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Regulatory and junctional proteins of the blood-testis barrier in human Sertoli cells are modified by monobutyl phthalate (MBP) and bisphenol A (BPA) exposure

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ABSTRACT

The blood-testis barrier (BTB) is responsible for providing a protected environment and coordinating the spermatogenesis. Endocrine disruptors (EDs) might lead to infertility, interfering in the BTB structure and modulation. This study aimed to correlate the actions of two EDs, monobutyl phthalate (MBP) and bisphenol A (BPA) in different periods of exposure, in a low toxicity dose to the human Sertoli cells (HSeC) and its effects on the proteins of the BTB and regulatory proteins involved in its modulation. HSeC cells were exposed to MBP (10 μM) and BPA (20 μM) for 6 and 48 h. Western Blot assay indicated that MBP was able to reduce the expression of occludin, ZO-1, N-cadherin and Androgen Receptor (AR), while BPA leads to a reduction of occludin, ZO-1, β-catenin and AR. TGF-β2 and F-actin were not modified. Phalloidin and Hematoxylin and Eosin assay revealed phenotically disruption in Sertoli cells adhesion, without changes in F-actin expression or localization. Our data suggested both EDs present potential for disrupting the structure and maintenance of the human BTB by AR dependent pathway.

1. Introduction

Infertility is defined as the inability of a couple to conceive naturally after one year of regular unprotected sexual intercourse (Kamel, 2010). Infertility affects 10–15% of couples at reproductive age and male factors may be responsible for 30–50% of cases (Boivin et al., 2007), with the reproductive tract being particularly susceptible to environmental factors, such as exposure to endocrine disruptors (EDs), capable of triggering morphological and functional changes (Sikka and Wang, 2008; Diamanti-Kandarakis et al., 2010).

Monobutyl Phthalate (MBP) has been highly used for being the active metabolite of Di-n-butyl phthalate (DPB), one of the most used EDs across the world (Chu et al., 2013; Frederiksen et al., 2011; Hu et al., 2013; Zhou et al., 2013). The levels of phthalates in household dust can reach as high as 400–700 mg/kg (Becker et al., 2004). Further, the estimated daily intake of phthalates in women is 41.7 μg/kg/day, a level that exceeds the tolerable daily intake level of 37 μg/kg/day (Koo and Lee, 2005) A study from Denmark found an estimated daily intake of 4.29 μg/kg body weight and a median concentration of 111 ng/mL of MBP in urinary samples of Danish children (Frederiksen et al., 2011). MBP was shown to affect Sertoli cell viability, disturb embryo development and embryonic pre-implantation, decrease testosterone production by Leydig cells and lead to cytoplasmic redistributions of membrane proteins in animals in *in vivo* and *in vitro* models (Chu et al., 2013; Frederiksen et al., 2011; Hu et al., 2013; Zhou et al., 2013).

Bisphenol A (BPA) is a chemical that is mainly used in combination with other chemicals to manufacture plastics and resins. Recent data from China estimated daily exposure dose for BPA is approximately 64.8 ng/kg · bw/day. Dietary intake was considered the primary exposure pathway in adults. The contribution of dietary intake, indoor dust, paper products and personal care products to BPA intake was 72.5%, 0.74%, 0.98%, 0.22% of the total exposure dose, respectively (Gao et al., 2016). BPA has also been used in animal models and is described as a reproductive toxicant in low doses, corresponding to daily human exposure, which can reach up to 200 μM released from plastic dental sealants in saliva (931 μg/30 mL) (Becker et al., 2004). Studies have showed that BPA can disrupt the blood-testis barrier in rats, and changes spermiogenesis and cellular development and functions (Izumi et al., 2011; Li et al., 2009; Monsees et al., 2000).

Germ cell development takes place in the seminiferous tubules and depends on Sertoli cells for growth factors and nutrient supply. Sertoli cells are responsible for providing a secure and highly specialized environment within the seminiferous tubules for germ cell development (França et al., 2012; Skinner and Griswold, 2005; Weber et al., 1983).

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Sertoli cells are connected by the gap junctions and tight junctions that establish the blood-testis barrier (BTB). Adhesion protein complexes such as the gap junction's N-cadherin/β-catenin and tight junction's occludin/ZO-1 work together to maintain the barrier, anchored in F-actin bundles that confer resistance to the BTB. Despite being one of the strongest blood-tissue barriers, the BTB undergoes extensive restructuring to allow the transit of preleptotene spermatocytes. Yet the immunological barrier conferred by the BTB cannot be compromised, even transiently, during the epithelial cycle to avoid the production of antibodies against meiotic and post meiotic germ cells (Mruk and Cheng, 2004).

Thus, the control of BTB dynamics is highly important to the spermatogenesis. The coordination of two classes of biomolecules, testosterone and transforming growth factor (TGF)-β2, regulates the opening and closing of the BTB. These molecules differentially affect the fate of the endocytosed integral membrane protein. Testosterone is important to cell adhesion, whereas cytokines such as the transforming growth factor (TGF)-s have been shown to disturb the BTB *in vitro* and *in vivo* by down-regulating the expression of integral membrane proteins in the BTB (Yan et al., 2008).

It is known that most people are exposed to low doses of DBP and BPA from the environment. Additionally, there are few studies simulating this exposition, especially in the human model. Thus, it is very important to elucidate their effects in order to understand the possible mechanisms capable of compromising health and human reproductive development and to collaborate with basic research for the comprehension of infertility cases associated with environmental exposure to EDs (Xiao et al., 2014).

This study was conducted to investigate the effects of MBP and BPA in different periods of exposure, from a dose of low toxicity on human Sertoli cells (HSeC) and their possible effects on the blood-testis barrier's integral and regulatory proteins, involved in spermatogenic process modulation.

2. Materials and methods

2.1. Sertoli cell culture

The human primary Sertoli cell line (HSeC) (Lonza, Walkersville, MD, USA), was maintained in Sertoli Cell Basal Medium (SeBM) (Lonza, Walkersville, MD, USA) supplemented with 10% fetal calf serum (Gibco®, Grand Island, NY, USA), and 500 μL of Antibiotic–Antimycotic (100×) containing 10,000 units/mL of penicillin, 10,000 μg/mL of streptomycin, and 25 μg/mL of Fungizone® Antimycotic (Gibco®, Grand Island, NY, USA),at 37 °C, 5% CO_2 and 95% humidity, as previously described (Ribeiro et al., 2014).

2.2. Endocrine disruptors dose definition

The doses used in this study were defined based on the MTT assay. MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl bromide tetrazolium (Sigma-Aldrich®, St Louis, MO, USA) assay was used as an indicator of cell viability as determined by the transformation of tetrazolium salt MTT to an insoluble formazan salt, as previously described (Mosmann, 1983).

Beyond, literature for MBP (Chu et al., 2013; Frederiksen et al., 2011; Hu et al., 2013; Zhou et al., 2013) and BPA (Izumi et al., 2011; Li et al., 2009; Monsees et al., 2000; Salian et al., 2009; Wang et al., 2012) was used for tested dose choices.

2.3. Cell treatment

HSeC were cultured in triplicates, until a confluence of 70% was observed. The different test toxicants were then added in fresh SeBM medium and cells were cultured for 6 or 48 subsequent hours, in order to observe the immediate and delayed effects of toxicants on BTB. Cells were resuspended in trypsin/EDTA 0.25% and centrifuged in PBS 2×. Pellets were then subjected to protein extraction for analysis by Western Blot. Bisphenol A and MBP were purchased from Sigma-Aldrich® (St Louis, MO, USA) and diluted in dimethyl sulphoxide (DMSO, Sigma-Aldrich®, St Louis, MO, USA). The maximum concentration of DMSO in SeBM was 0.05%. Control triplicates were performed using SeBM with DMSO (0.05%).

2.4. Western Blot

Protein lysates were prepared in RIPA lysis buffer (Millipore, Billerica, MA, EUA) (1 mL for ~5×10^6 cells) plus protease inhibitor cocktail (Sigma-Aldrich®,St Louis, MO, USA) (~10 μL/mL). Cells were transferred to a plastic tube and kept on ice for 5 min. Then, they were homogenized in a vortex mixer and centrifuged at 14,000 rpm for 15 min at 4 °C, and the supernatants were collected. The protein quantification was performed by the Bradford method in ELISA plates with 96 wells and read with an ELISA reader (595 nm). Aliquots (30–100 μg of protein) were treated with buffer solution (Laemmli Sample Buffer – BIO-RAD®) and β-mercaptoethanol at 100 °C for 5 min. Proteins were resolved by SDS-PAGE (triplicates for each treatment) and, after electrophoresis, transferred to nitrocellulose membrane. Nonspecific binding proteins were blocked by incubating the membrane in 3% BSA in Tris Buffered Saline with Tween® 20 (TBST) buffer for 1 h and 30 min at room temperature. Membranes were incubated overnight at 4 °C with the respective primary antibody: anti-occludin (clone ab31721); anti-ZO-1 (clone ab96594); anti-N-cadherin (clone ab12221); anti-βcatenin (clone ab32572); anti-F-actin (clone ab205); anti-TGF-β2 (clone ab36495) from Abcam® Inc., Cambridge, MA, USA; Androgen Receptor (sc-816, Santa Cruz® Biotechnology, Santa Cruz, CA, USA). Cytoskeletal protein β-actin (antibody anti-β-actin clone sc-47778, Santa Cruz® Biotechnology, Santa Cruz, CA, USA) was used as a protein loading control. Following four washing cycles in TBST, the membranes were incubated with specific HRP secondary antibody (IgG goat-anti rabbit, ab97051 or IgG goat-anti mouse, ab97023; Abcam® Inc., Cambridge, MA, USA). Immunoreactive components were revealed by chemiluminescence (Amersham™ ELC Select™ Western Blotting Detection Reagent, GE Healthcare®, UK). To calculate the mean and SEM, the optic density of the band was used as the unit of measure.

2.5. Morphology evaluation

For morphology evaluation, Hematoxylin and Eosin assay was performed for cell behavior, adhesion complexes and morphology observations. Cells were fixed in cold methanol for 10 min, passed fastly in ethanol 95%, 80%, 70%, washed in water and exposed to hematoxylin for 15 s. Cells were then washed in water, exposed to eosin for 40 s and washed in water again. Then, exposed for 1 min in ethanol 70%, 80%, 90%, 3 times in ethanol 100%, ethanol + xylene and 2 times in xylene. A drop of Permount was used to fix coverslip and analyses were performed using an image analyzing system (Axio Vision 4.8) coupled to the photomicroscope AxioLab.A1, Zeiss®.

Phalloidin, Tetramethylrhodamine B isothiocyanate (TRITC) (Sigma-Aldrich, St Louis, MO, USA), is a fluorescent conjugate used to label actin filaments. Stock solution was made in methanol, at 500μg/mL. Procedures were made according to producer guideline for cell staining. Cells were washed with phosphate buffered saline (PBS) and fixer for 5 min in 3.7% formaldehyde solution in PBS, then washed extensively in PBS. Cell permeabilization was made with 0.1% TRITON® X-100 in PBS, and washed again in PBS. Cells were then stained with a 500μg/mL fluorescent phalloidin conjugate solution in PBS for 40 min at room temperature. To remove unbound phalloidin conjugate, cells were washed several times with PBS and then analyzed in Zeiss® Axioskop 40 fluorescence microscope.

2.6. Statistical analysis

Results are expressed as mean ± SEM. Statistical analyses were performed using Mann–Whitney's test to compare experimental exposed groups to a Control group. P values ≤ 0.05 were considered significant.

3. Results

3.1. MTT assay

The MTT assay for MBP in HSeC Sertoli cells showed that doses above 10 μM inhibited more than 50% of cell viability after 48 h of exposure (Fig. 1-A). When cells were exposed to BPA selected doses, it was observed that doses above 20 μM inhibited more than 50% of cell viability after both exposure periods (Fig. 1-B). Based on the MTT results, the chosen doses were 10 μM for MBP and 20 μM for BPA.

3.2. Effects of MBP and BPA in blood-testis barrier proteins

3.2.1. MBP and BPA induce tight junction disruption

When human HSeC Sertoli cells were exposed to MBP, occludin expression declined 20% after a 6 h exposure, and significant occludin reduction was observed after 48 h of exposure, compared with the control group. ZO-1 expression showed significant reduction in both exposure periods (Figs. 2 and 3).

Similarly, a significant reduction in the level of occludin was observed at 48 h after BPA treatment, but only a 19% reduction after 6 h of exposure. Significant expression reduction was observed for ZO-1, in both exposure periods (Figs. 2 and 3).

3.2.2. MBP and BPA induce gap junction disruption

After 6 h of MBP treatment, comparison with the control group showed no significant changes in N-cadherin or β-catenin expressions, although N-cadherin declined 17%. Meanwhile, the expression of N-cadherin significantly decreased after 48 h of MBP treatment (Figs. 2 and 3), combined with a decrease of 36% of β-catenin after the same exposure period.

On the other hand, BPA exposure showed no significant decline in N-cadherin expression, however β-catenin expression decreased compared to the control group. After 6 h of exposure, β-catenin declined 35% and was significantly down-regulated after 48 h of treatment (Figs. 2 and 3).

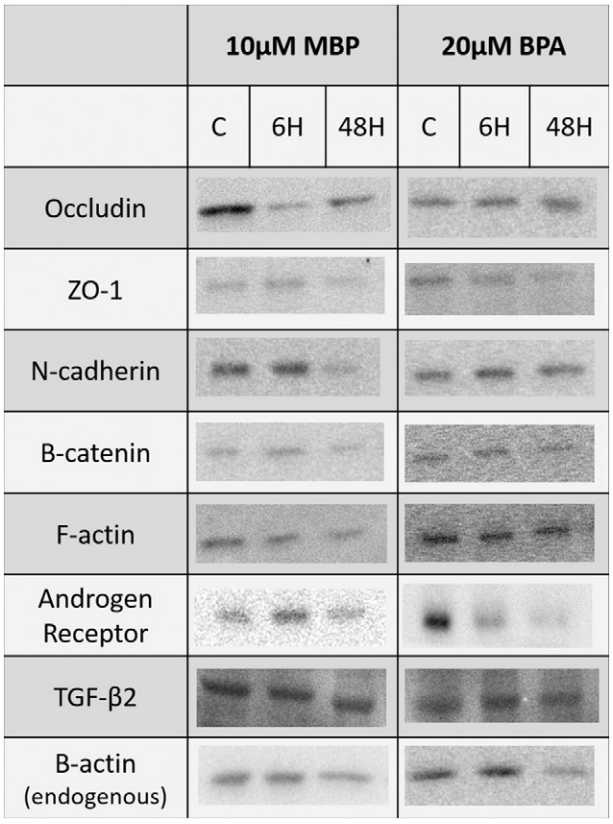

Fig. 2. Representative bands for junction and mediators proteins from experimental groups treated with 10 μM monobutyl phthalate or 20 μM bisphenol A exposure for 6 and 48 h from the protein extracts of cell cultures (each band representing a pool with samples of three cell cultures) obtained by Western Blot assay.

3.2.3. F-Actin does not seem to play a role in MBP or BPA BTB modulation

6 and 48 h of exposure of MBP or BPA to human HSeC Sertoli cells did not lead to significant changes in F-Actin expression (Figs. 2 and 3).

3.2.4. MBP and BPA seem to induce blood-testis barrier disruption through Androgen Receptor down-regulation

Exposure to MBP and BPA leads to a down-regulation of AR in both exposure times, while no changes were observed for TGF-β2 under the same conditions (Figs. 2 and 3).

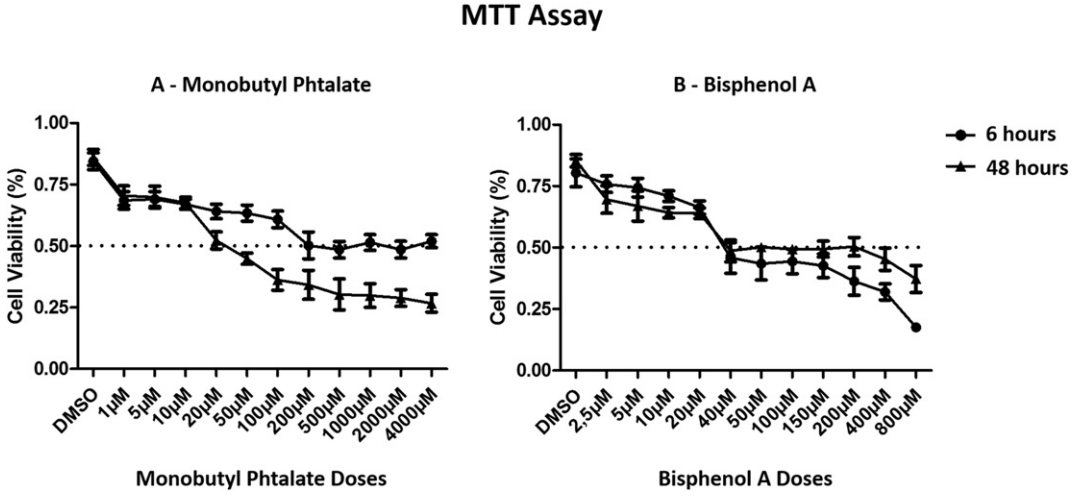

Fig. 1. MTT assay for monobutyl phthalate (A) and bisphenol A (B). The dose effect curve shows concentration-dependent toxicity in HSeC cells exposed to eleven doses of MBP or BPA. The graph represents relative cell viability after 6 and 48 h of exposure, expressed as mean ± SEM of seven plate's measurements. Results are representative of two independent experiments.

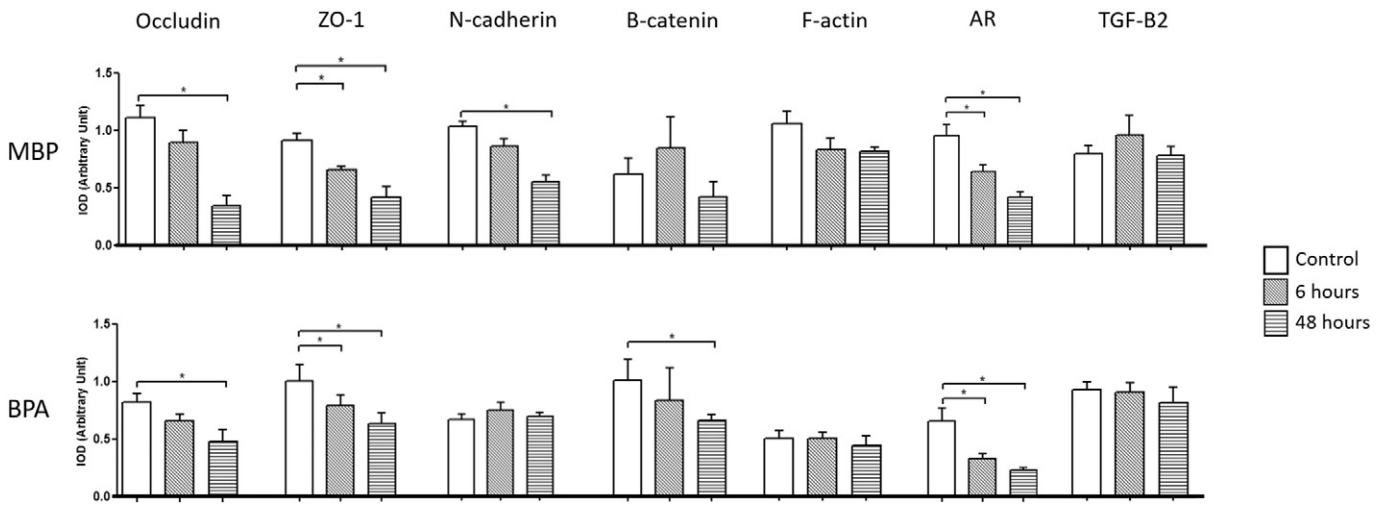

Fig. 3. The graphs represent the relative expression of integrated optical density for junction and mediators proteins from experimental groups treated with 10 μM monobutyl phthalate or 20 μM bisphenol A exposure for 6 and 48 h from the protein extracts of cell cultures (triplicates for each treatment). Optical density for all proteins was normalized by β-actin and was expressed as mean ± SEM. The asterisk represents a statistically significant difference ($P \leq 0.05$).

3.3. Effects of MBP and BPA on cellular interactions

3.3.1. MBP and BPA lead to a lower number of cells and loss of cell adhesion

After MBP and BPA exposure, cell monolayer was compromised, with reduced cell number and appearance of spaces between cells, showing reduction of cell adhesion (Fig. 4).

4. Discussion

In this study, we showed that the endocrine disruptor monobutyl phthalate and bisphenol A lead to a down-regulation of Androgen Receptor and junctional proteins in human Sertoli primary cell line (HSeC).

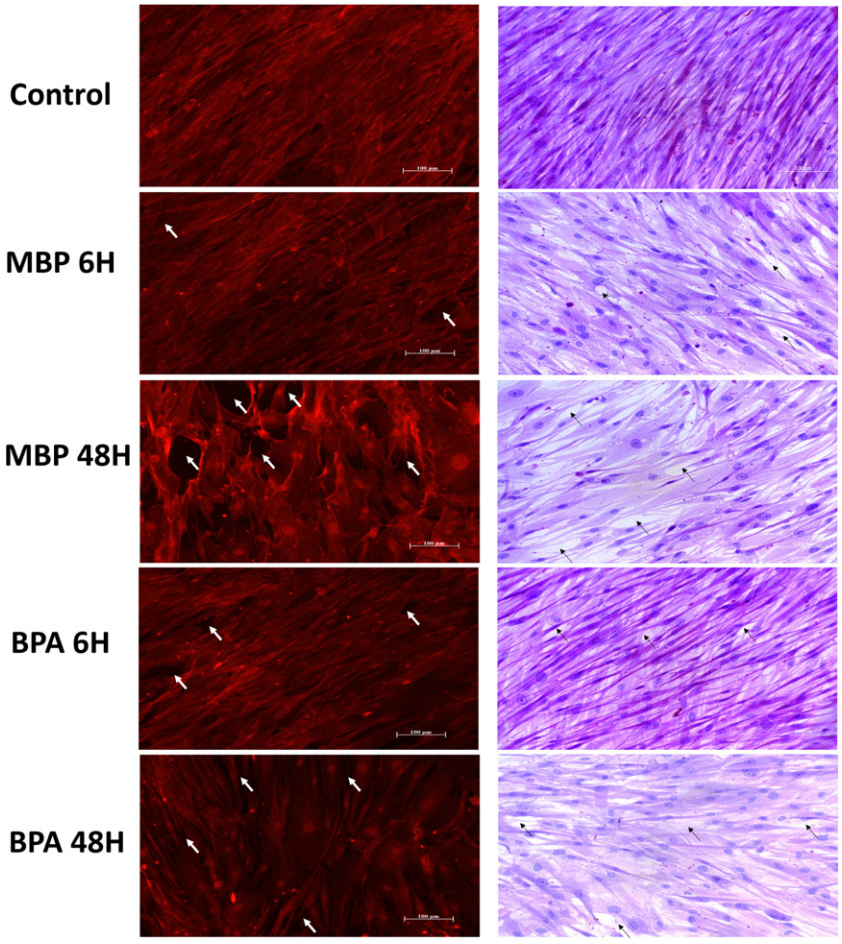

Fig. 4. HSeC Sertoli cells stained by Hematoxylin and Eosin (Optical microscope) and Phallodin-TRITC conjugated (Fluorescence microscope) from different treatments. Arrows are highlighting regions where loss of cell adhesion is evident. Magnification: ×200.

Although Cheng & Mruk (Cheng and Mruk, 2012) recently illustrated that studies using Sertoli cell lines may not reflect the physiological function of the Sertoli cell *in vivo*, this study is in agreement with Fiorini et al. (Fiorini et al., 2004), who reported native Sertoli cell features, like morphology, particularly tight junctions and some functional features in rat Sertoli cell line SerW3. The ability to form junctional interactions and express major and specific proteins involved in cell adhesion was seen in this study. Similarly, Chui et al. (Chui et al., 2011) showed that human Sertoli cells, when cultured *in vitro*, establish a functional epithelium that is associated with a physiological, tight junction-permeability barrier that mimics the Sertoli cell BTB *in vivo*.

Tight junctions are the only types of occlusion junction in the mammalian epithelium and endothelium, characterized by adhesion protein complexes formed mainly by occludin and ZO-1 connection (Lui et al., 2003).

The data presented in this study showed that MBP, at 10 μM, downregulated occludin expression in 48 h of exposure and leads to a significant reduction in ZO-1 expression after 6 and 48 h exposure periods.

Similarly, Li et al. (Li et al., 2010) showed that the metabolite and main toxicants of DBP and MBP induced damage to tight junctions between adjacent Sertoli cells at doses of 150 μM and 600 μM. Furthermore, Defamie et al. (Defamie et al., 2001) observed a similar expression reduction for ZO-1, after lindane (an organochlorine ED, used as pesticide and in some shampoos and lotions) exposure, but not for occludin in 42GPA9 Sertoli cells.

BPA has also been shown to disturb the tight junction in the blood-testis barrier. Studying BPA effects on immature rats, Li et al. (Li et al., 2009) found that 200 μM of BPA in a 24-hour exposure was able to reversibly disturb the tight junction barrier by the redistribution of BTB-associated proteins. BPA leads to a decline of N-cadherin and occludin at the Sertoli cell interface, which would affect cell adhesion, thereby compromising the tight junction barrier function and leading to BTB disruption (Chapin et al., 2008). Likewise, this study reported a significant reduction in occludin expression after 48 h of BPA exposure and in ZO-1 expression after both exposure periods.

Fiorini et al. (Fiorini et al., 2004) also reported that BPA at 45 μM, when exposed to rat Sertoli cell line SerW3, significantly reduced the steady-state protein levels of occludin and N-cadherin.

In the primary cell culture of immature rats, BPA caused a dose-dependent reduction in several junction proteins, at doses from 0.01 to 800 μM. These include tight junction proteins, occludin, and ZO-1, and basal proteins, N-cadherin and β-catenin (Li et al., 2009). Interestingly, no change was observed in N-cadherin expression followed by BPA exposure, but the results are in agreement with the observed decrease in occludin, ZO-1 and β-catenin. Xiao et al. (Xiao et al., 2014) also reported that BPA at 200 μM in human Sertoli cells reduced ZO-1 expression.

Likewise, this study found that MBP and BPA induced gap junction disruption. Gap junctions are cell–cell communication junctions formed by proteins such as N-cadherin and β-catenin, which form a protein complex responsible for cell adhesion. It can be found in the basal region of the blood-testis barrier (Lie et al., 2011a).

Using the 24GPA9 Sertoli cell line, Defamie et al. (Defamie et al., 2001) showed that Lindane, a chemical endocrine disruptor widely used as pesticide, at a non-cytotoxic dose (50 mM), abolished gap junction intercellular communication between adjacent cells. This change was associated with a time-related reduction and redistribution of Cx43 from the membrane to the cytoplasmic perinuclear region. Instead, this study analyzed N-cadherin and β-catenin, also from gap junctions, showing significant decreases after 48 h of exposure to MBP and BPA, respectively.

In two previous *in vitro* studies, it was indicated that phthalates disturbed the energy transfer chain existing between germ cells and Sertoli cells to lead to adverse changes in cell polarization, which affects membrane proteins (Foster et al., 1982; Li, 1997). The results of this study with MBP also showed the potential of phthalate to disturb adhesion junctions.

Xiao et al. (Xiao et al., 2014), in a study involving cadmium and BPA, reported no differences in N-cadherin or β-catenin expression after cadmium exposure. Contrarily, they reported that BPA at 200 μM downregulated the expression of these proteins, which was also observed in this study after 20 μM BPA exposure.

Fiorini et al. (Fiorini et al., 2004) showed strong evidence that these specialized junctional proteins are essential for the development and maintenance of spermatogenesis and those alterations of Sertoli–Sertoli cell interactions may lead to sterility in the male.

Specialized junctions are anchored by F-actin bundles and the disruption of the TJ barrier has been associated with a disruption of the actin network at the BTB (Lie et al., 2011b). However, this study did not find significant changes in F-actin expression followed by cell treatments, suggesting that the synthesis and degradation process were not modified. Similarly, Li et al. (Li et al., 2009) showed that F-actin bundles distribution in Sprague–Dawley primary cell culture was not compromised after 40 μM or 200 μM BPA exposure.

Testosterone promotes BTB assembly and its maintenance *via* its action on the production of proteins, such as occludin, and their proper localization at the Sertoli–Sertoli cell interface, proper protein–protein interactions (*e.g.*, N-cadherin–β-catenin), proper recycling of endocytosed proteins back to the cell surface to maintain cell adhesion and maintenance of actin filament bundles within the Sertoli cell (Xiao et al., 2011), which are necessary to maintain BTB integrity and function during the seminiferous epithelial cycle of spermatogenesis (Cheng and Mruk, 2012).

Furthermore, spermatogenesis maintenance depends on blood-testis dynamics control by the Androgen Receptor, which mediates the kinetics of integral junction proteins, including their distributions, internalization, degradation and recycling (Li et al., 2009).

Recent studies have suggested that "new" tight junction-fibrils may be formed below a migrating preleptotene spermatocyte *via* the action of testosterone, which is known to promote BTB integrity (Chung and Cheng, 2001) by mediating *de novo* synthesis of integral membrane proteins (*e.g.*, occludin) at the site. This occurs before "old" tight junction-fibrils found above migrating spermatocytes are completely disassembled, which is likely mediated by cytokines (TGF-β2, TGF-β3 and TNFα) that disturb BTB integrity (Su et al., 2010).

Our study shows that both MBP and BPA, at 10 μM and 20 μM, respectively, after 6 or 48 h of exposure, lead to a decline in Androgen Receptor expression in the HSeC cells (Figs. 2 and 3). Li et al. (Li et al., 2009) suggested that the disruptive effects of BPA on the Sertoli cell BTB function appear to be mediated *via* changes in the kinetics of integral junction proteins. These include their redistribution at the Sertoli–Sertoli cell interface and an increase in their turnover rate. Indeed, BPA was shown to enhance the turnover of occludin and N-cadherin in Sertoli cells with an established tight junction barrier.

Li et al. (Li et al., 2009) also suggested that the alteration of the distribution of integral membrane proteins and their peripheral adaptors, seen in this study, is likely due to a significant increase in the turnover from the cell surface. This increase in the protein turnover represents the net result of their internalization, degradation and recycling.

In views of BTB modulation, the data in this study did not show significant changes in TGF-β2 expression, which could mean that the endocytosis process is maintained after MBP or BPA exposure, leading to BTB disruption in association with a decrease in AR expression.

Ablation of AR (Willems et al., 2010) affects Sertoli cell maturation, results in delayed and incomplete formation of the blood-testis barrier, and changes the expression pattern of several genes related to cell adhesion in Sertoli cell (Fig. 5). Furthermore, after establishing the blood-testis barrier, AR is required for the process of opening the barrier for primary pre-leptotene spermatocytes passage, which means that the structurally normal barrier may not be functional without regular AR expression, leading to infertility (Willems et al., 2010).

Su et al. (Su et al., 2010) showed that TGF-β2, but not testosterone, appeared to direct endocytosed proteins for intracellular degradation

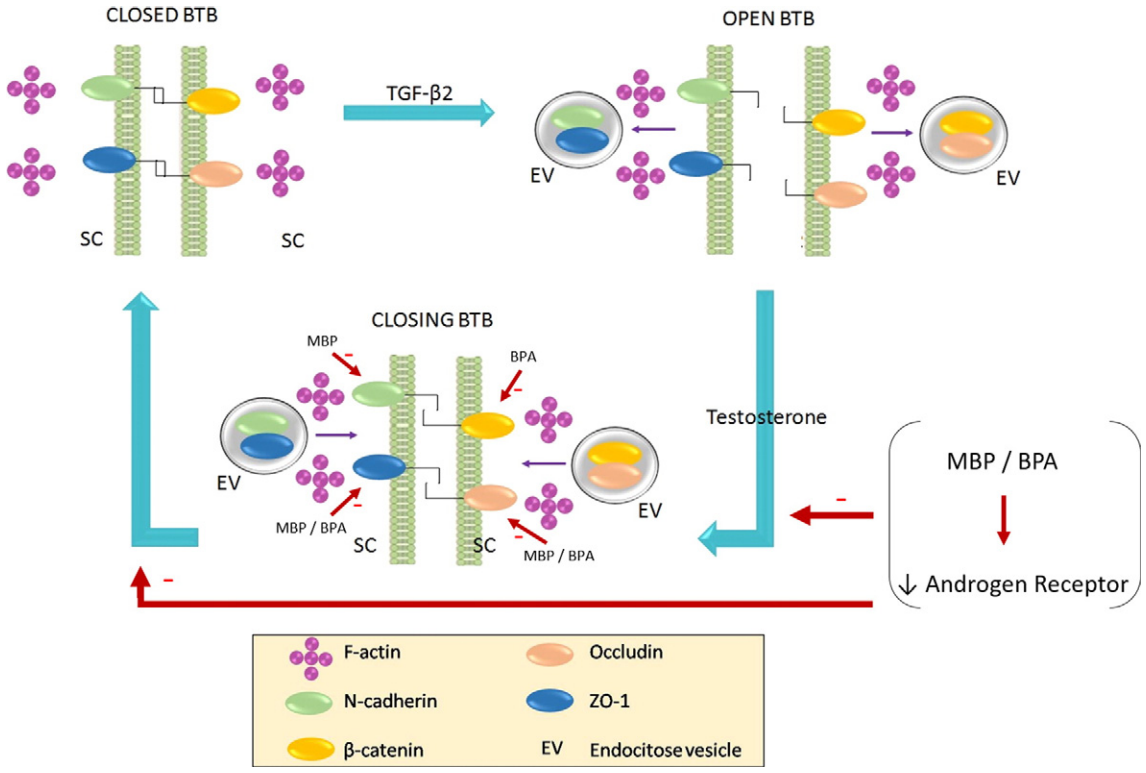

Fig. 5. A schematic drawing that illustrates the molecules that interact with each other to regulate the opening and closing of the normal BTB and the disruptive effects of MBP and BPA. Exposure to endocrine disruptors leads to a decline in occludin/ZO-1 and cadherin/catenin complex due to Androgen Receptor impairment, disrupting tight and gap junctions formed between adjacent Sertoli cells at the basal compartment of the seminiferous epithelium near the basal lamina.

since TGF-β2-treated Sertoli cells was shown to sequester internalized occludin. For instance, TGF-β2 signaling can disrupt either BTB and germ cell adhesion or BTB function only (Lui et al., 2003). More studies have shown that TGF-β2 also enhances clathrin mediated protein endocytosis at the Sertoli cell BTB (Xia et al., 2009; Yan et al., 2008), but instead of recycling the endocytosed proteins back to the cell surface, TGF-β2-induced endocytosed proteins is targeted for endosome- and/or ubiquitin-mediated intracellular degradation (Li, 1997; Yan et al., 2008).

We showed that a reduction of AR expression combined with a regular TGF-β2 expression also leads to an expression change of several junctional proteins. However, the precise mechanisms whereby these toxicants may exert their effects on intercellular junctions in the testis are not entirely known (Fiorini et al., 2004).

Likewise, Wakui et al. (Wakui et al., 2014) could not elucidate the precise mechanism of phthalates repression of AR expression after reporting that DBP at 100 mg/kg/day on gestational day 12 to 21 reduced the offspring AR expression level to 55% in Leydig cells. Elucidation of the AR repression mechanism needs to be researched in further experiments.

Phalloidin and HE staining provided a view of *in vitro* environment, showing loss of cell adhesion and reduced number of cells in monolayer. Phalloidin staining is in agreement with our Western Blot results for F-Actin, showing that expressions are not compromised after treatments. Besides, F-Actin expression doesn't seem to be able to maintain cell adhesion without tight or gap junctions' proteins, leading to loss of adhesion complex. Li et al. (Li et al., 2009) didn't observe changes in tight or gap junction protein localization after BPA exposure, but also reported changes in protein expression after Western Blotting assay, corroborating our findings.

Using HSeC cells as an experimental model of the blood-testis barrier, it was found that both the endocrine disruptors studied, monobutyl phthalate and bisphenol A, were able to reduce the Androgen Receptor expression in both the periods of exposure studied, interfering with the modulation of the blood-testis barrier and reducing the expression of integral barrier proteins. In summary, this study identified that MBP and BPA have the potential to disturb the blood-testis barrier in human Sertoli cells. Thus, considering the importance of the blood-testis barrier for male gametogenesis maintenance, further investigation will be necessary to fully understand the effects of MBP and BPA on male reproductive biology.

Transparency document

The Transparency document associated with this article can be found, in online version.

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