Effect of chlorpromazine on rat placenta development

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ARTICLE INFO

ABSTRACT

We examined the sequential histopathological changes in the placentas from rats exposed to chlorpromazine. Chlorpromazine was intraperitoneally administered on GD 14 at 50 and 100 mg/kg and the placentas were sampled on GDs 14.5, 15, 17 and 21. The incidence of dams with complete fetal resorption was increased from GD 17 up to 20% at 50 mg/kg and 44.4% at 100 mg/kg. The embryo/fetal weights reduced on GDs 15 and 17 at 50 mg/kg and during GDs 15–21 at 100 mg/kg. The placental weights reduced on GD 17 at 50 mg/kg and during GDs 14.5–21 at 100 mg/kg. Histopathologically, in the labyrinth zone, apoptotic cells were scattered in the trophoblastic septa without inhibition of cell proliferation on GDs 14.5 and 15 at 50 and 100 mg/kg in a dose-dependent manner. A decrease in trophoblasts led to labyrinth zone hypoplasia. In the basal zone, apoptotic cells were scattered on GDs 14.5 and 15 at 100 mg/kg, and most of them appeared to be glycogen cells. A decrease in glycogen cells induced the delayed development of glycogen cell islands and the subsequent remaining glycogen cell islands, and led to the cystic degeneration of glycogen cells. In addition, failure of development of the glycogen cell islands led to the impaired interstitial invasion of the glycogen cells, and then metrial gland hypoplasia occurred.

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1. Introduction

The placenta is an interface between dam and developing embryo/fetus, and has a major role in regulating maternal–fetal O_2/CO_2, nutrient and waste-product exchange during embryonic development with high blood flow. It also secures the embryo/fetus to the endometrium as a protective barrier of xenobiotics and releases a variety of steroids, hormones and cytokines. Although the placenta is a temporary organ, its growth and function play important roles in the maintenance of pregnancy, and the influence on fetal growth and development. The chorioallantoic placenta in rats is histologically divided into a fetal part and a maternal part (Furukawa et al., 2011b). The fetal part of the placenta is mainly involved in facilitating nutrient and waste exchange, and glycogen metabolism. The fetal part of the placenta originates from the trophectoderm of the embryo and consists of the basal zone and labyrinth zone. These constitutive cells of the fetal part differentiate and undergo morphological changes with the close relation to the maternal part of the placenta according to the development sequence. The fetal part of the placenta has high proliferative activity and becomes a major part of the placenta with pregnancy

progression in a short pregnancy period (Davies and Glasser, 1968). It has been reported that there are some toxicants that target the fetal part of the placenta and involve in cytostatic activity and apoptosis induction. Many of them are anticancer drugs, such as busulfan (Furukawa et al., 2007), cisplatin (Furukawa et al., 2013b), 6-mercaptopurine (Furukawa et al., 2008), methotrexate (Sun et al., 2013), 1-b-D-arabinofuranosylcytosine (Yamauchi et al., 2007), etc.

Chlorpromazine, a phenothiazine derivative known to be a tranquilliser, induces a remarkable reduction in weight and volume of the placenta, length of the umbilical cord and volume of the amniotic fluid showing possible correlation with fetal growth retardation in rats (Singh and Padmanabhan, 1979). A single chlorpromazine-treatment on gestation day (GD) 14 in rats induces a reduction of maternal weight and fetal weight, prolonged gestation, high fetal mortality and a decreased litter size of live fetuses (Singh and Padmanabhan, 1978). In addition, these placentas show poor development of the labyrinth zone and the cystic degeneration in the basal zone on GD 20 (Singh and Padmanabhan, 1980). Therefore, it has been known that chlorpromazine has an effect on the development mainly of the fetus part of the placenta. However, there are no reports describing the time-dependent changes of placenta in chlorpromazine-exposed rats, and the mechanisms of its placental toxicity have not been known. In order to clarify the effects of chlorpromazine on the development of each component part of the placenta, we examined the sequential histopathological

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Table 1
Effect of chlorpromazine on resorption and fetal mortality.

Autopsy	Treatment	No. of dams	Litters with completed resorption (%)	Living fetuses per litter[a]	Fetal mortality (%)[a]
GD14.5	Control	4	0.0	11.3 ± 1.9	2.1 ± 4.2
	50	4	0.0	13.5 ± 0.6	4.2 ± 8.3
	100	4	0.0	11.3 ± 2.8	12.4 ± 14.5
GD15	Control	4	0.0	12.0 ± 1.4	5.5 ± 6.9
	50	4	0.0	10.3 ± 1.0	8.0 ± 9.3
	100	4	0.0	13.0 ± 0.8	1.8 ± 3.6
GD17	Control	4	0.0	12.3 ± 1.8	3.1 ± 3.1
	50	5	20.0	9.2 ± 5.7	23.6 ± 43.4
	100	5	20.0	6.6 ± 4.2	32.7 ± 43.9
GD21	Control	4	0.0	10.8 ± 1.5	6.7 ± 8.2
	50	5	20.0	9.2 ± 5.3	20.0 ± 44.7
	100	9	44.4	4.7 ± 5.6	61.9 ± 45.1

Mean ± SD.

[a] Mean of individual litter values.

*, ** Significantly different from control at $P < 0.05$, <0.001, respectively (Dunnett's test).

changes in the placentas from the rats exposed to chlorpromazine on GD 14.

2. Materials and methods

2.1. Animals

Non-pregnant specific pathogen-free Wistar Hannover GALAS rats (CLEA Japan, Inc., Fujinomiya, Japan) were purchased at approximately 10–14 weeks of age. A female rat was housed together with a male rat of the same strain and source for mating. The occurrence of copulation was established by daily inspection for a vaginal plug. Mated female rats were assigned in this study. GD 0 was designated as the day when the presence of vaginal plug was identified. The animals were single-housed in plastic cages on soft-wood chip bedding in an air-conditioned room ($22 \pm 2°C$; humidity, $55 \pm 10\%$; light cycle, 12 h/day). Feed (CRF-1:Oriental Yeast Co., LTD., Tokyo, Japan) and water were available *ad libitum*.

2.2. Experimental design

Chlorpromazine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in 0.9% saline solution. The 56 pregnant rats were randomly allocated to 3 groups of 16–22 rats each (Table 1). Chlorpromazine was intraperitoneally administered to the groups at doses of 0 mg/kg with 0.9% saline solution, 50 and 100 mg/kg with a volume of 0.5 mL/100 g body weight on GD 14. Treatment of pregnant rats with chlorpromazine on GD 14 at 100 mg/kg was previously reported to have an effect on placenta and fetus (Singh and Padmanabhan, 1980). All treatments were made between 9 and 11 a.m. Maternal body weight was recorded on GDs 0, 6 and 14–21. The dams from each of the control and chlorpromazine groups were sampled on GDs 14.5, 15, 17 and 21.

The dams were euthanized by exsanguination under anesthesia, and necropsied. All fetuses were removed from the placentas. Half of the placentas were separated between the basal zone and decidua basalis, and removed from the uterus wall. The fetuses and removed placentas were weighed, and the fetal-placental weight ratio was calculated individually. The fetuses on GD 21 were macroscopically examined for external malformations. All placentas were fixed in 10% neutral buffered formalin. These experiments were conducted according to the Guidelines for Animal Experimentation, Japanese Association for Laboratory Animal Science.

2.3. Histopathological examination

Four placentas per litter were randomly obtained from live embryos/fetuses in all dams in all groups by sampling time points (except for dams with complete fetal resorption). The placentas were embedded in paraffin, sectioned at 4-μm thickness, and stained routinely with hematoxylin and eosin (H&E) for histopathological examination. Immunohistochemical staining, *in situ* TdT-mediated dUTP nick end labeling (TUNEL) (*In Situ* Cell Death Detection Kit, POD) (Roche Applied Science, Penzberg, Germany) and Phospho-Histone H3 (Ser10) (Cell Signaling Technology, Boston, USA), were performed on all selected placentas. The thickness of the labyrinth zone, basal zone, decidua basalis and metrial gland close to the central portion were measured in placentas from each dam with the aid of an image analyzer (WinROOF, Mitani Corporation, Tokyo, Japan). The numbers of phospho-histone H3 positive cells in the labyrinth zone, basal zone, decidua basalis and metrial gland, and the numbers of apoptotic cells in the labyrinth zone and basal zone were counted in 20 sections by light microscopy with a 40 objective, with the aid of an image analyzer.

2.4. Statistical analysis

Means and standard deviation (SD) of the individual litter values were calculated. Dunnett's multiple comparison test was performed (Pharmaco Analyst I, Three S Japan, Tokyo, Japan). The level of significance was set at $P < 0.05$ and <0.01.

3. Results

3.1. Effects on dams

Declining body weight gain (%) of dams (based on the body weight on GD 14 as 100%) is observed during GD 15 to GD 18 at 50 mg/kg and from GD 15 onwards at 100 mg/kg (Fig. 1). All treated animals exhibited prone position, hypothermia, loss or decrease of locomotor activity, vaginal hemorrhage, eye discharge, and incontinence of urine. These clinical signs disappeared by GD 17 at 50 mg/kg and by GD 19 at 100 mg/kg at the latest. The incidence of dams with complete fetal resorption was increased from GD 17 up to 20% at 50 mg/kg and 44.4% at 100 mg/kg (Table 1). The
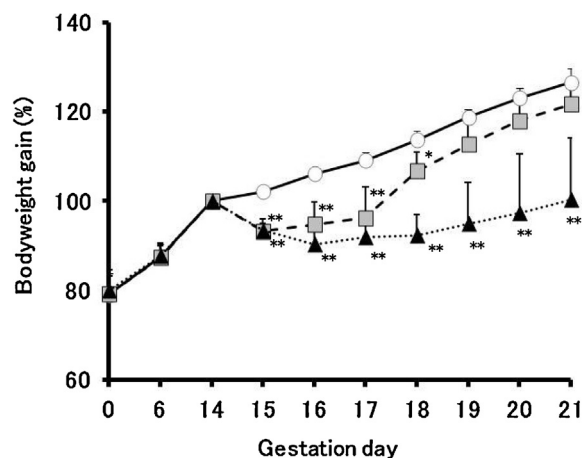

Fig. 1. Maternal bodyweight gain (%). Declining body weight gain of dams (based on the body weight on GD 14 as 100%) is observed during GD 15 to GD 18 at 50 mg/kg and from GD 15 onwards at 100 mg/kg. (○) Control; (▨) 50 mg/kg; (▲) 100 mg/kg. Each value represents mean ± SD. *, ** Significantly different from control at $P < 0.05$, $P < 0.01$ (Dunnett's test).