Bisphenol A and Obesity

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Lines of evidence have shown the embryogenic and transgenerational impact of bisphenol A (BPA), an endocrine-disrupting chemical, on immune-metabolic alterations, inflammation, and oxidative stress, while BPA toxic effects in adult obese mice are still overlooked. Here, we evaluate BPA's worsening effect on several hepatic maladaptive processes associated to high-fat diet (HFD)-induced obesity in mice. After 12 weeks HFD feeding, C57Bl/6J male mice were exposed daily to BPA (50 µg/kg per os) along with HFD for 3 weeks. Glucose tolerance and lipid metabolism were examined in serum and/or liver. Hepatic oxidative damage (reactive oxygen species, malondialdehyde, antioxidant enzymes), and mitochondrial respiratory capacity were evaluated. Moreover, liver damage progression and inflammatory/immune response were determined by histological and molecular analysis. BPA amplified HFD-induced alteration of key factors involved in glucose and lipid metabolism, liver triglycerides accumulation, and worsened mitochondrial dysfunction by increasing oxidative stress and reducing antioxidant defense. The exacerbation by BPA of hepatic immune-metabolic dysfunction induced by HFD was shown by increased toll-like receptor-4 and its downstream pathways (i.e., NF-kB and NLRP3 inflammasome) amplifying inflammatory cytokine transcription and promoting fibrosis progression.

obesity | reactive oxygen species | inflammatory cytokines | toll-like receptor-4 | liver fibrosis | mitochondrial respiratory capacity | NLRP3 inflammasome signaling pathway

1. Introduction

Bisphenol A (BPA) is considered one of the most widespread endocrine-disrupting chemicals (EDCs), substances affecting human health and impacting not only the endocrine system but also immune and metabolic functions [1][2]. Many lines of evidence have highlighted the link between impaired immunity and obesity and their relationship with EDC exposure [3][4].

An increase in oxidative stress-associated inflammation has been hypothesized to be one of the major mechanisms in the pathogenesis of obesity-related diseases [5]. As known, oxidative stress and inflammation are mutually dependent and connected. In fact, a rise in inflammatory cytokine levels drives a further increase in oxidative stress, sustaining a vicious cycle. In this regard, many toxic xenobiotics affect mitochondrial function and cause pro-oxidative conditions [6].

BPA, like several EDCs, can affect mitochondrial function, targets hepatic mitochondria [7], and predisposes to liver mitochondrial oxidative damage, altering the complex I activity of the mitochondrial electron transport chain [8], and ATP synthesis [9]. However, the involvement of mitochondrial dysfunction associated with BPA hepatotoxicity is disregarded and, even if critical periods of development have been analyzed, few pieces of evidence have been collected on the hepatotoxic effects of BPA on adult obese animals. However, BPA causes liver damage by the production of reactive oxygen species (ROS) and endoplasmic reticulum stress, and decreases fatty acid b-oxidation as shown in both in vitro and in vivo studies [10][11][12].

Increasing data have reported the adverse effects of BPA exposure, particularly during gestation and development [13][14]. BPA induces transgenerational obesity in rats [15] and following embryogenic exposure generates metabolic disturbances later in life, including diabetes and obesity both in offspring and in mothers themselves [16][17].

Perinatal exposure to BPA worsens hepatic alterations caused by a high-fat diet (HFD) in rat offspring [18]. Moreover, long-term exposure to HFD, simultaneously to BPA, induces insulin resistance (IR) in growing mice without affecting obesity and body adiposity [19]. Conversely, other authors have reported that perinatal BPA administration combined with HFD exacerbates dyslipidemia and obesity, and significant changes were observed in the expression of main factors involved in fatty acid metabolism [20].

Similar evidence has emerged in humans, where higher urinary concentrations of BPA were associated with morbidity in an adult population characterized by IR or central obesity [21]. In an exploratory study performed in healthy subjects, Stahlhut and co-authors [22] reported that oral administration of BPA (50 µg/kg) may induce an alteration of glucose-stimulated insulin response in humans. Notably, the dose of 50 µg/kg bw/day was considered the first safety reference dose (tolerable daily intake, TDI) for BPA [23] from 1988 by the Environmental Protection Agency and then adopted by Food and Drug Administration until 2015. More recently, the European Food Safety Authority (EFSA) has suggested a re-evaluation of TDI, indicating 4 µg/kg body weight daily as temporary TDI (t-TDI) (EFSA, 2017).

2. Discussion

In this study, we demonstrate that a sub-chronic BPA exposure in HFD-induced obese mice worsens most of the features related to obesity in adulthood. We investigate the mechanisms underpinning immune-metabolic impairment focusing on oxidative stress and hepatic mitochondrial dysfunction related to inflammasome activation causing the progression of tissue damage.

Previous evidence describes BPA, similarly to other EDCs, as an inducer of epigenetic transgenerational inheritance of obesity [1]. Embryonic, perinatal, and early life exposure to BPA generates metabolic alterations later in life predisposing to obesity and diabetes [1][2], evidencing its role in the etiology of metabolic disorders. Here, we have determined the mechanisms underlined to liver toxic effect of BPA in adult obese mice, regardless of perinatal exposure, since obesity-related disorders are associated with BPA exposure in both children and adults [24][25]. Moreover, in healthy subjects, a single oral administration of BPA (50 µg/kg) causes an altered insulin response to

glucose stimulation [22]. Generally, the toxic effects of the xenobiotics depend on concentration/dose and time of exposure. In preclinical in vivo and in vitro studies planned to explore the molecular mechanisms of EDCs, high doses or concentrations are usually preferred [26][27]. In our experimental condition, we chose 50 µg/kg based on literature data considering the short time of mice exposure to BPA (3 weeks).

Here, BPA did not modify body weight and food intake but significantly increased fat mass, as well as serum hepatic enzymes and triglycerides. Moreover, BPA altered adipokine and hormone profile as evidenced by the increased leptin/adiponectin ratio and HOMA-IR. As known, leptin and adiponectin are inversely involved in glucose and lipid metabolism, through AMP-activated protein kinase (AMPK) activation [28]. Here, BPA exposure of obese mice markedly reduces oral glucose and pyruvate tolerance and increases key enzymes or mediators involved in gluconeogenesis (i.e., G6Pase and PCK1), de novo lipogenesis (i.e., fatty acid synthase or FAS, and sterol regulatory element-binding protein-1c or SREBP-1c), and triglycerides accumulation in the liver. Previous data have demonstrated that BPA exposure in offspring fed with HFD reduced peroxisome proliferator-activated receptor (PPAR)-α and CPT1 expression, leading to hepatic lipid accumulation through the inhibition of fatty acid oxidation [18]. Consistently, we observe a reduction of mitochondrial CPT activity in the liver from BPA-treated adult obese mice, which causes the accumulation of toxic lipid-derived metabolites in hepatocytes. Excessive free fatty acid amounts in the liver can activate the signaling pathways that promote oxidation, inflammation, and fibrosis [29].

To date, no evidence regarding BPA toxicity on hepatic mitochondria in adult obese animals is available. Our previous studies showed that HFD causes the alteration of liver mitochondrial function and dynamics in mice, also reducing the activity of antioxidant scavengers [30][31]. Here, we prove the worsening effect of BPA on HFD-induced hepatic mitochondrial damage. Indeed, BPA further reduced HFD-impaired respiratory capacity, enhancing ROS and MDA levels, and inhibiting the activity of detoxifying enzymes (i.e., SOD and aconitase); more interestingly, BPA also dampened CPT activity, leading to an increase of hepatic ectopic lipid storage, as demonstrated by triglycerides' accumulation. Consistent with the BPA-induced alteration of redox balance, Khan et al. [7] observed a decrease in glutathione (GSH) levels and an increase of superoxides in BPA-treated rats.

Our data agree with previous studies reporting that BPA interferes with mitochondrial functions in the liver and other tissues [7][32] compromising the respiratory chain, reducing OXPHOS capacity, and increasing oxidative stress [26][32]. Moreover, the alteration of mitochondrial bioenergetics, dynamics, and apoptosis by BPA has been recently demonstrated [7][33]. In three-week-old offspring, perinatal exposure to BPA decreases mitochondrial respiratory complex activity and modifies the expression of genes involved in fatty acid metabolism, without alteration of liver morphology and function [34].

In our experimental condition, BPA increases systemic inflammation induced by fat overnutrition. Indeed, higher levels of LPS and MCP1, and the reduction of anti-inflammatory IL-10 were observed in serum. LPS is one of the most crucial factors contributing to low-grade inflammation, also called "metainflammation", associated with HFD feeding, responsible for the induction of inflammatory cytokines by immune cells and adipocytes [31].

Several lines of evidence have previously demonstrated BPA's capability of inducing the dysregulation of cytokines, hepatocyte apoptosis, and oxidative stress in the liver [11][35]. Moreover, Moon et al. [19] showed that long-term simultaneous exposure to HFD and BPA induced the alteration of glucose homeostasis and insulin sensitivity in growing mice without modifying adiponectin and inflammatory cytokines. On the other hand, adiponectin level was suppressed in prenatal BPA-treated animals even if they did not show steatosis features [17]. Recently, clinical evidence on the adult male population revealed the association among BPA plasma levels and inflammatory markers, visceral obesity, and IR [36].

During lipid overnutrition, circulating free fatty acids, whose levels are commonly increased in obesity, accumulate in the liver as fat storage, and at the same time, in concert with LPS, trigger TLR4/NF-κB pathway [37]. Therefore, as a consequence, the massive production of pro-inflammatory cytokines in hepatic cells (i.e., hepatocytes and Kupffer cells) leads to the alteration of the leptin/adiponectin ratio and promotes the conversion of steatosis into steatohepatitis causing liver damage progression [45]. Consistently, the deleterious effect of BPA results in the exacerbated activation of innate immune response as evidenced by the further increase of hepatic TLR4, its adaptor protein MyD88, as well as the downstream target NF-κB and excessive cytokine production. It is known that in nonalcoholic fatty liver disease (NAFLD) patients as well as animal models of NAFLD, NF-kB activation is observed in liver cells, including hepatocytes, hepatic stellate cells, and Kupffer cells [38][39]. Among these, hepatocytes respond minimally to TLR ligands suggesting that also other mediators can activate NF-kB in hepatocytes. On the other hand, these TLR ligands directly activate NF-kB in Kupffer cells, sustaining the vicious cycle of the inflammatory response.

3. Conclusions

In summary, BPA aggravates liver immune-metabolic and mitochondrial dysfunction in adult obese mice magnifying the cross-talk among hepatic oxidative stress, cytokine network, and fibrosis progression, and highlighting the critical role of inflammasome activation. Therefore, our data point out that BPA exposure represents an additional risk factor for the progression of fatty liver diseases and the other pathological features strictly related to obesity in adulthood.

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