Bisphenol A promotes hepatic lipid deposition involving Kupffer cells M1 polarization in male mice

Qiong Lv1,*, Rufei Gao1,2,*, Chuan Peng1, Juan Yi1, Lulu Liu1, Shumin Yang1, Danting Li1, Jinbo Hu1, Ting Luo1, Mei Mei1, Ying Song1, Chaodong Wu4, Xiaoqiu Xiao3 and Qifu Li1

1Department of Endocrinology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China
2School of Public Health and Management, Chongqing Medical University, Chongqing, China
3Laboratory of Lipids and Glucose Metabolism, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China
4Department of Nutrition and Food Science, Texas A&M University, College Station, Texas, USA
*(Q Lv and R Gao contributed equally to this work)

Abstract

Bisphenol A (BPA), one of the most common environmental endocrine disruptors, is considered to promote hepatic lipid deposition. However, the mechanism has not been fully elucidated. The polarization of Kupffer cells (KCs) plays an important role in hepatic inflammation by promoting pro-inflammatory M1 phenotype (M1KCs), which contributes to dysregulated lipid metabolism. The purpose of this study is to investigate the role of KC polarization in BPA-induced hepatosteatosis in male mice. In this study, we examined hepatic lipid contents and quantified M1KC in BPA-treated CD1 mice, and further explored the interaction between KCs and hepatocytes using conditional HepG2 cell culture. BPA treatment significantly increased hepatic fat contents in CD1 mice, accompanied by increased number of pro-inflammatory M1KCs and enhanced secretion of inflammatory cytokines. Increased lipid contents were also observed in HepG2 cells treated with BPA. Interestingly, higher TG contents were observed in HepG2 cells treated with conditional media from BPA-treated KCs, compared with those treated with BPA directly. Incubation of KCs with BPA promoted the polarization of KCs to pro-inflammatory M1 dominant subtypes, which was blocked by estrogen antagonist ICI182780. Taken together, our results revealed that M1KCs polarization is involved in BPA-induced hepatic fat deposition, which is possibly associated with the estrogen receptor signaling pathway.

Key Words
- Kupffer cell
- bisphenol A
- polarization
- NAFLD
- inflammation

Introduction

Excess hepatic deposition of lipids, especially triglyceride (TG), is one of the important features of nonalcoholic fatty liver disease (NAFLD). Over-nutrition, metabolic diseases and genetic factors were widely considered to contribute to the development of NAFLD (Cohen et al. 2011). Recently, environmental pollutants have become a new focus of NAFLD etiology (Rochester 2013). Some environmental chemicals, known as environmental endocrine disruptors (EEDs), could interfere with the process of hormones metabolism, which induces a wide...
range of adverse effects (Gorelick et al. 2014). EEDs include a variety of chemicals, such as bisphenol compounds, phthalate esters and polychlorinated biphenyls, among which bisphenol compounds have been proved to be closely related to the morbidity of NAFLD (Cave et al. 2010, Vandenberg et al. 2012).

Bisphenol A (BPA), one of the most studied EEDs, belongs to bisphenol compounds. BPA is widespread in human’s life, such as in bottles, food packaging, insurance box, medical devices, etc. Under the condition of high temperature or high acid, BPA could easily seep into human body along with food or drink or even be absorbed through the skin (Rochester 2013, Hormann et al. 2014, Lin et al. 2015). Numerous studies have shown that, BPA can be detected in urine from 93% to 95% of the natural population (Yang et al. 2009, Carwile & Michels 2011, Hormann et al. 2014). The chemical structure of BPA is similar to estrogen, so BPA binds to estrogen receptor and competes with estrogen, and further affects the reproductive system and development (Vandenberg et al. 2009). Moreover, increasing evidence from clinical observation and laboratory studies suggests that BPA is involved in the pathogenesis of NAFLD (Lang et al. 2008, Melzer et al. 2010), with mechanisms that have not been fully elucidated.

Macrophages consist of a heterogeneous population differentiated from myeloid-derived mononuclear cells, which are the critical components of the innate immune system. Resident macrophages enriched in livers are named as Kupffer cells (KCs) (Bouwens et al. 1986). They act as the first responder to pathogens, toxins, and tissue damage by producing a panel of M1 pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFN-γ) prototypically (Sica et al. 2014). It was reported in rodent models of diet-induced hepatosteatosis that the activities of KCs were increased and KCs were polarized to pro-inflammatory M1 dominant subtypes, while depletion of KCs polarization prevented the development of hepatic steatosis (Huang et al. 2010, Chen et al. 2012). Furthermore, blocking the anti-inflammatory or alternatively activating M2 program of KCs decreases hepatocyte fatty acid oxidation (Odegaard et al. 2008). These studies suggested that M1 KCs polarization play an important role in hepatic lipid metabolism. However, it is enigmatic whether M1 KCs polarization is involved in BPA-induced lipid accumulation in liver, which is investigated in this study.

Materials and methods

Animal care and maintenance

All procedures were approved by the Animal Care Committee of Chongqing Medical University. All experiments were performed in accordance with the relevant guidelines and regulations, including any relevant details. Four-week-old male CD1 mice were purchased from Vital River (Beijing, China). After arrival, mice were maintained on a constant 12-h light/12-h darkness cycle with free access to water and ad libitum fed with a standard chow diet and allowed to acclimate for two weeks. BPA (Sigma-Aldrich) was incorporated with standard diets at doses of 0, 0.05, 0.5 and 5μg/g diet. All mice were randomly divided into four groups (n=12/group), and fed with different BPA containing diets, which corresponds to an oral exposure of BPA at 0(control), 5, 50 and 500μg/kg BW/day respectively, in considering a diet consumption of 10% of the body weight (BW) per day (Marmugi et al. 2012, Weinhouse et al. 2014). BW and food intake were measured daily during the whole periods of experiments. At the end of the experiment, mice was killed by CO₂ inhalation and the livers (n=7/group) were removed, weighed and either fixed in 4% paraformaldehyde or snap-frozen in liquid nitrogen. Formalin-fixed samples were stored at 4°C and the rest of the samples were stored at −80°C until use. Another five livers of mice in each group were used for flow cytometry analysis.

Isolation of primary KCs

Primary KCs culture was prepared as described by Kitani and coworkers (Kitani et al. 2011). Firstly, the liver of male mice was perfused in situ through the portal vein with Ca²⁺–Mg²⁺-free Hank's balanced salt solution (HBSS), containing 0.5 mM EGTA, 10mM HEPES and 4.2 mM NaHCO₃ at a rate of 10mL/min. When the liver turned pale, the perfusion was continued with another HBSS solution, containing 10mM HEPES, 4.2mM NaHCO₃, 0.05% type IV collagenase (Sigma-Aldrich) and 50μg/mL trypsin inhibitor (Sigma-Aldrich) for 10–20min at a rate of 10mL/min until the liver slightly swelled. Secondly, the liver was transferred into a culture dish and minced into small pieces by scissors, and then filtrated through a cell strainer (100μm) to remove connective tissues and undigested tissue fragments, centrifuged twice at 50g for 5 min at 4°C.
The suspension of six-week-old C57BL/6J mice, purchased from the Animal Center of Chongqing medical university, was seeded to three tissue culture flasks in Dulbecco’s modified eagle medium (DMEM/F12) (Gibco, BRL) with 10% FBS, 1 μg/mL insulin and 100 μM β-mercaptoethanol (Sigma-Aldrich). Then, the cells were incubated at 37°C in an atmosphere of 5% CO₂, 95% air and washed with fresh growth medium every 2–3 days intervals gently. About 12 days later, cells proliferated fully and were incubated with TrypLE Express solution (Invitrogen), collected and centrifuged at 180 g for 5 min, and then dissociated into single cells with growth medium and cultured in 6-well plates. After 30 min, the adherent macrophages were ready for further study.

The suspension from BPA-treated CD1 mice was added with Percoll (Sigma-Aldrich) for differential centrifugation (Louvet et al. 2011, Ge et al. 2014). Briefly, after cells were centrifuged twice at 50 g for 5 min at 4°C, suspended and centrifuged again at 800 g for 10 min at 4°C. The pellet obtained was re-suspended in PBS and centrifuged at 800 g for 15 min through a Percoll gradient at 4°C. The KCs-enriched fraction (the 25–50% Percoll layer) was transferred and centrifuged at 800 g for 10 min to obtain the KCs, which were used for flow cytometry detection.

**Treatment of cultured KCs in vitro**

The cells were treated with BPA (10 μM, Sigma-Aldrich), lipopolysaccharide (LPS) (100 ng/mL, Sigma-Aldrich) and estrogen receptor (ER) antagonist ICI 182780 (ICI, 10 μM, Tocris, Cookson, Ellisville, MO, USA) respectively or treated with BPA combined with ICI for 24 h. ICI was added to the cell culture media 1 h before BPA treatment.

**Culture and treatment of HepG2 cells**

The human hepatocellular carcinoma cell line HepG2 cells were obtained from the American Type Culture Collection and maintained in a RPMI-1640 medium (Gibco) with 10% FBS (Gibco) at 37°C in an atmosphere of 5% CO₂–95% air. HepG2 cells at 70% confluence in 6-well plates were switched to another medium containing 0.2% β-mercaptoethanol (Sigma-Aldrich), collected and centrifuged at 180 g for 5 min, and then dissociated into single cells with growth medium and cultured in 6-well plates. After 30 min, the adherent macrophages were ready for further study.

Briefly, liver sections and HepG2 cells were evaluated by Oil Red O staining. Briefly, liver sections and HepG2 cells were treated with the supernatant from 0, 10 μM BPA or 100 ng/mL lipopolysaccharide (LPS) (100 ng/mL, Sigma-Aldrich) for 12 h. The cells were incubated with TrypLE Express solution (Invitrogen), collected and centrifuged at 180 g for 5 min, and then dissociated into single cells with growth medium and cultured in 6-well plates. After 30 min, the adherent macrophages were ready for further study.

**Oil Red O staining**

The lipid accumulation in liver of CD1 mice and HepG2 cells were evaluated by Oil Red O staining.

**Quantitative measurement of triglycerides (TG) contents**

Quantitative measurement of TG contents was performed using commercial kits (jiancheng, Nanjing, China). Briefly, liver tissue of BPA-treated CD1 mice and HepG2 cells from the 6-well plates were collected and lipids were extracted by adding 1 mL solvents (n-hexane/isopropanol=2/3.5). The lipid phase was collected and dried in vacuum. The concentration of TG contents were analyzed with the kits (mg/mL) and normalized by total protein from tissues (mg/mL).

**Immunohistochemistry of liver tissues**

The sections of livers were subjected to immune histochemical staining using a monoclonal antibody against mouse anti-mouse F4/80 (Abcam). Briefly, 5 μm paraffin sections were deparaffinized with xylene (100%) for 1 h, rehydrated in a graded ethanol series, permeated by Triton (3%) for at most 30 min, followed by microwave heating in 0.01 M citrate sodium buffer (pH 6.0) at 92–98°C for 10 min in order to retrieve the antigens and incubated in H₂O₂ (3%) for 30 min to quench endogenous peroxidase activity. After washing with PBS thrice, the sections were then incubated in the anti-mouse F4/80 (1:1000, Abcam) overnight at 4°C (Lopez et al. 2011). The next day, after washed with PBST thrice, the sections were incubated in biotinylated secondary mouse anti-goat IgG (Boster Biological Technology, Wuhan, China) for 1 h at
Table 1 Primers of quantitative real-time PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward and reverse primers</th>
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<tbody>
<tr>
<td>GAPDH (mouse)</td>
<td>5’GCTTCCGCTTGTGTCTACC3′</td>
</tr>
<tr>
<td>IL-1β (mouse)</td>
<td>5’GCAAGAATGCTGGGACCCCTT3′</td>
</tr>
<tr>
<td>IL-6 (mouse)</td>
<td>5’TCTCCTCTCTGTGTCTG3′</td>
</tr>
<tr>
<td>TNF-α (mouse)</td>
<td>5’GAGTAACTCGGAGCTGGG3′</td>
</tr>
<tr>
<td>MCP-1 (mouse)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>IL-1α (mouse)</td>
<td>5’CTCGTCAGGGAGAACTGTG3′</td>
</tr>
<tr>
<td>IL-10 (mouse)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>SREBP1 (mouse)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>FAS (mouse)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>CD36 (mouse)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>PPARα (mouse)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>ATGL (mouse)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>MTP (mouse)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>FATP1 (human)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>GAPDH (human)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
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<td>SREBP1 (human)</td>
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<td>FAS (human)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>CD36 (human)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
<tr>
<td>PPARα (human)</td>
<td>5’GATCTTGCTGTGTACTGTTG3′</td>
</tr>
</tbody>
</table>

37°C. The sections were washed in PBS and incubated with 3,3′-diaminobenzidine tetrahydrochloride (DAB, Zhongshan Golden Bridge, Beijing, China) for 5 min. Counter-staining was conducted by Harris’s hematoxylin for 30 s. Dehydrated by another graded ethanol series, and finally mounted by neutral resins. The numbers of F4/80-positive cells were counted by light microscopy in three randomly selected fields of liver sections (n=5/group).

Flow cytometry detection

The identification of KCs and quantification of M1KCs were performed by flow cytometer analysis. Briefly, cells were harvested and blocked by 5% rat serum for 1 h at 4°C, washed twice by PBS. For validation of the purity of KCs, cells were labeled for 1 h at 4°C with F4/80/PE (≤1.0μg/10⁶ cells in 100μL). For analysis of the percentage of M1KCs subsets, the cells were labeled with PE Anti-Mouse F4/80 Monoclonal Antibody (F4/80-PE) (≤1.0μg/10⁶ cells in 100μL) and APC Anti-Mouse CD11c Monoclonal Antibody (CD11c-APC) (≤0.25μg/10⁶ cells in 100μL) for 1 h at 4°C (Lumeng et al. 2007). All the antibodies were purchased from Sungene Biotech, Tianjin, China. Isotype-matched IgG (Rat IgG2a-PE and Hamster IgG-APC) were used as a negative control (Southern Biotech).

ELISA analysis for cytokines

ELISA for tumor necrosis factor-α (TNF-α), IL-1β, IL-6, monocyte chemotactic protein-1 (MCP-1), IL-10 and IL-1α in the supernatants of BPA- and LPS-treated KCs and the BPA-induced CD1 mice liver tissue were performed with the protein chip of Direct Detect (Merck) respectively. Each sample was assayed on triplicate wells according to the manufacturer’s instructions.

Statistical analysis

Statistical significance was determined by performing a two-tail Student’s test or one-way ANOVA using SPSS13.0. P values <0.05 were considered statistically significant.

Results

BPA promoted hepatic lipid deposition in CD1 mice

There were no obvious changes in BW, liver weight or food intake after BPA treatment at 3 different doses for 8 weeks (Table 2). However, quantitative TG test showed that hepatic TG content was significantly increased after BPA exposure in CD1 mice (Fig. 1A). Meanwhile, Oil Red O staining showed BPA-induced hepatic steatosis significantly (Fig. 1B). Figure 1C illustrated the effects of BPA on mRNA levels of key components involved in hepatic lipid metabolism and transport, including sterol regulatory element-binding transcription factor 1 (SREBP1), fatty acid synthase (FAS), peroxisome proliferator-activated receptor alpha (PPARα), microsomal triglyceride transfer protein (MTP), adipose triglyceride lipase (ATGL), cluster of differentiation 36 (CD36) and fatty acid transport protein 1 (FATP1). Overall, BPA promoted hepatic lipid synthesis without changing lipid transport in transcription level.
BPA-induced hepatic inflammation in CD1 mice

To investigate whether BPA exposure affects the expression of inflammatory mediators in CD1 mice, we examined the mRNA and protein levels of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6 and MCP-1 and anti-inflammatory cytokines such as IL-10 and IL-1α by qRT-PCR and ELISA. The mRNA and protein levels of TNF-α, IL-1β, IL-6 and MCP-1 were significantly increased in the mice exposed to BPA 5 or 50 μg/kg/day compared with the control mice, and mRNA levels of these genes were also enhanced at the dose of 500 μg/kg/day (Fig. 2). Both IL-10 and IL-1α protein levels were significantly increased in BPA 5 μg/kg/day group. IL-1α was also increased in BPA 50 μg/kg/day group in transcription level. Altogether, these data demonstrated that BPA induced the inflammatory response in liver.

BPA promoted M1KCs polarization in vivo

KCs are the major source of liver inflammation, and macrophages polarized to M1 synthesis and secrete more inflammatory factors, so the number of M1 KCs in mouse liver was quantified. The number of KCs stained by F4/80 was significantly increased in the mice exposed to 50 or 500 μg/kg/day of BPA (Fig. 3A and B) and the amount of M1 KCs were further characterized by positive staining for both F4/80 and CD11c, which was determined by flow cytometry. It was found that, compared with the control group, the number of M1 KCs were significantly increased after the exposure of BPA at the concentration of 5 or 50 μg/kg/day (Fig. 3C and D), which suggested that BPA promoted the transformation of KCs toward a pro-inflammatory M1 phenotype in vivo.

BPA-regulated KCs polarization in vitro

Moreover, we investigated whether BPA altered M1 polarization in primary KCs in vitro, and LPS was used to treat primary KCs as a positive control, since it was widely considered to promote M1 polarization of KCs. Primary KCs were successfully isolated, cultured and identified from C57BL/6J mice (Supplementary Fig. 1, see section on supplementary data given at the end of this article). Flow cytometry assay showed that M1 KCs was accounted for about 20% at the basal condition. However, after the

Table 2  Body weight and liver weight in BPA-exposed mice for 8 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>LW/BW (%)</th>
<th>Food intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA0</td>
<td>43.05 ± 3.82</td>
<td>2.13 ± 0.21</td>
<td>5.13 ± 0.36</td>
<td>6.1 ± 0.33</td>
</tr>
<tr>
<td>BPA1</td>
<td>42.61 ± 2.60</td>
<td>2.14 ± 0.12</td>
<td>4.97 ± 0.44</td>
<td>6.55 ± 0.25</td>
</tr>
<tr>
<td>BPA2</td>
<td>43.00 ± 4.67</td>
<td>2.24 ± 0.31</td>
<td>5.06 ± 0.12</td>
<td>6.52 ± 0.30</td>
</tr>
<tr>
<td>BPA3</td>
<td>42.86 ± 3.82</td>
<td>2.11 ± 0.16</td>
<td>4.87 ± 0.20</td>
<td>6.67 ± 0.20</td>
</tr>
</tbody>
</table>

Male CD1 mice were exposed to different doses of BPA (0, 5, 50 and 500μg/kg/day) for 8 weeks, which are named BPA0, BPA1, BPA2 and BPA3 groups respectively. The body weight (BW), liver weight (LW) and LW/BW were recorded and data are expressed as mean ± s.e.m. from 7 animals by each group.
treatment with BPA (10μM) or LPS (100 ng/mL) for 24 h, M1 KCs increased profoundly and reached 50.7% and 65.6% respectively (Fig. 4A and B).

We further examined the protein concentration of inflammatory mediators in the supernatant and the mRNA expression of inflammatory factors in KCs. Compared with the control group, the protein levels of TNF-α, IL-1β, IL-6 and MCP-1 were significantly increased in BPA or LPS groups (Fig. 4C). The mRNA levels of TNF-α, IL-1β, IL-6 and MCP-1 were obviously enhanced after BPA or LPS treatment (Fig. 4D). Overall, these data revealed that the percentage of M1 KCs was significantly increased in BPA-induced KCs along with the enhanced expression of inflammatory cytokines.

**Conditioned medium from BPA-treated KCs promoted lipid accumulation in hepatocytes**

To evaluate the impact of BPA-induced M1 KCs on fat accumulation in hepatocytes, conditioned medium
was collected from control KCs, BPA-exposed and LPS-exposed KCs. Then, HepG2 cells were incubated with vehicle, BPA or the condition medium from vehicle or BPA or LPS induced KCs for 24 h, which was named as Ctrl, BPA, Ctrl-KCs, BPA-KCs and LPS-KCs group respectively. TG content quantification test showed that, compared with Ctrl group, intracellular TG content was increased in BPA, BPA-KCs and LPS-KCs groups, but not in Ctrl-KCs group. Interestingly, TG content was increased more in BPA-KCs group compared with BPA group (Fig. 5B). In accordance with that, Oil Red O staining displayed more obvious intracellular fat in BPA-KCs and LPS-KCs groups compared to that in BPA or Ctrl-KCs group (Fig. 5A). Moreover, the mRNA expression of the key genes involved in lipid metabolism, such as SREBP1, FAS, ACC and CD36 were upregulated, but PPARα was decreased in HepG2 cells cultured with the conditioned medium obtained from BPA-exposed or LPS-exposed KCs (Fig. 5C). Altogether, these data demonstrated that the conditioned medium containing inflammatory mediators secreted by BPA-treated KCs could induce fat accumulation in hepatocytes.
Estrogen receptor signaling was associated with BPA-induced KCs polarization

Previous studies demonstrated that BPA might interfere with the normal estrogen signaling by interacting with ERs (Ge et al. 2014). In our study, ER antagonist ICI was used in BPA-treated KCs to address whether ER is involved in the process of BPA-induced KCs polarization. Compared with BPA-treated cells, the ratio of M1 KCs was significantly decreased after the treatment with ICI as assayed by flow cytometry analysis (Fig. 6A and B). The mRNA levels of TNF-α, IL-1β and IL-6 were significantly inhibited in ICI-treated groups in the same time (Fig. 6C). These results suggested that ER signaling pathway might be involved in BPA-induced KCs polarization.

Discussion

The aim of our study was to address the potential role of KCs polarization in BPA-induced hepatic lipid accumulation and its underlying mechanism. Our data demonstrated that in addition to direct induction of de novo lipogenesis, BPA promoted KCs polarization to pro-inflammatory M1 type with increased inflammatory factors, which may also participate in hepatic lipid deposition both in vivo and in vitro.

In our study, 5, 50 and 500 µg/kg/day of BPA treatment for 8 weeks induced hepatosteatosis in male CD1 mice, which was consistent with Marmugi’s report (Marmugi et al. 2012). Meanwhile, we observed that 10 µM BPA increased intracellular lipid content in HepG2 cells,
confirming the effect of BPA on hepatic lipid accumulation. It was known that abnormal fatty acid uptake, synthesis, oxidation, secretion and decomposition contributed to increased hepatic TG contents. Our study clearly demonstrated that BPA enhanced the expression of SREBP1 and FAS and reduced the expression of PPARα, MTP and ATGL, suggesting that increased de novo lipogenesis and inhibited fatty acid oxidation and export may contribute to BPA-induced hepatic steatosis collectively, which was in accordance with previous literatures (Somm et al. 2009, Marmugi et al. 2012).

One key finding of our study is that KCs polarization to M1KC and subsequent release of inflammatory mediators may participate in BPA-induced hepatic steatosis. Previous studies with isolated macrophages from Zebra fish demonstrated that low doses of BPA exposure induced a pro-inflammatory effect possibly through ERα and NF-κB pathways (Yang et al. 2015). Our results confirmed and extended these studies using primary KCs, conditional culture and mammalian models. It was shown that BPA-treated CD1 mice displayed obvious hepatosteatosis and M1KCs infiltration in the liver, which was responsible for hepatic inflammatory status. Hepatic KCs were isolated from C57BL/6J mice and challenged with BPA to demonstrate that BPA significantly enhanced M1 KCs polarization and promoted the production of inflammatory factors including TNF-α, IL-1β and IL-6.

BPA exposure may trigger multiple organ alteration and complicated pathophysiological processes. For instance, BPA enhanced hepatic and myocardial lipid accumulation and subsequently led to organ injuries accompanied by endoplasmic reticulum stress and inflammatory responses and dysregulated lipid metabolism in WHHL rabbits livers and cultured HUVECs (Fang et al. 2014). Recent animal studies have shown that M1 KCs polarization increased hepatic lipid deposition (Borgeson et al. 2015, Liu et al. 2015), while inhibition of M1 KCs polarization ameliorated lipid accumulation in the liver (Huang et al. 2010, Wan et al. 2014). To investigate whether BPA-induced M1 KCs polarization contributed to hepatic TG accumulation, we treated HepG2 cells with 10μM BPA or with different conditioned medium collected from KCs exposed to 10μM BPA or vehicle. It turned out that there was more lipid accumulation in hepatocytes in the group treated by BPA-exposed KCs supernatant compared with that in the group treated by BPA directly, while no obvious lipid accumulation was detected in hepatocytes exposed to vehicle-exposed KCs supernatant. These results implied...
that in addition to modulating hepatic lipid metabolism directly, BPA could possibly trigger KCs polarization to pro-inflammatory M1 subtype, which also contributed to the abnormal lipid accumulation in the liver. Some studies believed that pro-inflammatory cytokines inhibit triglyceride synthesis and storage (Carbo et al. 2001, Ye 2015). However, some studies showed that lipid accumulation in liver was increased in the pro-inflammatory state. Zhang X’s group found that Reactive oxygen species-induced TXNIP drives fructose-mediated hepatic inflammation and lipid accumulation through NLRP3 inflammasome activation (Zhang et al. 2015).

BPA is considered as a weak environmental estrogen analog because of its relatively low affinity for the nuclear ERs compared with estradiol (10,000–100,000-fold lower affinity) (Delfosse et al. 2012, 2014). Several studies indicated that BPA is a partial agonist (Routledge et al. 2000, Ge et al. 2014). The estrogen antagonist ICI was reported to significantly decreased the levels of TNF-α and IL-6 and increased the TGF-β and IL-10 production in THP1 macrophages (Liu et al. 2014). By using ICI in BPA-induced KCs, we detected that M1 KCs proportion were reduced, leading to reduced pro-inflammatory cytokines including TNF-α, IL-1β and IL-6 compared to BPA-treated group. Our results are agreeable to Kou’s data, which showed that estrogen promoted M1-like macrophage activation to aggravate joint inflammation in female rats, and this effect is related to the increased expression of cadherin-11, a well-known cell adhesion molecule that is responsible for tissue morphogenesis and architecture (Kou et al. 2015). However, further studies are needed to clarify the molecular events involved in BPA-induced M1 KCs polarization.

In this study, we observed that chronic exposure to BPA aggravated the development of NAFLD, and M1 KCs polarization to M1 KCs was involved in BPA-induced hepatic lipid accumulation in addition to the direct effect of BPA on de novo lipogenesis. Activation of ER and its downstream signaling pathway in the KCs was possibly associated with the hepatic impact of BPA.

Figure 6
Effects of the estrogen receptor antagonist on BPA-induced KCs polarization. KCs were incubated in medium containing BPA (0 µM), BPA (10 µM), BPA (10 µM) plus ICI (10 µM) (BPA + ICI) respectively, for 24 h. (A and B) The contents of M1KCs were stained by F4/80 and CD11c, and analyzed by flow cytometry as described in ‘Materials and methods’ section. (C) The mRNA levels of the pro-inflammatory factors TNF-α, IL-1β, IL-6 were assayed by qRT-PCR. All data were normalized to GAPDH mRNA expression levels. Values shown are the mean ± S.E.M. from at least six samples in each group. * means P<0.05 vs BPA group.
These results may be beneficial for our understanding of the development of NAFLD and other related metabolic disorders caused by EEDs.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-17-0028.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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