Effect of Bisphenol-A on the Morphology of Small Intestine in Pregnant Rats

Jesmine Khan¹, Siti Hamimah Binti Sheikh Abdul Kadir¹,², Wan Nor I'zzah Wan Mohamad Zain¹, Roziana Kamaludin³, Zatilfarihah Rasdi², Amirah Abdul Rahman¹

ABSTRACT

Introduction: Bisphenol A (BPA) is an environmental pollutant. Human beings are exposed to BPA through food and water. Due to its prolonged contact with the intestinal tract (IT), it might have harmful effects on the IT particularly in pregnant women and the fetuses. Objective: The aim of this study was to investigate the effect of BPA on the morphology and tight junction protein expression of the small intestine of pregnant rats.

Materials and Methods: Twelve Pregnant Sprague Dawley rats were divided into group 1 (control, n = 6) and group 2 (BPA treated, = 6). Group 1 received tween 80 and group 2 received BPA (0.2 mg/ml) in drinking water.

Results: There were no significant differences in the villus height, crypt depth and the number of goblet cells in the jejunum and ileum between the two groups. Intestinal tight junction protein (ITJP) claudin 2 expression was similar in both groups. ITJPs claudin 3 and 4 were expressed less intensely in the ileum of group 2 as compared to group 1.

Conclusion: Chronic low dose BPA throughout pregnancy in rats did not affect the morphology of villi, crypt, goblet cells and ITJP claudin 2 but reduced the expression of claudin 3 and 4 in the ileum, which might compromise intestinal barrier.

KEY WORDS

Bisphenol A, villi, crypt, goblet cells, claudin 2,3,4

INTRODUCTION

Bisphenol A (BPA) is one of the most widely used industrial compounds worldwide. It is mainly used in consumer products, such as epoxy resin lining food and beverage cans, plastic food containers, tablewares, baby feeding bottles and toys. Under normal condition, BPA leaks from the polymers into food. It leaks and spreads faster when exposed to elevated temperature such as heating and boiling. BPA is found in the blood, urine, fetal tissues and umbilical cord blood of human beings. Human beings are largely exposed to BPA through food and water. Presence of BPA in the water is either due to contamination at the source of water or leaching from the water pipes. Moreover, there is a global concern for human health as BPA is known to bind estrogen receptors (ERs), have endocrine disruptor activity and can interfere with normal sex hormone balance.

US Environmental Protection Agency and European Food Safety Agency established the tolerable daily intake (TDI) of BPA at 50 μg/kg/day. Therefore, exposure to BPA in different populations particularly during pregnancy, has raised an alarming public health concern where animal studies revealed that in utero exposure to BPA can alter the development and a wide range of function of different organs in the offspring including mammary gland, reproductive tract, bone development, neurological function, cardiac function and accelerate the onset of puberty. Liver and kidney are also found to be highly susceptible to the toxicity of BPA. BPA present in foods and drinks act as antigens and are in prolonged contact with the small intestinal mucosa before their absorption. Prolonged contact of potentially harmful ligands and antigens in the intestinal tract may cause chronic mucosal injury. Any layer of the GI mucosa eg. the mucus layer, mucus secreting goblet cells, villi, crypts, tight junction proteins such as claudins has the potential to be affected. Claudins are a group of transmembrane proteins which play a critical role in the proper functioning of epithelial tight junctions.

Disruption of the above components will permit excessive passage of harmful materials which might initiate intestinal inflammation in pregnant woman and transport both BPA and the inflammatory metabolites through placenta to the fetus throughout the intrauterine life and cause immediate and/or delayed harmful effects in the fetus. BPA was found to dose-dependently decrease basal intestinal permeability in one study. In this study, we investigated the effect of BPA on the morphology of different components of IB in pregnant rats. BPA level used in...
this study was chosen based on its level in the water supply system of the local area (unpublished data). Finding of this study will trigger researchers to identify tools or measures to prevent BPA contamination in water.

**MATERIALS AND METHODS**

**Chemicals and reagents**

All chemicals and reagents were purchased from Thermo Fisher Scientific USA and Sigma Aldrich USA, unless otherwise stated. All primary and secondary antibodies used were from Thermo Fisher Scientific USA with the dilution stated in each particular method.

**Animal care and BPA exposure**

The animal work was performed in accordance to the regulation of the approval of Universiti Teknologi MARA Committee of Animal Research and Ethics (UiTM CARE). Rats received standard rat food ad libitum. Six male and eighteen female Sprague-Dawley rats aged between 150-180 days were used in this study.

Rats were acclimatized for 7 days to the standard conditions in the laboratory as follows: room temperature 24°C; a 12 hour light and dark cycle and exchange of room air 12 times per day.

Control food was obtained from the local supplier (Gold coin, Selangor, Malaysia).

Twelve female rats were mated with six male rats. When sperm was observed in the vaginal smears, that day was noted as pregnancy day 1. Pregnant rats were divided into 2 groups: Group 1 (Vehicle; Tween 80 control group, n = 6) and Group 2 (Treated with BPA 0.2 mg/ml, n = 6).

Six female non pregnant and six pregnant rats were used to observe any differences of the intestinal morphology between them. Rats were euthanized by cardiac puncture. Fetuses were observed for any macroscopic anomalies and the whole small intestine was collected for further analysis.

**Tissue processing for hematoxylin and eosin stain and immunohistochemistry**

Ileum and jejunum of the mother were processed in the automatic tissue processor for 24 hours and subsequently embedded in paraffin-wax (Surgipath Paraplast Plus, Leica Biosystems, Germany). After tissue processing, wax blocks containing tissues were sectioned into three micrometer thickness using microtome, taken on glass slides for H and E staining following the standard procedure.

Immunohistochemical staining was done for the jejunum and ileum using an evaluation technique based on the one used by the Sydney Missionary Hospital, Malaysia. The slides were incubated with primary anti-claudin antibodies for Claudin 2, Claudin 3 and Claudin 4 (1:500 dilution) overnight. Then slides were incubated with secondary antibodies; HRP Goat anti-Rabbit IgG (H + L) Cross-Adsorbed Secondary Antibody and Goat anti-Mouse IgG (H + L) Secondary Antibody HRP (1: 5000 dilution;). Then, slides were rinsed and nuclei counterstained with hematoxylin. Slides were rinsed under running tap water, dehydrated in graded alcohols and the whole small intestine was collected for further analysis.

**Image analysis**

The slides were viewed under light microscope Nikon Eclipse 80i (Nikon, Japan). Villus height, villus width and crypt depth were measured by image analyser. A total of twenty slides were analyzed, 10 for group 1 and 10 for group 2. From each slide, 10 longitudinally oriented villi were measured. Villus height was measured from the tip to the base of each villus. Width of the villus was measured at the widest part of each villus from left to the right. Goblet cells were counted manually from each villus. Ten villi were used from each slide. To avoid repeated counting, we started counting from the left side of each slide and gradually moved to the right side of the slides. All the measurements were done blindly by two researchers. The expression of immunohistochemical (IHC) staining for claudin-2, claudin-3 and claudin 4 were graded using an evaluation technique based on the one used by the Sydney Missionary Hospital, Malaysia.
Classification System\(^{10}\). The number of positive cells stained with dark brown colour in the the villi are observed with 10X and 40X. Microscopic assessment of the grade was scored semi-quantitatively according to a previously the above scoring system as absent (0), mild (+), moderate (++) or severe (+++).

Statistical analysis

Analyses were performed using GraphPad Prism version 7.04. Data were presented as mean ± S.E.M. Two-way ANOVA with Sidak’s multiple comparisons test was used to compare means of each group of pregnant and non-pregnant rats.

RESULTS AND DISCUSSION

On macroscopic examination of the small intestine of the mother, there was no signs of abnormality such as wall thickening, bruise, hemorrhagic spots, ulceration or inflammation of the small intestine externally. When dissected longitudinally, such changes were also absent on the inner surface.

In the fetus, we could not find any macroscopic anomalies but the whole intestine was amalgamated, identification of different parts of the small intestine was impossible. Although we wanted to observe the morphology of jejunum and ileum of the fetus but this feature prevented us from doing H and E and IHC staining. The small intestine was so tiny and thread like that we could not gain sufficient mRNA for western blot analysis to observe the TJPs. We will try to pull out the samples for western blot in our next batch of animals.

There were no significant differences between the height (Jejunum 531 ± 7 vs 328.7 ± 25, Ileum 273 ± 13.5 vs 267 ± 9.2) and width of the villi (Jejunum 125.59 ± 2.4 vs 96.70 ± 7.36, Ileum 125.4 ± 9.9 vs 102.3 ± 7.8), crypt depth (Jejunum 131 ± 10 vs 89 ± 5.3, Ileum 121.5 ± 8.6 vs 129.2 ± 9.1) and the number of goblet cells (Jejunum 36 ± 7 vs 40 ± 9, Ileum 35 ± 4 vs 30 ± 6) between the non-pregnant and pregnant rats.

In group 2 (BPA treated), there was disruption of the villus tips in few of the slides but on measurement, there were no significant differences between the height (Jejunum, 445 ± 20.3 vs 423 ± 90, Ileum 325 ± 21.4 vs 246.5 ± 9) and width of the villi (Jejunum 124 ± 7.4 vs 108.70 ± 7, Ileum 125.3 ± 15.5 vs 114.8 ± 11.4), crypt depth (Jejunum 91 ± 4.7 vs 125 ± 22, Ileum 121.3 ± 4 vs 102.3) and the number of goblet cells (Jejunum 45 ± 9 vs 40.4 ± 5, Ileum 38.2 ± 11 vs 36 ± 8) between group 1 and group 2. This results suggest that BPA exposure in a low dose throughout the pregnancy did not cause any significant changes on the villi, crypt and goblet cells which represent no or very negligible harmful effects of low dose BPA exposure on these components of intestinal barrier of healthy pregnant mothers.

Claudin 2:

Claudin 2 expression was very faint in both ileum and jejunum in both groups (group 1 and 2). Claudin 2 is a pore forming tight junction protein\(^{19}\). It is minimally expressed in control group as we have found in both jejunum and ileum. In normal condition, there is less claudin 2 expression, so less pores in the tight junction area and less transport of macromolecules across the intestinal barrier to trigger any inflammatory process in the small intestine and in the body. It seems that the dose used in this study did not cause any changes of claudin 2 TJPs.

Claudin 3:

Both ileum and jejunum of group 1 had higher expression (+++) of claudin 3, which is an expected finding in control groups. Claudin 3 is an intestinal barrier tightening protein and highly expressed in control subjects\(^{20}\). In this experiment it is highly expressed in both control jejunum and ileum. Higher expression of claudin 3 represents a strong intestinal barrier which prevents harmful materials entering the body. Both ileum and jejunum had moderate (+) expression of claudin 3 in group 2 (BPA treated group). This finding suggests that low dose BPA exposure for prolonged period might have some harmful effect on the expression of claudin 3 which represents a compromised IB.

Claudin 4

Both jejunum and ileum had very fade expression of claudin 4 in group 2 (BPA group) as compared to group 1. Same as claudin 3, claudin 4 is also a barrier tightening protein\(^{21,22}\). This finding also suggest that tight junction integrity is compromised in prolonged BPA exposure throughout pregnancy.

CONCLUSION

Finding of this study indicates that prolonged low dose BPA exposure during pregnancy have the tendency to compromise intestinal barrier function. Compromised IBF will initiate different inflammatory reactions and along with BPA, these inflammatory biomolecules have the potential to transport to the placenta and have harmful effects in the fetus.

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