doi: 10.1111/obr.12471

Etiology and Pathophysiology/Toxicology

Molecular targets of developmental exposure to bisphenol A in diabesity: a focus on endoderm-derived organs

I. Porreca,[1,†] L. Ulloa-Severino,[1,2†] P. Almeida,[3] D. Cuomo,[1] A. Nardone,[5] G. Falco,[1,6] M. Mallardo[7] and C. Ambrosino[1,4]

[1]IRGS, Biogem, Ariano Irpino, Italy, [2]PhD School in Nanotechnology, University of Trieste, Trieste, Italy, [3]STAB VIDA-Investigação e Serviços em Ciências Biológicas, Madan Parque, Caparica, Portugal, [4]Department of Science and Technology, University of Sannio, Benevento, Italy, [5]Department of Public Health, University of Naples 'Federico II', Naples, Italy, [6]Department of Biology, University of Naples 'Federico II', Naples, Italy, and [7]Molecular Medicine and Medical Biotechnologies, University of Naples 'Federico II', Naples, Italy

Received 1 June 2016; revised 8 August 2016; accepted 23 August 2016

Address for correspondence: I Porreca, IRGS, Biogem, Via Camporeale, 83031 Ariano Irpino, Avellino, Italy.
E-mail: immacolata.porreca@biogem.it
C Ambrosino, Department of Science and Technology, University of Sannio, via Port'Arsa 11, 82100 Benevento, Italy.
E-mail: coambros@unisannio.it
[†]Both authors contributed equally to this work.

Summary

Several studies associate foetal human exposure to bisphenol A (BPA) to metabolic/endocrine diseases, mainly diabesity. They describe the role of BPA in the disruption of pancreatic beta cell, adipocyte and hepatocyte functions. Indeed, the complexity of the diabesity phenotype is due to the involvement of different endoderm-derived organs, all targets of BPA.

Here, we analyse this point delineating a picture of different mechanisms of BPA toxicity in endoderm-derived organs leading to diabesity. Moving from epidemiological data, we summarize the *in vivo* experimental data of the BPA effects on endoderm-derived organs (thyroid, pancreas, liver, gut, prostate and lung) after prenatal exposure. Mainly, we gather molecular data evidencing harmful effects at low-dose exposure, pointing to the risk to human health. Although the fragmentation of molecular data does not allow a clear conclusion to be drawn, the present work indicates that the developmental exposure to BPA represents a risk for endoderm-derived organs development as it deregulates the gene expression from the earliest developmental stages.

A more systematic analysis of BPA impact on the transcriptomes of endoderm-derived organs is still missing. Here, we suggest *in vitro* toxicogenomics approaches as a tool for the identification of common mechanisms of BPA toxicity leading to the diabesity in organs having the same developmental origin.

Keywords: Bisphenol A, endoderm-derived organs, epigenetics, perinatal exposure.

Abbreviations: BPA, bisphenol A; ER, oestrogen receptor; GD, gestation day; GPR30, G protein coupled receptor 30; 0mER, membrane-associated ER; NF-kB, nuclear factor kappa B; NHANES, National Health and Nutrition Examination Survey; PND, postnatal day; T2D, type 2 diabetes; T_4, tetra-iodothyronine; TH, thyroid hormone; XME, xenobiotic-metabolizing enzyme.

Introduction

Human exposure to bisphenol A (BPA), used in the manufacturing of several products as polycarbonate plastics, is continuous and widespread. Ingestion is thought to be the primary source of human exposure (1–4). Several studies showed its rapid transformation into an inactive form (5). The residual active form is in small amounts, not

raising concern if we exclude that metabolites can also exert toxic activity. BPA acts with a nonmonotonic dose–response curve (6,7), typical of natural hormones and other endocrine-disrupting compounds, complicating the extrapolation of a threshold daily exposure dose from experimentally determined high-dose effects (8).

Human exposure to BPA has been associated with different diseases including the diabesity (obesity associated to type 2 diabetes [T2D]) (9). Indeed, BPA differentially interferes with several hormonal signalling pathways. The ability of BPA to interfere with oestrogen signalling was accepted from the early beginning. It was considered a weak oestrogen because its binding affinity to the oestrogen receptors (ERs) alpha and beta was estimated to be over 1,000–10,000-fold lower than the natural hormone E2 (10,11). However, more recent studies have demonstrated that BPA oestrogenicity can be exerted through ER-dependent extra-nuclear mechanisms, at similar or stronger levels than E2 (12,13). It acts by binding to membrane-associated ER (mER) and the G protein coupled receptor 30 (GPR30), for which it shows higher binding affinities than for ERs (14–16). In addition, at low dose, BPA affects the pathways related to the thyroid receptor (17) and to the nuclear oestrogen-related receptor γ (18). At higher concentrations, BPA can also act on the androgen receptor (19). Other mechanisms of low-dose BPA toxicity include the pathways related to oxidative stress (20), such as the Nuclear Factor kappa B (NF-*k*B) (21,22) and, finally, epigenetic modifications (23). The last is an important issue because the adverse effects of a foetal/neonatal exposure can remain undetected till diseases develop in adulthood.

The BPA has been detected in amniotic fluid, neonatal blood, placenta, cord blood and human breast milk (2). Infants and foetuses are more susceptible \to its effects because of their rapid development and reduced detoxification ability during pregnancy observed in both rodents (24) and humans (25). Indeed, BPA shows limited binding to alpha fetoprotein, a protein that binds oestrogens and protects the developing tissues from excessive exposure, resulting in an increased access for BPA to oestrogen-sensitive tissues (26,27). Altogether BPA can exert greater effects on development than expected.

The BPA toxicity data are often contrasting, and they have been already reviewed specifically for some endoderm-derived organs (28–33). These organs develop from a specific position of the primitive gut in response to inductive signals from surrounding tissues, activating specific transcriptional programmes. Although not confirmed in the Good Laboratory Practice (GLP) studies (34,35), academic research provided evidence that BPA can alter the function of endoderm-derived organs like liver, thyroid and pancreas as well as, recently, prostate, gut and lung (36–47).

Considering the recent advances in the understanding of stem cell biology and transdifferentiation processes, here, we analyse the published research to explore the effects of developmental exposure to BPA, via maternal intake, exerted on the endoderm-derived organs and playing a direct or indirect role in the diabesity phenotype in the adulthood. The effects of BPA exposure on the metabolic–endocrine functions of some of these organs have been partially summarized at epigenetic/molecular level (9). As molecular data are limited, we analysed in detail an *in vitro* toxicogenomic analysis, conducted in our laboratory on thyrocytes, that suggests mechanisms of BPA toxicity also in other endoderm-derived tissues involved in diabesity.

Impact of developmental exposure to bisphenol A on endoderm-derived organs

The metabolic imbalance and diabesity associated to early exposure to BPA are the object of different reports, often focused on two endoderm-derived organs: liver and pancreas (9). Recently published works underline the involvement of other endoderm-derived organs in diabesity, although it is debated if their impairment is a cause or an outcome of the pathological condition. Here, we analyse those works describing the damages of endoderm organs upon *in utero* exposure to BPA in animal models, referring to the diabesity phenotype. We will discuss molecular data to highlight 'phenotype anchoring' aspects.

Liver

The liver plays a relevant part in the metabolic balance impaired in the diabesity phenotype (48). Indeed, the increased expression of hepatic genes involved in glycolysis and lipogenesis is pivotal in the enhancement of insulin resistance (49).

Human foetal exposure has been confirmed by the detection of BPA in foetal liver samples, at concentrations ranging between 1.3 and 27 $ng\,g^{-1}$ (50,51). The BPA molecular targets in foetal liver have been investigated in animal models. It altered the expression of key markers of hepatocyte maturation (i.e. glycogen synthase), of immature hepatocytes (alpha fetoprotein) and of CCAAT/enhancer binding protein alpha (C/EBP-α), a hepatocyte-specific transcription factor, impairing the maturation of mouse livers in female foetuses exposed via maternal diet (E7.5–E18.5). Increased expression of the xenobiotic-metabolizing enzyme (XME), Cyp1a1 and Gst, was described only in livers of murine embryos exposed to 200 $\mu g\,kg^{-1}\,d^{-1}$ of BPA, although other doses were tested (52). In contrast, the inhibition of XME transcripts and the hypermethylation of their promoters were reported in foetal human livers specimen (BPA range: 35.4–56.1 $ng\,g^{-1}$) (53). The described results were not really contrasting (52) as different genes/proteins of the XMEs family were monitored and the liver or plasma level of BPA was not determined in the mouse study. In addition, the perinatal exposure to BPA determined liver

damages in adult rats exposed to 50 $\mu g\,kg^{-1}\,d^{-1}$ of BPA (reference dose) from conception to weaning. Increased apoptosis of adult hepatic cells, because of altered expression of Bcl-2 family genes, was observed (54). In similar experimental settings, a decrease in the global hepatic DNA methylation and the specific hypermethylation of the hepatic *glucokinase* promoter were described in 3- and 21-week old rats. The resulting inhibition of *glucokinase* transcript could contribute to insulin resistance (55).

The modifications of hepatic methylome were investigated by genome-wide analysis of liver DNA, conducted at postnatal day (PND) 22, in mice exposed to multiple doses of BPA through the maternal diet (56). Interestingly, they involved well-known targets of BPA, such as Myh7b and Slc22a12. These results were not confirmed in another work, similarly conducted, in which less sensitive techniques were used (global DNA methylation – High-performance liquid chromatography) (57). Sex-specific epigenetic mechanisms have been recently described for the inhibition of *Cpt1a* and other fatty acid β-oxidation transcripts in liver of male rats exposed to 100 $\mu g\,kg^{-1}\,d^{-1}$ of BPA from gestation day (GD) 6 to PND21, developing the steatosis in adulthood (58).

The XME enzymes are considered factors of progression in diabesity (59) and in the imbalance of the energetic metabolism (60); therefore, they are the molecular targets of BPA in diabesity. In addition, the analysis of mice metabolome suggested that BPA could affect the energetic metabolism impairing the mitochondrial pathways (61). The results were confirmed at molecular level in a study characterizing the fatty liver disease developed during adulthood in rats exposed to 40 $\mu g\,kg^{-1}\,d^{-1}$ of BPA from GD0 to PND21. The upregulation of genes involved in lipogenesis pathways, Reactive Oxygen Species generation and cytochrome C release from mitochondria in liver was detected already at 3 weeks, when liver morphology and function were still normal (62).

In summary, prenatal and perinatal exposure to BPA can damage liver activity deregulating gene expression by sex-specific epigenetic mechanisms, implied in the diabetes (63). The summarized molecular modifications are involved in the diabesity phenotype progression, in particular, the ones related to mitochondrial dysfunctions and resulting in damage of redox homeostasis (64).

Pancreas

Exposure to BPA has been epidemiologically associated with T2D, insulin resistance and obesity (65,66). BPA administration (10 $\mu g\,kg^{-1}\,d^{-1}$, GD9–GD16) could predispose adult male offspring to T2D development (36). The pancreatic beta cells, isolated from exposed animals, exhibited enhanced insulin secretion in response to basal level of glucose, altered calcium signalling and reduced proliferation rate (36). The results are in agreement with other *in vitro* and *in vivo* studies demonstrating that BPA could damage pancreatic beta cells or other pancreatic cell types (37), whose impairment is strictly connected to T2D (67). The impairment of glucose homeostasis dependent on BPA has been documented in several other studies (68–70). The development of hyperglycaemia, hyperinsulinemia and glucose intolerance in adulthood was described in rats exposed to 50 $\mu g\,kg^{-1}\,d^{-1}$ of BPA from conception to weaning (71). Morphological (swollen mitochondria, dilated rough reticulum, etc.) and molecular changes (inhibition of specific transcripts such as *Pdx-1* and *Nkx6.1*) were described in beta cells isolated from exposed animals. Recently, the role of BPA exposure in proliferation and differentiation of beta cells has been confirmed in a report showing an altered α:β-cell ratio in islets prepared from the foetal pancreas of exposed animals (72). The critical window of susceptibility to BPA exposure on the development of dysglycaemia was characterized by exposing pregnant mice to 100 $\mu g\,kg^{-1}\,d^{-1}$ of BPA at different times during foetal/neonatal life and analysing the offspring at 3, 6 and 8 months of age. The study confirmed that male mice were more prone to developing T2D and identified the preimplantation period as the less vulnerable. All the morphological and functional damages of beta cells were confirmed, and a reduced rate of beta cells turnover within the islet was observed (73). BPA exposure accelerated spontaneous diabetes development in non-obese diabetic mice developmentally exposed from conception to weaning, through the dams' drinking water (0.1, 1 and 10 $mg\,L^{-1}$). Increased apoptosis of beta and glucagon-secreting cells was described, caused by damages of the immune system (37). Recently, Angle *et al.* have demonstrated the existence of a nonmonotonic relationship between BPA foetal exposure (from GD9 to GD18) and insulin sensitivity using a full range (from 5 to 50,000 $\mu g\,kg^{-1}\,d^{-1}$) of BPA doses. Insulin sensitivity decreased at the other tested doses, 500 and 50,000 $mg\,kg^{-1}\,d^{-1}$ of BPA (69).

Molecular pathways have been investigated in beta cells isolated from humans, mice and rats (12,14,74–77). BPA increased the insulin content and its release in the mouse pancreatic islets. The insulin gene expression was induced, in an inverted U-shape dose–response manner (14), by mechanisms involving nuclear ERs, mER and GPR30 (12,74). Other *in vitro* studies confirmed the role of mitochondria alteration (mass, morphology, etc.) and of *Bax* (induced) and *Bcl2* (reduced) gene expression in beta cells failure upon exposure (75). Similar results were shown in *ex vivo* mouse and rat pancreatic islets (76,77).

In summary, BPA is directly involved in islets failure, which is considered a critical aspect of diabesity in youth (78). Although the molecular mechanisms of BPA activity in beta cells have been described, they need further verification *in vivo*.

Thyroid

Thyroid hormones (THs) regulate glucose and lipid metabolism; therefore, diabesity and thyroid diseases appear to be closely linked (9,79,80). TH disruptors, such as BPA, can perturb the TH action in all body tissues through different mechanisms, in particular, antagonizing the activity of the thyroid receptor (17,44,81). Epidemiological data from the National Health and Nutrition Examination Survey (NHANES) study suggested an inverse relationship between BPA exposure and total tetra-iodothyronine (T_4) concentrations (82). Some aspects related to diabesity were also found to be impaired in the NHANES cohort (83,84). The thyroid effects were strengthened by another epidemiological study following women and their children (Center for the Health Assessment of Mothers and Children of Salinas cohort). High maternal urinary concentrations of BPA were significantly associated with lower maternal serum T_4 and negatively associated with neonatal thyroid-stimulating hormone in boys but not in girls (85).

Contradictory results have been reported in animal models. No alteration in serum T_4 levels, dosed at 1, 3 and 9 weeks of age, was retrieved in rat offspring of pregnant dams orally exposed to BPA (4–400 mg kg^{-1} d^{-1}, GD6–PND20) (81). In contrast, BPA exposure (1–50 mg kg^{-1} d^{-1}) from GD6 resulted in an increase of T_4 serum level only at PND15 in rat offspring. The increase resulted in an augmented expression of *RC3/neurogranin*, a TH target gene (38). A study executed at lower BPA doses (1 and 0.1 mg kg^{-1} d^{-1}), administered from GD11 to PND 21, reported a transient hyperthyroidism at PND7 followed by hypothyroidism at PND21 in male offspring (87).

In a model of a long-gestation species, with regulation and ontogenesis of thyroid function similar to humans (Lacaune sheep), the exposure to BPA (5 mg kg^{-1} d^{-1}, GD28 end of pregnancy) decreased the total T_4 blood level in pregnant sheep and in newborns (30% decrease), disappearing at 2 months of age (88).

The BPA might alter thyroid homeostasis antagonizing TH signalling pathways (17,89). However, it is not possible to exclude a direct action of BPA on thyrocytes. This point was assessed *in vivo* (zebrafish embryos) and *in vitro* (immortalized rat thyrocytes) analysing the transcription of thyroid specific genes such as *thyroglobulin* (codifying for the precursor of TH) and *Pax8*, its main transcriptional regulator, in both experimental models exposed to low-dose BPA (21). This was the first evidence of a direct effect of BPA on thyroid cells, suggesting the involvement of the NF-*k*B and the Retinoic Acid Receptor/Retinoid X Receptor pathways in this activity. Other mechanisms of thyroid BPA toxicity as well as the deregulation of several pathways involved in the diabesity phenotype were evidenced in an *in vitro* transcriptomic analyses conduced on thyrocytes (90), among them insulin receptor signalling (p-value = 0.025) and 1D-myo-inositol hexakisphosphate biosynthesis II and superpathway of inositol phosphate compounds (p-value = 0,035) (unpublished results) (91). The transcriptomic study suggested that the gene expression profiling of immortalized thyrocytes exposed to 1 nM of BPA could evidence the impairment of pathways and mechanisms of toxicity in other endoderm-derived organs, in which they have not been directly assessed.

Gut

The digestive tract is the largest endocrine-related organ system in the body, secreting several metabolic hormones (i.e. gherlin and leptin). Among the endoderm-derived organs, it is the only one characterized by a continuous and rapid renewal of its epithelial cells. Gastrointestinal morphology and function are affected in diabesity, in particular, with intestinal barrier impairment (92,93). Recent evidence describes 'leaky gut' as a factor involved in the development of diabesity. The destruction of the intestinal barrier and its permeability may enhance the natural interactions between intestinal bacterial products and hepatic receptors (e.g. toll-like receptors) promoting oxidative stress, insulin resistance and so on. The alterations of the junction systems in the intestinal epithelial cells are involved in the process (94).

Clinical and experimental evidence points to oestrogen and xenoestrogen (BPA among them) role in the development and regulation of the intestinal barrier (95). The impact of BPA on the intestinal function remains poorly explored although the gut is in direct contact with BPA when orally absorbed. BPA affects the gut permeability reducing Ca^{++} adsorption in pregnant mice (39,40). Perinatal exposure (GD15–PND21) to BPA (5 mg kg^{-1} d^{-1}) impaired intestinal permeability and, consequently, increased the inflammatory response after colitis induction in female rats during adulthood. The increased transcription of tight junction proteins, occludin and junctional adhesion molecule A, was described in the mother's colon whereas ER beta transcript was found to be increased in the mothers and reduced in male offspring (39). In the same study, the reduction of intestinal permeability in the colon of neonates and adult female offspring was documented (39). Thus, the intestinal barrier is a target of BPA, and related damages, such as impairment of cell–cell junctions, can contribute to progression of inflammatory diseases and diabesity (94).

More detailed molecular mechanisms on modulation of junctions in intestinal mucosa are not available although it was characterized in the blood–testis barrier (96). We tried to gain further insights on this aspect by looking for cell junction-related information in the previously described *in vitro* toxicogenomics study (90). We evidenced the BPA effects on the cellular junction systems in immortalized thyrocytes (Table 1). Similar to gut epithelial cells, these cells are polarized and strictly connected. In thyrocytes,

Table 1 Tight junction-related genes deregulated in immortalized thyrocytes exposed to 1 nM of BPA as determined by microarray

Gene symbol	Fold change (BPA-treated vs. untreated cells)	Corrected *p*-value	Gene description
Utrn	−2.01	2.66E-05	Utrophin ([113])
Ctnna1	−2.07	1.41E-03	Catenin (cadherin-associated protein), alpha 1
Vcl	−2.09	2.81E-03	Vinculin ([114])
Camk2d†	−2.11	8.02E-04	Calcium/calmodulin-dependent protein kinase II delta
Nf2	−2.12	1.69E-04	Neurofibromin 2 (merlin) ([115])
Shroom3	−2.13	3.68E-04	Shroom family member 3 ([116])
Lrp4†	−2.13	5.00E-03	Low-density lipoprotein receptor-related protein 4
Csk	−2.15	1.90E-04	c-src tyrosine kinase ([117])
Snta1†	−2.24	3.80E-04	Syntrophin, acidic 1
Dlg1	−2.25	1.45E-04	Discs, large homolog 1 (Drosophila) ([118])
Akap1†	−2.26	1.50E-04	A kinase anchor protein 1
Tjp3	−2.26	1.43E-04	Tight junction protein 3 ([119])
Ptprm	−2.27	1.45E-04	Protein tyrosine phosphatase, receptor type ([120])
Itga5	−2.31	5.11E-04	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
Pard3	−2.39	3.00E-05	Par-3 (partitioning defective) ([121])
Amotl2	−2.45	7.46E-04	Angiomotin like 2 ([122])
Numb	−2.48	1.58E-04	Numb homolog (Drosophila) ([123])
Dsg2	−2.52	6.28E-05	Desmoglein 2
Tjp2	−2.53	1.61E-04	Tight junction protein 2
Arhgef2	−2.63	1.97E-06	Rho/rac guanine nucleotide exchange factor 2 ([124])

Genes deregulated in immortalized thyrocytes after 7 d of exposure to 1 nM of BPA and selected for their involvement in cell junctions formation (the whole gene list is available at www.ebi.ac.uk/arrayexpress under the accession number E-MTAB-4458) (90).
†Gene involved in cytoskeleton organization and characterized only in the neuromuscular junction.
BPA, bisphenol A.

genes involved in tight junction (i.e. Tjp3 and Tjp2), adherens junctions (i.e. Vcl and Ctnn1a) and signal transduction and related cytoskeleton remodelling (i.e. Nf2) were inhibited. Although describing the BPA effects on junction transcripts in thyrocytes, the results summarized in Table 1 are different to the ones described *in vivo* (39). The discrepancy can depend on the short exposure time considered for *in vivo* experiments not reflecting continuous and rapid renewal of the intestinal epithelium. These data are just suggestive of the possible mechanisms responsible for establishment of the leaky gut phenotype upon BPA foetal exposure. They need to be specifically investigated *in vivo*.

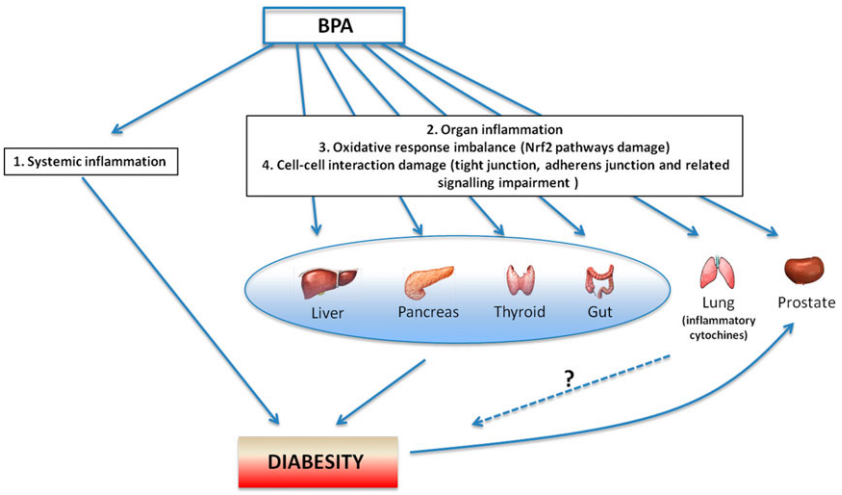

Figure 1 Schematic drawing of the multiple bisphenol A (BPA)-induced effects contributing to the diabesity development. BPA involvement in diabesity is exerted through different mechanisms: (i) induction of systemic inflammation; (ii) organ-specific inflammation; (iii) impairment of cell–cell interaction; and (iv) dysfunction of oxidative stress control pathways. When these alterations occur in the endoderm-derived organs such as pancreas, gut, thyroid and liver, it results in diabesity. The lung involvement in the disease development is not clearly stated, but the local inflammatory cytokines production associated to BPA exposure could have a role in the diabesity progress.

Lung

The BPA is reported as a risk factor for childhood respiratory problems (asthma) and chronic obstructive pulmonary disease (COPD) (97), both exhibiting an increase of pro-inflammatory cytokines potentially associated to T2D risk. Furthermore, COPD, as well as diabesity, is associated to oxidant/antioxidant imbalance and systemic inflammation (98). BPA might have a role in the etiopathogenesis of COPD through a mechanism involving Nrf-2, a characterized target in nonendodermal cells (99,100). This result was confirmed by *in vitro* toxicogenomics analyses in immortalized thyrocytes exposed to 1 nM of BPA for 3 d, in which the activation of Nrf2-mediated oxidative stress response pathway (p-value = 0.024, z-score = 1.4) was observed (90).

Evidence of BPA toxicity in lungs came from epidemiological studies that, although not describing identical results, underline a positive correlation between maternal urinary BPA concentrations and odds of the child's wheeze (41,42).

The possible mechanisms through which prenatal exposure to BPA affected the health of the lungs have been investigated in rhesus macaques, in which BPA (GD100–GD150) altered the development of airway cells. Higher expression of *Muc5B* and *Ccsp*, genes codifying two secretory proteins, was retrieved contributing to the excessive secretion and storage of mucus. Notably, BPA exposure was conducted in order to obtain BPA levels in serum (from 2.2 to 3.3 ng mL^{-1}) within the range measured in humans (101). BPA exposure during the prenatal and postnatal phases in mice led to a predisposition to allergic asthma as well (102). BPA exposure (10 μg mL^{-1} in drinking water, from GD0 to weaning) resulted in bronchial hyperresponsiveness to allergens and eosinophilic airway inflammation in PND17 offspring (103). The asthma BPA-related phenotype was sex dependent, with women more prone to its development (104). No effect was detected after postnatal exposure to BPA by breast milk (105). Effects of BPA developmental exposure on the expression of key molecular markers of lung maturation, such as aquaporin 5, were studied in mice exposed via mother's diet (E7.5–E18.5). It was altered, and the involvement of glucocorticoid signalling was suggested (106).

Overall, the effects of developmental exposure to BPA on the lung have been analysed only recently and poorly at molecular level, a point that should be specifically addressed.

Prostate

Here, we will briefly discuss the molecular aspects of BPA effects on the prostate epithelium as not directly involved in diabesity, although diabesity markedly increases the risk of benign prostatic hyperplasia (107,108).

The BPA developmental exposure (20 μg kg^{-1} d^{-1}, GD13–GD16) in mice induced 17 beta-oestradiol levels impairing the expression of *Cyp19a1* and *Cyp11a1* during gland development. The increase of P450 aromatase (Cyp19a1) has been also associated with obesity and diabetes (108). *In utero* exposure to BPA deregulates the expression of different genes involved in prostate hyperplasia such as *Nr5a1*, *androgen receptor* and *prostatic acid phosphatase* expression (109,110). The role of BPA in prostate hyperplasia has been evidenced also in the previously mentioned *in vitro* toxicogenomic study, in which the biofunction 'hyperplasia of prostate gland' (p-value = 0.00858) was evidenced at 3 d of exposure (90).

The reports on the effects of *in utero* BPA exposure mainly focus on the alteration of the prostatic epithelium (43) and are related to the susceptibility of the prostate gland to adult-onset carcinogenesis following hormonal exposures. Its molecular aspects have been analysed even at epigenetic levels but will not be discussed here as far beyond the scope of this review (45,111).

Conclusion and needs

This review represents the first attempt to parallel the phenotypic and molecular effects of BPA in organs having the same developmental origin (i.e. liver, pancreas and thyroid), whose activities are strictly interplayed in diabesity. Although the molecular data are fragmentary, the available ones illustrate an important role for transcriptional regulators and epigenetic changes induced by BPA in the development of diabesity (20). This suggests that epigenetic changes, inheritable through the germ line even in the absence of continued exposure, should be considered for a proper evaluation of BPA risks. Notably, a systematic analysis of the effects on the histones code is actually missing, and a better evaluation of transgenerational effects related to endoderm-derived organs should be conducted (112). In addition, more molecular data are needed to have an *in vivo* phenotype anchoring for the endoderm-derived organs.

We suggest the gene expression profiling analysis as a valid instrument to clarify the molecular pathways involved in BPA activity in endoderm-derived organs in a first-line approach. Although the use of toxicogenomics is strongly increased in the assessment of xenobiotic activity, we found few papers applying it in molecular dissection of BPA modes of action in the endoderm-derived organs. We strongly suggest this approach because the effects of the low-dose BPA exposure can hardly be detected at phenotypic level as depending on the experimental conditions (i.e. animal model, strains and administration route). This gives rise to the strong debate on the effects of BPA at its environmental doses. Furthermore, the paucity of the molecular data negatively influences the possibility to find molecular hallmarks

of exposure or effect (i.e. genes and pathways), needed to set up correct procedures in the risk evaluation.

In our opinion, *in vitro* toxicogenomic represents a starting point, a good compromise between the costs, higher for *in vivo* toxicogenomics, and the need for pathways discovery. Indeed, we have reported here how *in vitro* toxicogenomics data from a model of endoderm-derived cells (rat follicular cells) highlight genes (i.e. codifying for junction proteins) and pathways (Nrf2 pathway, 1D-myo-inositol hexakisphosphate biosynthesis II, etc.) that play a key role in damaging endoderm organs involved in diabesity (Fig. 1). This is the evidence that the analysis of molecular data sets in organs having the same developmental origin and known to be targets of developmental exposure to BPA could lead to the identification of common/specific deregulated pathways. These pathways are hopefully usable in the risk assessment procedures as well as in the establishment of alternative methods.

Acknowledgements

This work was supported by the Italian Workers' Compensation Authority (grant no. 12010), the Italian Ministry of Health (grant no. RF 2010-2309484) and Sensor Regione Campania (grant no. 23).

We wish to thank R. De Felice for linguistic editing of the manuscript and IRGS (Biogem) for support.

Conflict of interest statement

The authors declare that they have no competing financial interests.

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