

Bisphenol-A Exposure Imprints Expression of Genes (Hoxa-10 & Hoxa-11), Bcl-2 Protein And Incidence of Resorption in Early Gestation Albino Rat



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KEYWORDS : BPA, Resorption, Hoxa-genes, Bcl-2

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ABSTRACT

The widespread use of Bisphenol-A (BPA) in polycarbonate plastics has raised concerns regarding potential estrogenic effects on normal development and adult function of the reproductive system in humans and animals. The BPA is the most estrogenically active molecule present in plastics. Human infants may also be exposed to environmental estrogens. Bottle fed infants are most likely exposed to BPA. This study tested the hypothesis that maternal BPA exposure cause female reproductive tract defects. Pregnant rats were orally treated from gestational day 8th to 15th with a dose of BPA (50 mg/kg & 500 mg/kg) that resulted in resorptions, changes the expression levels of anti apoptotic protein Bcl-2 in placenta, uterus and alter the gene expression levels (HOXA-10 & HOXA-11 genes) in uterus. The results of the current study indicate that environmental estrogens, such as the BPA at physiological concentrations, cause adverse affects on the developing and adult female reproductive system.

INTRODUCTION

BPA, an environmental estrogen, is widespread in our living environment, because of its production and use increased; exposure of humans to BPA is becoming a significant issue. The ubiquitous & extensive use of BPA containing products results in a high human exposure worldwide (Vandenberg et al., 2010). Exposure of low levels of endocrine disrupting chemicals to human being may be of great concern. They interfere with many metabolic, molecular processes and cause widespread damage to body tissue (Humblet et al., 2008). It is thought that human exposure mainly occurs through diet as polymers containing BPA can be hydrolyzed under high temperature and acidic or basic drink containers. Thus, in humans, BPA is detected not only in serum and urine but also in the placenta and amniotic fluid (Cafalat et al., 2005).

BPA is omnipresent in the environment and widely distributed and unavoidable. It accumulates in pregnant adult females and its continued exposure during gestation is likely to have an impact on the development of the fetus. Developmental exposure to estrogenic chemicals induces morphological, functional, and behavioral anomalies associated with reproduction.

Prenatal exposures were investigated to reveal the significance of maternal transfer of BPA to fetus during gestational period. In vivo study, the minimum concentration of BPA that was found to cause statistically significant effects on reproductive performance was 50 mg BPA /kg/day (NTP, 2004). Accordingly the acceptable human BPA intake was calculated to be 50 mg/ kg/day. However recent studies have revealed that human exposure to BPA could be considerably greater than this acceptable level and daily intake of BPA is not restricted to the diet reported by Stahlhut et al. More importantly trans-placental transport of BPA was observed and has been demonstrated in both rodents and humans. Hence fetus may act as a sink of BPA and would be mostly affected during gestational development. Adverse effects of BPA on developmental and reproductive processes in rodents and primates were reported by Richter et al., (2007). Kim et al, reported that, foetal death and resorptions were increased in pregnant Sprague-Dawley rats administrated a high BPA level during the entire gestational period.

HOX genes have a necessary role in the development of the mouse and human reproductive tract and may be a common target of endocrine disruption. Altered HOXA-10 expression in women is seen in association with a number of common medical conditions. Diminished HOXA-10 & HOXA-11 expression has

been reported in polycystic ovary syndrome, endometriosis, hydrosalpinx, and improper implantation.

The Bcl-2 family of proteins has been demonstrated to play an important role in the regulation of apoptosis in a variety of cells. For example, BPA at low doses induced apoptosis and up regulation of Bax and down regulation of Bcl-2 in murine ovarian granulosa cells, indicating that the intrinsic apoptotic pathway is involved.

In this study we examined the effect of BPA on fetal development, genes and antiapoptotic protein Bcl₂ expression changes in BPA treated rats.

MATERIALS AND METHODS

1. Gestational period, at day 15th of pregnancy, females were sacrificed and the number of resorptions were counted & the fetuses were removed by caesarean operation.
2. The RT-PCR was used to analyses Hoxa-10 & Hoxa- 11 m- RNA expression levels.
3. The expression profile of antiapoptotic protein (Bcl-2) in placenta & uterus by western blot analysis.

RESULTS

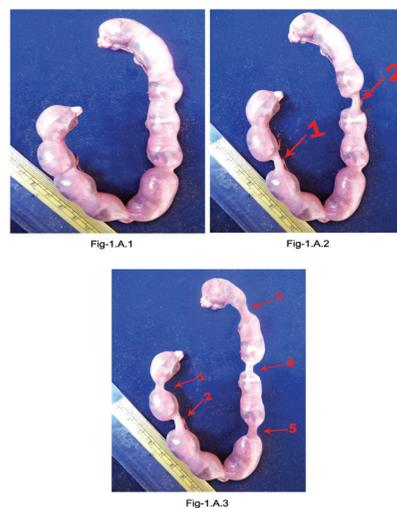


Fig-1.A.1: Gravid uterus of control female rats. Uterus having normal embryos.

Fig-1.A.2: Gravid uterus of lower dose BPA (50 mg/kg) exposed female rats showed that reduced number of embryos. Arrows 1, 2 indicates resorption sites seen in uterus compared to control rats.

Fig-1.A.3: Gravid uterus of higher dose BPA (500 mg/kg) exposed female rats showed that highly reduced number of embryos. Arrows 1,2,3,4 and 5 indicates more number of resorption sites were observed in uterus compared to control rats.

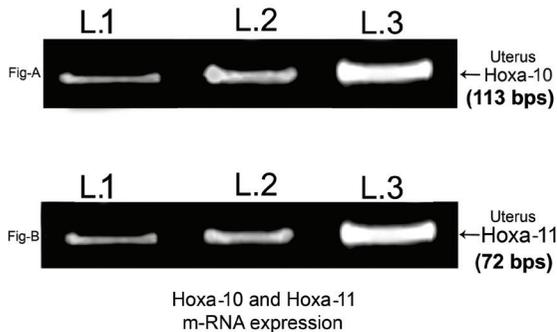


Fig-2: The reverse transcription-PCR was performed to screen the effect of BPA treatment on HOXA-10&HOXA-11 genes expression of uterus in treated groups. Rats were treated with concentrations of BPA from 50mg, 500mg/kg/d.b.wt. RNA was subsequently isolated, reverse-transcribed, and amplified using previously reported RT-PCR protocol. As shown in Fig-A and B, control diluents exerted no effect on HOXA-10& HOXA-11 expression. An increase in HOXA-10&HOXA-11 gene expressions were seen with increasing concentrations of BPA treatment compared with controls.

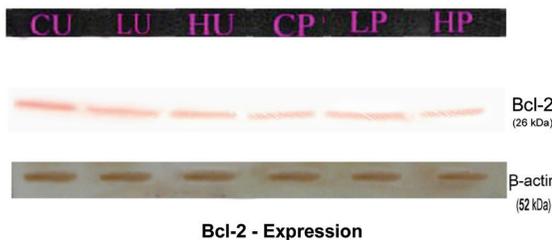


Fig-3: shows the changes associated with BPA treatment in expression of Bcl-2 protein in placental & uterine tissues. There is a significant decrease in the expression of the antiapoptotic Bcl-2 protein was observed in the BPA treated groups compared with the controls.

DISCUSSION

In recent years, the association between alterations in hormonal regulation and exposure to estrogenic endocrine disrupting chemicals (EEDs), such as xenoestrogens (BPA) has led to increasing public and scientific concern. EEDs reportedly have the potential to produce widespread adverse effects through their endocrine disrupting activity, such as carcinogenicity, immunotoxicity, reproductive abnormalities, developmental toxicity, and so on (Witorsch, 2002). Many studies in animal models have been reported that prenatal exposure to EEDs could induce the embryo toxicity and birth defects.

Therefore in the present study we evaluated the role of BPA on fetal development by administration of BPA orally through gavage on 8th to Gestational Day 15th caused significant decreases in fetal weights and induced some abnormal changes in gestation such as resorption of fetuses than those in control group. This decrease in the fetal and gravid uterine weights was due to increased resorptions after BPA administration. Data of the

present investigation revealed that administration of BPA (50, 500mg/kg) significantly elevated the rate of resorption and significantly decreased fetal growth when compared to controls (Fig-1.A.1 to 1.A.3). This effect of the BPA on implantation is marked by the presence of resorption sites through the uterine horns of rats sacrificed on the 15th day of gestation and by the absence of pups to those left to term. These results were similar those of Kim et al, who observed a reduction in the number of embryonic implantation sites. It is known that, implantation takes place normally three to four days after fertilization in female rats, which suggests that administration of the BPA from the 8th day of gestation would have hindered the process of implantation at dose 50 mg/kg & 500mg/kg. The dose dependent increase of resorption sites observed to the treated animal could be related to presence of chemical, uterine activity or hormonal disturbance.

In fact, chemical agents can disrupt pregnancy possibly by interfering with the mitotic process of the embryo development then led in embryonic loss (Elbetieha, et al., 2000). It is likely that inactivation and poor placement of the blastocytes linked to uterine activity are harmful to embryo implantation. Therefore, the decrease of the uterine weight is an indication of failure in the development of the embryo in this study.

In this study of results revealed that, the HOXA-10 & HOXA-11 gene expression levels were altered in BPA treated groups when compared to corresponding controls. The results were in agreement with Smith et al., (2007). He was reported that, in utero BPA exposure resulted in an alteration of uterine HOXA-10 & HOXA-11 expressions, mice were treated with 0.5-5.0 mg/kg BPA on gestational days 9-16.

In the present study of results revealed that, the increased expression profiles of HOXA-10 & HOXA-11 transcripts in the uterus treated with low and high doses of BPA. As shown in Fig-2, significant increases were observed in both genes of HOXA in the uterus of treated groups compared to control. Although changes in transcript levels or activity of these genes indicate that the effects of BPA on uterus may involve the HOXA genes (HOXA-10&HOXA-11). The cellular and morphological changes were observed in the BPA treated rat uterus in my previous study; suggest that prenatal exposure to BPA may act directly by altering the expression of HOXA-10& HOXA-11 genes in the target organs (uterus), because environmental estrogens (BPA) can directly alter estrogen-sensitive genes during fetal development. Alterations in the normally precise temporal regulation of HOXA genes, either increased or decreased, may have implications for reproductive success. Estrogenic compounds exhibit profound and lasting effects on essential developmental genes in the reproductive tract. These changes are likely to influence reproductive competence.

In this investigation, we examined the effect of BPA on the expression profile of antiapoptotic protein is Bcl-2 in placenta & uterine tissues by western blot analysis. These results were in accordance with the Benachour and Aris, 2009. The Bcl-2 family of proteins, containing anti-apoptotic members, is known to regulate mitochondrial mediated apoptosis (Shimizu et al., 1999). However, we observed significant changes in the expression of antiapoptotic Bcl-2 protein (decreased the BCL-2 protein activity levels) in BPA-treated group rats, because prenatal exposure to BPA may act on the placental, uterine tissues, causing release of apoptogenic proteins and activation of caspase-3 and altered Bcl-2 activation. Kluck et al, observed that, an alteration in the ratio of anti-apoptotic protein-Bcl₂, resided on the outer membrane of mitochondria, may modulate the release of apoptogenic proteins in rats.

CONCLUSION

In this study we can say that, resorptions are formed by alterations of genes and antiapoptotic protein expressions in BPA treated pregnant rats.

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