochemical staining, in situ TdT-mediated dUTP nick end labeling (TUNEL) (ApopTag®: Chemicon International, U.S.A.), was performed. The thickness of the labyrinth zone, basal zone and decidua basalis close to the central portion were measured with the aid of an image analyzer (IPAP; Processor for Analytical Pathology, Sumika Technoservice Co. Japan). The number of mitotic cells and apoptotic cells (except the giant cells) in the labyrinth and basal zone were counted in ten different fields (× 400) for each placenta on GDs 15, 17 and 21.

Statistical analysis: Means and standard error (SE) of the individual litter values were calculated. Continuous data were analyzed with the F test. When variances were homogeneous, the Student t-test was performed. The Aspin-Welch t-test was performed when variances were not homogeneous. The Wilcoxon rank sum test was employed for nonparametric data, such as the dead embryo/fetus ratio. The level of significance was evaluated at $P<0.05, P<0.01$ or $P<0.001$.

These experiments were conducted according to the Guidelines for Animal Experimentation, Japanese Association for Laboratory Animal Science, 1987.

RESULTS

All dams showed neither effects on body weight nor any abnormal clinical signs during the experimental period.

Table 1 shows the total number and weight of live embryo/fetus, dead embryo/fetus ratio and placenta weight at each sampling time point. There were no effects on the dead fetus ratio on each sampling time and no fetal abnormalities macroscopically on GD 21 in the treated group. In the treated group, the fetus weight on GD 17 were significantly higher than those in the control group. The placental weight, diameter and thickness on GDs 15, 17 and 21 were statistically higher than those in the control group, and the placentas macroscopically exhibited hypertrophy on GD 21 (Fig. 1a).

Histopathologically, in the treated group, increased in thickness was noted significantly in the labyrinth zone and basal zone on GDs 17 and 21, while on GD 15 the change had been already evident in the former zone (Fig. 2). Mitosis of the trophoblasts was increased significantly in the labyrinth zone on GD 15 (Fig. 1b), while on the contrary it was significantly decreased on GD 17 (Fig. 3). However, there was no change in the number of apoptotic cells during GDs 15 to 21 in both labyrinth and basal zone. In the labyrinth zone, a multiple cystic dilatation of maternal sinusoids was observed in some placentas on GDs 15, 17 and 21 (Fig. 1c). There also was a deposition of fibrin in the dilated maternal sinusoids at the central region under the amnion. In the basal zone, an increase in spongiotrophoblasts and clusters of glycogen cells was seen on GDs 17 and 21, which was particularly remarkable at the edge of the basal zone (Figs. 1d, 1e). In the decidua basalis, there were no changes in morphology and no significant differences in thickness during GDs 15 to 21 between the control and treated group.

DISCUSSION

The present results indicated that placental hypertrophy was found following oral administration of ketoconazole to dams at 25 mg/kg/day during GDs 12 to 14. Histopathologically, ketoconazole treatment mainly evoked an elevated mitotic activity of trophoblasts in the labyrinth zone on GD 15, and an increase in spongiotrophoblasts and clusters of glycogen cells in the basal zone on GDs 17 and 21.

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Table 1. Effect of ketoconazole on embryos/fetuses and placentas

<table>
<thead>
<tr>
<th>Gestation day</th>
<th>Group</th>
<th>No. of dams</th>
<th>Total No. of live embryos/fetuses</th>
<th>Dead embryo/fetus ratio (%)</th>
<th>Embryo/fetus weight (g)</th>
<th>Weight (g)</th>
<th>Major axis (mm)</th>
<th>Minor axis (mm)</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Control</td>
<td>4</td>
<td>60</td>
<td>1.6 ± 1.6</td>
<td>0.265 ± 0.006</td>
<td>0.220 ± 0.009</td>
<td>11.4 ± 0.12</td>
<td>10.6 ± 0.13</td>
<td>2.6 ± 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>4</td>
<td>63</td>
<td>7.1 ± 2.7</td>
<td>0.268 ± 0.006</td>
<td>0.257 ± 0.014$</td>
<td>12.4 ± 0.13**</td>
<td>11.3 ± 0.23*</td>
<td>3.0 ± 0.27*</td>
</tr>
<tr>
<td>17 Control</td>
<td>4</td>
<td>62</td>
<td>3.3 ± 3.3</td>
<td>0.765 ± 0.014</td>
<td>0.368 ± 0.012</td>
<td>13.5 ± 0.28</td>
<td>12.4 ± 0.22</td>
<td>3.5 ± 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>4</td>
<td>56</td>
<td>5.0 ± 3.2</td>
<td>0.854 ± 0.029*</td>
<td>0.540 ± 0.035**</td>
<td>14.8 ± 0.35*</td>
<td>13.4 ± 0.32*</td>
<td>3.9 ± 0.03*</td>
</tr>
<tr>
<td>21 Control</td>
<td>4</td>
<td>56</td>
<td>4.7 ± 4.7</td>
<td>5.165 ± 0.174</td>
<td>0.449 ± 0.019</td>
<td>14.2 ± 0.14</td>
<td>12.6 ± 0.15</td>
<td>3.4 ± 0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>4</td>
<td>62</td>
<td>0.0 ± 0.0</td>
<td>5.455 ± 0.048</td>
<td>0.663 ± 0.029***</td>
<td>15.7 ± 0.31**</td>
<td>13.7 ± 0.28*</td>
<td>4.2 ± 0.12***</td>
</tr>
</tbody>
</table>

Mean ± SE.
a): Mean of individual litter values.
*,**,**,**: Significantly different from control at $P<0.05, P<0.01, P<0.001$, respectively (Student t-test).
$^5$: Significantly different from control at $P<0.05$ (Aspin-Welch t-test).
As mentioned in the introduction, some chemicals as well as various stressful situations may cause placental hypertrophy, and there are some known mechanisms. Placentas have potential to become hypertrophic under the conditions of an unfavorable maternal environment and fetal demand to catch-up with normal weight. Placental hypertrophy in spontaneously hypertensive rats (SHRs) was considered to be a compensatory reaction to a poor capacity of the SHR uteroplacental unit for transferring glucose to the fetuses [17]. Maternal hemorrhage [3], uterine vessel ligation [4],
carbon monoxide exposure [18], and indomethacin treatment [27] can also elicit placental hypertrophy to facilitate compensatory recovery of fetal-growth retardation. In addition, ethanol-induced placental hypertrophy in rats might be an adaptive response to meet placental damage and repair such as hemorrhage, stagnated maternal blood, fibrin deposition and inflammation in the labyrinth zone [1, 10]. Estrogen and progesterone are both essential for the initiation and maintenance of pregnancy and closely related to placental growth. Estrogen is known as an inhibitor of placental growth and its deficiency may induce placental hypertrophy [2, 8]. In this study, we found that ketoconazole treatment evoked increased mitosis of trophoblasts in the labyrinth zone on GD 15 without giving any unfavorable effects on maternal condition or severe damage of trophoblasts in the labyrinth zone, although cystic dilatation of maternal sinusoids was observed. Decline in mitotic activity was observed on GD 17, which seemed to be an inverse rebound to trophoblastic proliferation. On the other hand, ketoconazole is shown to inhibit 17α-hydroxylase/C17,20-lyase and aromatase activity in the steroid biosynthesis pathway [19, 23]. It is also known that administration of estrogen inhibits ketoconazole induced-placental hypertrophy in rats [16]. Furthermore, in the early placental development, the trophoblasts differentiate from the ectoplacental cone and exhibit high proliferative activity until GD 16 in the labyrinth zone [22]. The volume of the whole placenta grows linearly, which is induced by caused by increased contribution of the labyrinth zone to the placenta [9]. Thus, we might suggest that ketoconazole-induced hypertrophy of the labyrinth
zone can be attributable to the proliferation of trophoblast as a response to the inhibition of estrogen synthesis during the early placental development.

In the normal placental development [9], the basal zone is composed of small cytotrophoblastic elements on GD 12. The cytotrophoblastic elements differentiate into glycogen cells and spongiotrophoblasts after GD 14, and the size of basal zone consequently becomes to reduce in accordance with development of the placenta after GD 15, and then finally glycogen cells will be diminished by glycogenolysis until GD 21. The basal zone is the site of production of steroids and peptide hormones, which play an important role for maintenance of pregnancy [20, 25]. Overgrowth of the basal zone is induced in pregnant rats which are ovarioctomized and supplied with estrogen and progesterone, and it may be a response to the hormonal imbalance [6]. On the other hand, the basal zone is also functionally important in metabolizing xenobiotics [11]. CYPs are functionally very important in metabolizing a number of endogenous substrates and xenobiotics. CYP3A was detected as a major component of the CYP system in the basal zone of the rat placenta through pregnancy [11, 12]. Ketoconazole is known to inhibit CYP3A and enzymes which are responsible for dependent steroid biosynthesis in the hepatic and placental tissues in human [21]. In this study, ketoconazole treatment was found to induce an increase in the composed cells in the basal zone on GDs 17 and 21, while regression of the basal zone of the placenta was inhibited. The overgrowth of the basal zone might be attributed to the hormonal imbalance as a consequence of the inhibition of the steroid biosynthesis or possible rebound reaction of some enzymes following the withdrawal of ketoconazole treatment.

In this study, fetal weight showed a significant increase on GD 17, and increasing trends on GDs 15 and 21. It is known that a positive correlation between placental and birth weight was observed in the appropriate and large-for-gestational-age infants in human [24]. The placental size and structure as well as its development and pathological processes can be associated with subtle episodes of placental transport and metabolic mechanisms which may affect the placental-fetal nutrient exchange. Thus, an increase in embryonic/fetal weight by ketoconazole administration might have contributed to placental hypertrophy.

In conclusion, ketoconazole administration in pregnant rats during GDs 12 to 14 induced an increase in composed cells (trophoblasts, spongiotrophoblasts and glycogen cells) in the labyrinth and basal zone, leading to placental hypertrophy.

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