DEVELOPMENTAL TOXICITY IN RATS EXPOSED TO THE ENVIRONMENTAL ESTROGEN BISPHENOL A (BPA) during pregnancy

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Introduction

A number of recent studies have found increases in the incidence of hormone-dependent abnormalities such as cancers of breast, prostate and testis, decreased semen quality, and genitourinary defects. It has been hypothesized that environmental chemicals with estrogenic activity may play a role in the increasing incidence of endocrine-related abnormalities[1]. BPA is used principally as a monomer of polycarbonate plastics and a constituent of epoxy and polystyrene resins. The wide spread use of this compound is increasing the amount of BPA released into the environment. The possibility of this compound entering into biological systems has caused great concern about their reproductive and developmental toxicity. Although the toxicity of BPA has been extensively studied in short and long term animal tests, limited data have been published on the animal embryofetal effects of BPA. Moreover, there have been conflicting findings regarding alterations of pregnancy rate and embryofetal development in the literature[2,3]. Therefore, a further study needed to be carried out to determine whether or not treatment of BPA during pregnancy results in reduced pregnancy rate, delayed fetal development, and fetal dysmorphogenesis.

The present study was conducted to investigate the adverse effects of the environmental estrogen BPA on initiation and maintenance of pregnancy and embryofetal development.

Materials and Methods

Sprague-Dawley rats aged 10 weeks were obtained from a specific pathogen free colony at KRICT Breeding Facility, Daejeon, Korea. Eighty females successfully mated were randomly assigned to four experimental groups. BPA was administered by gavage to mated females from day 1 to 20 of gestation at dose levels of 0, 100, 300, and 1000 mg/kg. Clinical signs, mortality, body weight changes, and food consumption were checked and measured during the gestation period. All females were subjected to caesarean section on day 21 of gestation and their fetuses were examined for external, visceral, and skeletal abnormalities. The litter was used as the basis for analysis of fetal variables. Analysis of variance and Scheffe's multiple comparison test, Kruskal-Wallis test, or Fisher's exact test was used as appropriate.

Results and Discussion

At 1000 mg/kg, significant toxic effects including abnormal clinical signs, decreased body weight, and reduced food intake were observed in pregnant rats. An increase in the pregnancy failure was also found in the successfully mated females. In addition, increased pre- and postimplantation loss, reduced litter size and fetal weight, and decreased fetal ossification centers of several skeletal

districts were seen. On the contrary, no significant changes induced by BPA were detected in the number of corpora lutea and implantation sites. There were no abnormalities observed by fetal morphological examinations at any dose level. At 300 mg/kg, suppressed maternal body weight, decreased food intake, and reduced body weight of male fetuses were seen. Although the anogenital distance of male fetuses at 300 and 1000 mg/kg was significantly decreased when compared with that of the control group, it was of no toxicological significance because the normalized anogenital distance value with the cube root of body weight was comparable to the control value. There were no signs of either maternal toxicity or developmental toxicity at 100 mg/kg.

It is considered that the dose-dependent increase of maternal toxicity such as abnormal clinical signs, decreased body weight, and reduced food intake at 300 and 1000 mg/kg were induced by the administration of BPA. A dose-dependent reduction in the rate of successful pregnancy in mated females indicates that the pregnancy failure was caused by the administration of BPA. This finding is consistent with the previous report that intraperitoneal injection of BPA to mated female rats at dose level of 125 mg/kg during GD 1-15 significantly impaired the establishment of pregnancy[2]. A recent *in vitro* study also revealed that the rate of development to the blastocyst stage in 48-h cultures of two-cell mouse embryos was significantly decreased by exposure to BPA at a concentration of 100 uM[4]. The earlier study and our study clearly indicate that early embryos are highly sensitive to the environmental estrogen BPA. The developmental toxicity included increased embryonal deaths, increased postimplantation loss, reduced litter size and fetal weight, and decreased fetal ossification centers of several skeletal districts. This indicates that the developmental alterations were caused by treatment of the environmental estrogen BPA. However, the administration of BPA did not cause fetal malformations even at a significantly maternally toxic dose in rats.

The present study concluded that administration of the environmental estrogen BPA during the entire period of pregnancy resulted in increased pregnancy failure, increased pre- and postimplantation loss, decreased fetal body weight, and retarded fetal ossification at a maternally toxic dose, but did not induce embryofetal dysmorphogenesis in rats.

References

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Table 1. Maternal findings of mated females treated with BPA

Dose (mg/kg/day)	0	100	300	1000
Number of mated females	20	20	20	20
Number of pregnant females	20	20	18	14*
% pregnancy failure	0	0	10	30*
Body weight (mean ± SD g)				
GD 0	234.6±13.3	238.6±14.1	238.4±15.4	240.8±17.5
GD 3	250.9±15.7	251.9±11.2	250.8±15.1	245.6±23.8
GD 7	263.8±15.6	263.1±11.7	251.4±14.8	242.4±24.0**
GD 10	276.1±15.3	288.2±28.4	259.6±15.5	252.8±25.5*
GD 14	296.9±16.9	298.7±14.3	273.8±12.1**	269.4±28.7**
GD 17	327.0±18.0	330.3±17.4	292.6±15.6**	289.3±24.0**
GD 21	387.1±27.7	389.9±39.8	336.7±23.7**	313.5±35.8**
Body weight gain (g)[a]	152.5±22.4	151.4±35.0	98.4 ±27.8**	72.7 ±27.8**
Food consumption (mean ± SD g)				
GD 1	19.4 ± 2.1	22.0 ± 6.3	20.6 ± 3.4	18.5 ± 2.5
GD 4	16.0 ± 2.1	16.4 ± 2.9	12.1 ± 4.2**	6.9 ± 2.1**
GD 8	15.6 ± 2.2	18.1 ± 3.1	17.2 ± 9.6	12.8 ± 7.3
GD 11	22.5 ±10.2	16.8 ± 4.2	20.8 ± 9.6	16.0 ± 7.1
GD 15	18.6 ± 4.0	19.2 ± 4.5	22.5 ± 6.5	19.8 ± 8.4
GD 18	21.7 ± 3.1	22.2 ± 3.0	18.9 ± 8.6	17.8 ± 3.3
GD 21	19.1 ± 4.6	18.2 ± 5.1	17.7 ± 4.9	16.5 ± 6.4

[a]Body weight gain = body weight on GD 21 - body weight on GD 0.

Table 2. Reproductive findings of pregnant females treated with BPA

Dose (mg/kg/day)	0	100	300	1000
Number of pregnant animals	20	20	18	14
Number of corpora lutea[a]	16.4±2.6	17.2±2.6	17.2±1.7	15.7±3.0
Number of implantations[a]	14.2±3.3	15.1±3.8	15.4±3.1	11.7±4.5
% preimplantation loss[a]	13.6±14.5	12.8±17.0	11.2±14.9	24.9±25.1
Number of fetal deaths[a]	0.4±0.8	1.0±1.4	0.5±0.9	2.6±2.1**
Resorptions: Early	0.4±0.8	1.0±1.4	0.5±0.9	2.4±1.9**
Late	0	0	0	0
Dead fetuses	0	0	0	0.1±0.4
% postimplantation loss[a]	2.9±6.0	7.3±9.8	3.2±4.8	31.9±33.5**
Number of litters totally resorbed	0	0	0	1
Number of live fetuses per litter[a]	13.8±3.4	14.1±4.0	14.9±2.8	8.9±5.7**
Male/Female	146/130	157/125	125/143	52/73
Fetal body weight (g): Male[a]	4.94±0.26	5.08±0.25	4.23±0.71*	3.96±1.40**
Female[a]	4.69±0.20	4.80±0.37	4.05±0.65	3.71±1.38**
Anogenital distance (mm): Male[a]	5.02±0.32	4.85±0.23	4.49±0.36**	4.36±0.64**
Female[a]	3.19±0.28	3.06±0.16	3.00±0.24	2.96±0.30
Corrected distance (mm/g^3): Male[a]	3.06±0.27	2.87±0.12	3.26±0.55	3.02±0.21
Female[a]	2.04±0.20	1.91±0.13	2.29±0.49	2.19±0.29
Placental weight (g)[a]	0.55±0.07	0.56±0.08	0.49±0.07	0.55±0.13

[a]Values are presented as means ± SD.
* P<0.05, ** P<0.01.

Table 3. External, visceral, and skeletal findings in fetuses from dams treated with BPA

Dose (mg/kg/day)	0	100	300	1000
External anomalies				
Litters examined	20	20	18	13
Fetuses examined	276	282	268	125
Fetuses with malformations (%)	2 (0.7)	1 (0.7)	0	3 (2.4)
Litters affected (%)	2 (10.0)	2 (10.0)	0	2 (15.4)
Visceral anomalies				
Litters examined	20	20	18	11[a]
Fetuses examined	132	134	129	59
Fetuses with malformations (%)	1 (0.8)	0	1 (0.8)	0
Litters affected (%)	1 (5.0)	0	1 (5.6)	0
Fetuses with variations (%)	36 (27.3)	40 (29.9)	24 (18.6)	15 (25.4)
Litters affected (%)	18 (90.0)	14 (70.0)	11 (61.1)	7 (63.6)
Skeletal anomalies				
Litters examined	20	20	18	12[b]
Fetuses examined	144	148	139	64
Fetuses with malformations (%)	1 (0.7)	0	0	0
Litters affected (%)	1 (5.0)	0	0	0
Fetuses with variations (%)	20 (13.9)	24 (16.2)	28 (20.1)	11 (17.2)
Litters affected (%)	14 (70.0)	14 (70.0)	12 (66.7)	9 (75.0)
Ossification centers (mean ± SD)				
Cervical vertebra	2.53 ± 1.57	1.73 ± 0.74	1.77 ± 1.27	1.83 ± 1.72
Sternebra	5.97 ± 0.09	6.00 ± 0.00	5.84 ± 0.31	5.19 ± 1.39**
Metacarpals in both forelimbs	7.97 ± 0.09	8.00 ± 0.00	7.89 ± 0.23	7.33 ± 1.06**
First & 2nd phalanges in both forelimbs	9.81 ± 0.79	10.1 ± 0.77	8.84 ± 0.92	8.23 ± 2.12**
Metatarsals in both hindlimbs	8.80 ± 0.39	8.64 ± 0.56	8.43 ± 0.55	8.13 ± 0.94**
First & 2nd phalanges in both hindlimbs	12.5 ± 2.79	11.4 ± 3.25	12.1 ± 2.25	7.93 ± 7.14*
Sacral and caudal vertebra	8.49 ± 2.64	8.52 ± 3.11	8.78 ± 1.92	5.34 ± 4.63

[a]One litter with 2 fetuses was excluded due to the severe developmental delay. Another 1 litter with a single fetus was excluded because of the skeletal examination.
[b]One litter with 2 fetuses was excluded due to the severe developmental delay.
*P<0.05, **P<0.01.