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Original Article

Exacerbating lupus nephritis following BPA exposure is associated with abnormal autophagy in MRL/lpr mice

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Abstract: For the development of Lupus nephritis, environmental factors are reasoned to be one of the risk factors. In recent years, the role of bisphenol A (BPA) in kidney injury has attracted wide attention. In this study, we explored the nephrotoxicity and its possible mechanism of BPA exposure to lupus-prone MRL/lpr mice. Orally exposure of BPA increased serum anti-dsDNA level and urinary protein, and aggravated renal pathological injury in MRL/lpr mice. BPA increased the expression of NF-κB protein and activated the inflammatory response in both MRL/lpr and C57 mice. Unlike C57 mice, BPA exposure partially activated autophagy associated proteins, but the autophagy signaling pathway lacked the regulation of Becline1 and LC3-associated phagocytosis deficiency, and decreased Nrf2 protein expression in renal tissue of MRL/lpr mice. Therefore, exacerbating lupus nephritis induced by BPA exposure was associated with the activation of inflammation, abnormal autophagy and decreased antioxidant ability.

Keywords: Bisphenol A, lupus nephritis, autophagy, inflammation, antioxidant ability

Introduction

Systemic lupus erythematosus (SLE) is a chronic and heterogeneous autoimmune disease, which is characterized by progressive involvement of multiple organs and systems. The pathogenesis of lupus is not clear yet. SLE is the tenth leading cause of death among women aged 15-24 years in USA, according to data from the Centers for Disease Control and Prevention (CDC) [1]. Lupus nephritis (LN) is one of the most common and serious complications of SLE, which contributes to the progression and prognosis of SLE. Renal involvement occurs in 50-70% of lupus patients within 5 years after diagnosis, and 5-15% of them progress to end-stage renal disease within 10 years [2, 3]. A recent study in Hong Kong showed that the life expectancy of LN patients was reduced by 15.1-23.7 years [4]. Moreover, 27-66% of LN patients were at least one relapse during their follow-up treatment [5]. Therefore, it is urgent to explore the cause and mechanism of LN development, in order to find effective prevention and treatment methods.

The etiology of lupus is complicated, which is related to genetic, environmental and estrogen levels. Increasing evidences have indicated that exposure to environmental pollutants can lead to structural and pathophysiological changes of human and animal kidneys [6, 7]. Bisphenol A (BPA) is one of the most common environmental endocrine disruptors, as the main raw material for the production of baby bottles, food and beverage packaging containers, canned food lining and other consumer products, which is widely exposed to human body in daily life [8, 9]. The reproductive toxicity, metabolic disorders, neurodevelopmental abnormalities and immune abnormalities of BPA have attracted wide attention in recent years [10-12]. An epidemiological study including 3055 adults showed an association of BPA exposure with low-grade albuminuria in Shanghai [13], which is consistent with another study of American children [14]. A recent 6-year prospective study demonstrated a potential relation between high serum BPA levels and a five-fold increased risk of CKD3 stage in patients with primary hypertension [15]. A few

animal studies have also shown that BPA exposure can cause kidney damage [16, 17]. These results have aroused widespread concern that BPA exposure in daily life may have adverse effects on the kidneys. Especially for patients with immune deficiency, whether BPA can aggravate the degree of renal injury has not been reported at present, and the mechanism of toxicity of BPA on the kidneys is still unclear, which needs to be studied further.

It is now well established that inflammation is the basis of kidney diseases [18]. Nuclear factor κB (NF-κB) plays an important role in the inflammatory pathway, which is activated in glomerular epithelial and mesangial cells of LN patients accompanied by an increase of inflammatory cytokines [19, 20]. The inhibition of NF-κB pathway may prevent further development of LN [21, 22]. In addition, there is increasing evidence that oxidative stress is involved in the pathogenesis of LN [23]. Studies have shown that nuclear factor erythroid 2-related factor 2 (Nrf2) improves lupus nephritis by inhibiting oxidative damage and the NF-κB mediated inflammatory response [24]. Nrf2-deficient mice spontaneously develop lupus-like autoimmune nephritis in old age [25]. On the other hand, the accumulation of dead cells and autoantibodies cannot be effectively cleared in SLE patients [26], because the effective phagocytosis and removal of dead cells can prevent the occurrence of autoimmune diseases [27]. Mice lacking autophagy molecules associated with clearance of dead cells developed lupus-like manifestations [28]. Studies have found that LC3-associated phagocytosis (LAP) can eliminate apoptotic or necrotic cells and immune complexes, inhibit auto-inflammation, and play an important role in controlling the occurrence of lupus like symptom [29]. In the mice with LAP defect, there were increased lymphocytes, monocytes and neutrophils, and the levels of anti-dsDNA antibody and anti-nuclear antibody significantly increased, and large amounts of IgG and complement C1q deposition were found in the glomerulus [30].

Therefore, this was the first report on the effect of BPA on nephrotoxicity in mice with immunodeficiency (lupus prone mice). We explored the possible mechanisms of BPA from the aspects of inflammation, antioxidation and autophagy. Meanwhile, different from previous studies on the renal toxicity caused by high-dose BPA exposure, the lower dose of BPA (less than 50 μg/kg/d BPA) was used to investigate the renal toxicity in mice with lupus prone or C57BL/6 control mice in this study.

Materials & methods

Animal treatment

Six-week-old female MRL/lpr mice were purchased from SLRC Laboratory (Shanghai, China) and six-week-old female C57/BL6 mice were obtained from the animal center of China Medical University. Animal care was in accordance of the National Institutes of Health Guide for Care and Use of Laboratory Animals and local Laboratory Animal Care standards. After a 1-week adaptation period in a room with standard temperature (22±2°C) and illumination on a 12-h light-dark cycle, eighteen MRL/lpr mice were randomly divided into three groups (n=6/group): control group, 0.1 μg/mL BPA (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) group, 0.2 μg/mL BPA group. Twelve C57BL/6 mice were randomly divided into two groups (n=6/group): control group and 0.2 μg/mL BPA group. The control group was given water containing 1% ethanol, the concentration used as vehicle for BPA solution. BPA was exposed through free access of drinking water for six weeks. Water consumption was measured daily according to volume reduction in the bottles, and body weight and food consumption were measured weekly. Urine samples were collected once a week from the second week of BPA exposure, and centrifuged at 1500 rpm for 15 min at 4°C, and supernatant was stored at -80°C until urinary protein analysis. At the end of the experiment, mice were anesthetized with ether and blood samples were taken from the abdominal aorta. The kidneys were then dissected, with left kidney used for pathological analysis and right kidney used for western blot analysis. The coefficient of kidney was calculated (weight of kidney/weight of mice). Separated serum and kidney were stored at -80°C until subsequent analysis.

Biochemical assays

The concentrations of serum anti-dsDNA antibody were determined with ELISA kits (CUSBIO, Inc. USA) according to the manufacturer's pro-

tocol. The levels of urinary protein were measured by using a bicinchonininc acid (BCA) protein assay kit (Beijing Ding Guo Chang Sheng Biotechnology, Beijing, China) according to the manufacturer's instruction. The urine creatinine was detected by sarcosine oxidase method using a creatinine kit (Nanjing Jiancheng Bioengineering Institute, China).

Histological analysis

The kidneys were fixed in 4% paraformaldehyde at 4°C overnight for paraffin embedding, then sectioned longitudinally in 3-μm sections. Hematoxylin and eosin (H&E) (Baso Diagnostic, Inc.) staining was used for histological analysis. The National Institutes of Health (NIH) activity index (AI) scores were calculated by two pathologists. All images were obtained by using a microscope equipped with a color camera (Nikon Eclipse Ni, Japan).

Western blot analysis

The kidney tissue were homogenized with RIPA lysing buffer and smashed using disrupted ultrasonic cell disruptor, then centrifuged (1200×g, 4°C) for 15 min. Protein concentration from the supernatant was then determined using BCA protein assay kit. Samples were denatured with a SDS sample buffer, separated by 8% or 15% SDS-PAGE and then transferred onto PVDF membranes. After blocking in 5% BSA buffer for 1.5 h, membranes were incubated with primary antibodies against NF-κB, Nrf2, mTOR, LC3, Becline1, P62, Rubicon (1:1000, Cell Signaling Technology, USA), and ERα, TLR4 (1:1000, Santa Cruz Biotechnology, USA) and AhR (1:1000, Novus Biologicals, USA) and at 4°C overnight followed by HRP-conjugated anti-rabbit/mouse IgG antibody (1:5000, Absin Bioscience, China) for 1 h at room temperature. Specific bands were visualized by ECL detection and quantified via Image J software (Bio-Rad, Hercules, CA, USA). Relative protein expression of target band was equal to the ratio of gray value of target protein to that of internal control GAPDH. These protein bands and their corresponding GAPDH, and their quantitative analysis were shown in Figure S1 and Table S1 respectively.

Statistical analysis

All analyses were performed with the SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). Differences between groups were analyzed by one-way analysis of variance (ANOVA). A threshold of $P<0.05$ was defined as statistically significant.

Results

Effects of BPA exposure on body weight, food consumption, water intake and the coefficient of kidney in MRL/lpr and C57 mice

We evaluated the effects of BPA exposure on physiological status in both MLR/lpr and C57 mice. The body weight, food consumption and water intake increased with time in all groups, and the amount of food and water consumption was relatively stable after 9-week-old mice. While, there was no significant difference in body weight, the amount of food consumption and water intake, and the coefficient of kidney between BPA exposed female MRL/lpr mice and their controls ($P>0.05$, **Figure 1**), and the same for C57 mice.

Effects of BPA exposure on the levels of serum anti-dsDNA antibody in MRL/lpr and C57 mice

Serum anti-dsDNA antibody level is closely related to the disease activity of SLE. Therefore, we examined the effect of BPA exposure on serum anti-dsDNA antibody level in MRL/lpr and C57 mice to determine whether BPA exposure has an impact on the severity of lupus. The levels of anti-dsDNA antibody showed an increasing trend with the doses of BPA exposure. It was significantly higher in 0.2 μg/mL BPA group compared with control in MRL/lpr mice ($P<0.05$, **Figure 2**). However, there was no difference in serum anti-dsDNA antibody levels between control and 0.2 μg/ml BPA group in C57 mice ($P>0.05$, **Figure 2**).

Effects of BPA exposure on the ratio of urinary protein and creatinine in MRL/lpr and C57 mice

Large amount of urinary protein is an important clinical manifestation of LN, and the ratio of urinary protein and creatinine can accurately reflect the degree of LN. MRL/lpr mice exposed to low or high doses of BPA for 6 weeks resulted in an increased urinary protein/creatinine ratio, and there was a dose-effect relationship between urinary protein/creatinine ratio from 11 to 13 weeks after BPA exposure in MRL/lpr mice, and there was a statistically significant difference between the high-dose BPA group

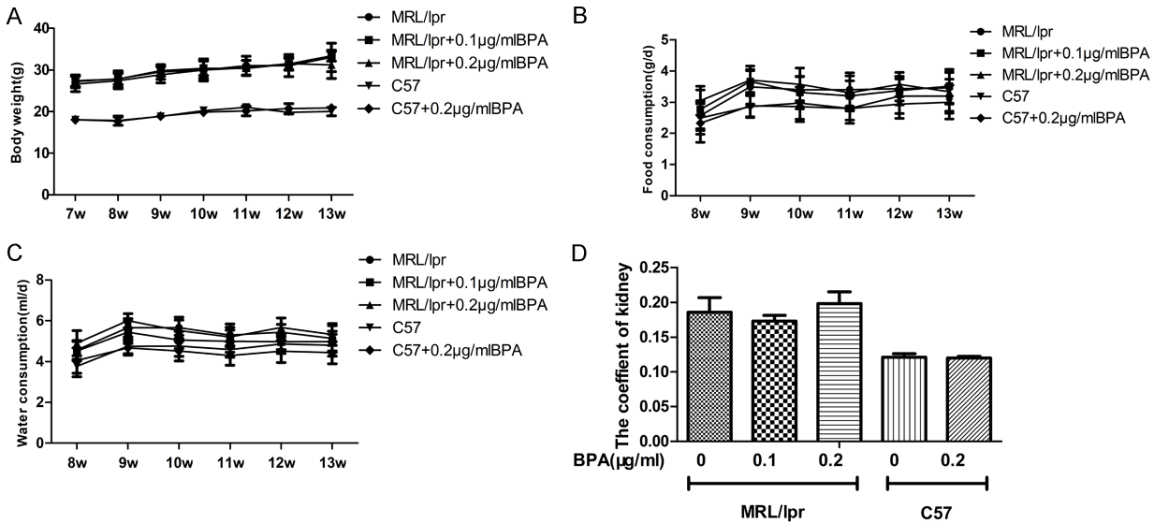

Figure 1. Effect of BPA exposure on the body weight (A), food consumption (B), water intake (C) and organ coefficient of kidney (D) in MRL/lpr and C57 female mice. MRL/lpr and C57 female mice were exposed to ethanol or 0.1 μg/mL or 0.2 μg/mL BPA through free access of drinking water for six weeks. Data are shown as mean ± SD (n=6) and were analyzed by one-way analysis of variance (ANOVA).

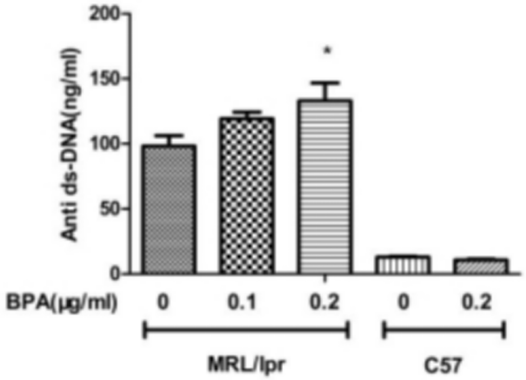

Figure 2. Effect of BPA exposure on serum anti-dsDNA antibody in MRL/lpr and C57 female mice. MRL/lpr and C57 female mice were exposed to ethanol or 0.1 μg/mL or 0.2 μg/mL BPA through free access of drinking water for six weeks. Data are shown as mean ± SD (n=6) and were analyzed by one-way analysis of variance (ANOVA). *, P<0.05 vs. MRL/lpr control.

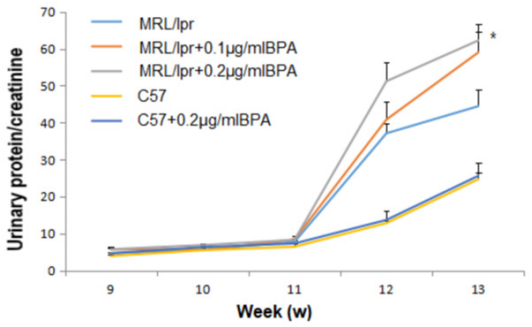

Figure 3. The effect of BPA exposure on the ratio of urinary protein and creatinine in MRL/lpr and C57 female mice. MRL/lpr and C57 female mice were exposed to ethanol or 0.1 μg/mL or 0.2 μg/mL BPA through free access of drinking water for six weeks. Data are shown as mean ± SD (n=6) and were analyzed by one-way analysis of variance (ANOVA). *, P<0.05 vs. MRL/lpr control.

(0.2 μg/ml) and the control group at the 13th week (P<0.05, **Figure 3**). However, urinary protein/creatinine ratio remained unchanged after exposure to 0.2 μg/ml BPA in C57 mice compared with the corresponding control group (P>0.05, **Figure 3**).

Effects of BPA exposure on renal pathology of MRL/lpr and C57 mice

MRL/lpr mice, as a classic model of LN, play an important role in the study of the occurrence and progression of SLE. We compared the renal pathological changes of MRL/lpr and C57 mice after BPA exposure to further clarify the causes of renal injury. There was a normal histological features with intact glomeruli and tubules in the kidney sections obtained from C57 mice (**Figure 4D**). No remarkable changes in glomeruli or tubule were observed in C57 mice after exposure to 0.2 μg/mL BPA for 6 weeks (**Figure 4E**). Mesangial cell proliferation and interstitial inflammatory cell infiltration were observed in MRL/lpr control group (**Figure 4A**). There were increased number of mesangial cells and inter-

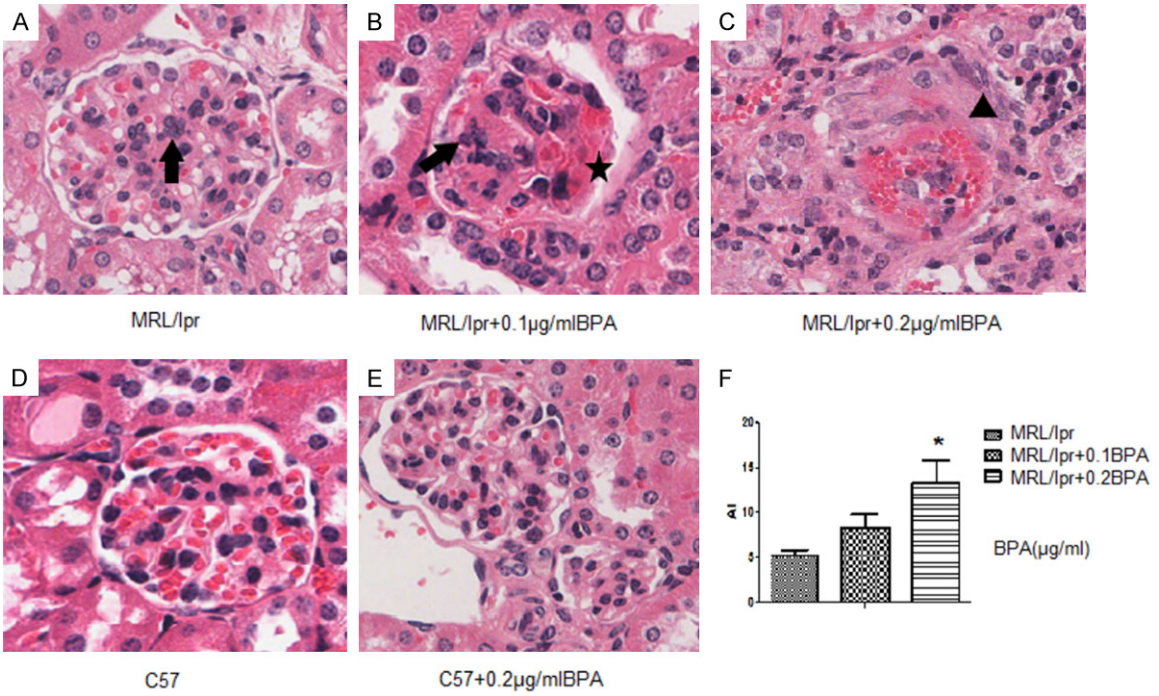

Figure 4. Photomicrographs of the kidney sections stained with hematoxylin and eosin (H&E, 20×). MRL/lpr and C57 female mice were exposed to ethanol or 0.1 μg/mL or 0.2 μg/mL BPA through free access of drinking water for six weeks. A. Renal histological changes in MRL/lpr control mice. B. Renal histological changes in 0.1 μg/mL BPA exposed MRL/lpr mice. C. Renal histological changes in 0.2 μg/mL BPA exposed MRL/lpr mice. D. Renal histological changes in C57 control mice. E. Renal histological changes in 0.2 μg/mL BPA exposed C57 mice. F. The pathological activity index (AI) score in BPA 0, 0.1 and 0.2 μg/mL BPA exposed MRL/lpr mice, Data are shown as mean ± SD (n=4) and were analyzed by one-way analysis of variance (ANOVA). *, P<0.05 vs. MRL/lpr control. The arrow showed mesangial cell proliferation in MRL/lpr mice. The asterisk showed microthrombosis in MRL/lpr+0.1 μg/mLBPA group. The triangle showed crescent body formation in 0.2 μg/mLBPA BPA exposed MRL/lpr mice.

stitial inflammatory cell infiltration in 0.1 μg/mL BPA exposed MRL/lpr mice, as well as microthrombosis and partial glomerular focal sclerosis (**Figure 4B**). Moreover, kidney injury was significantly aggravated and presented as multiple crescent body formation, interstitial inflammatory cell infiltration, partial glomerular sclerosis, renal tubular epithelial cell enlargement, and urinary protein tubular type in 0.2 μg/mL BPA exposed MRL/lpr mice (**Figure 4C**). In addition, the pathological activity index (AI) score of high-dose BPA (0.2 μg/mL) exposed MRL/lpr mice was significantly higher than that of the control group (P<0.05, **Figure 4F**).

Effects of BPA exposure on NF-κB and Nrf2 protein expression in renal tissues of MRL/lpr and C57 mice

NF-κB is the core factor of regulating inflammation response. Nrf2 is a key regulatory factor of various antioxidant genes expression in cells, and protects cells from oxidative damage. 0.2 μg/mL BPA exposure increased the expression of NF-κB protein significantly in renal tissues of MRL/lpr and C57 mice (P<0.05, **Figure 5B**). In addition, compared with MRL/lpr control group, the level of Nrf2 protein in the 0.2 μg/mL BPA exposed MRL/lpr group was significantly reduced (P<0.05, **Figure 5C**), however, the levels of Nrf2 protein expression increased in renal tissues of 0.2 μg/ml BPA exposed C57 mice (P<0.05, **Figure 5C**).

Effects of BPA exposure on mTOR and autophagy-related proteins in renal tissues of MRL/lpr and C57 mice

To further explore the mechanism of aggravating renal injury induced by BPA exposure in MRL/lpr mice, we examined the levels of mTOR and autophagy-related proteins expression in renal tissues. There were significantly decreased expressions of mTOR protein in kidney

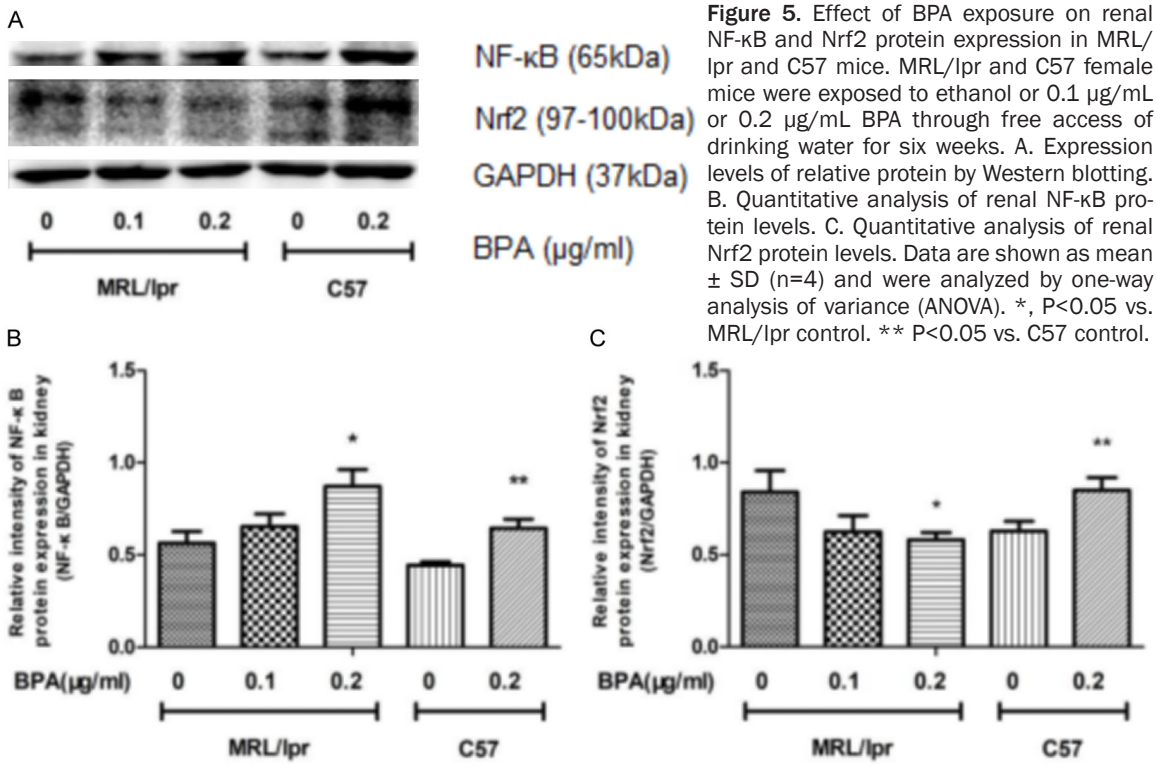

Figure 5. Effect of BPA exposure on renal NF-κB and Nrf2 protein expression in MRL/lpr and C57 mice. MRL/lpr and C57 female mice were exposed to ethanol or 0.1 μg/mL or 0.2 μg/mL BPA through free access of drinking water for six weeks. A. Expression levels of relative protein by Western blotting. B. Quantitative analysis of renal NF-κB protein levels. C. Quantitative analysis of renal Nrf2 protein levels. Data are shown as mean ± SD (n=4) and were analyzed by one-way analysis of variance (ANOVA). *, P<0.05 vs. MRL/lpr control. ** P<0.05 vs. C57 control.

tissues of MRL/lpr mice after continuous exposure to BPA at 0.1 μg/mL or 0.2 μg/mL for 6 weeks (P<0.05, **Figure 6B**). Significant increases in the levels of LC3II protein expression were observed in 0.2 μg/mL BPA exposed MRL/lpr or C57 mice, compared with their corresponding control group (P<0.05, **Figure 6C**). While no significant effects were observed on Becline1 and P62 following BPA exposure in MRL/lpr and C57 mice (P>0.05, **Figure 6D** and **6E**). The levels of Rubicon protein expression significantly decreased in kidney tissues of BPA exposed MRL/lpr mice, compared with MRL/lpr control (P<0.05, **Figure 6F**), while an increased level was found in 0.2 μg/mL BPA-treated C57 mice (P<0.05, **Figure 6F**).

Effects of BPA exposure on the levels of ERα, AhR, TLR4 protein expression in renal tissues of MRL/lpr and C57 mice

We examined the expression of ERα, AhR, TLR4 receptor proteins in renal tissues, through which BPA could exert its toxic effects. There were significant increases in the levels of ERα and AhR protein expression in 0.2 μg/mL BPA exposed MRL/lpr group when compared with MRL/lpr control group (P<0.05, **Figure 7B** and **7C**). 0.2 μg/mL of BPA caused significant increase in the levels of renal AhR protein, rather than ERα in C57 mice (P<0.05, **Figure 7B** and **7C**). BPA exposure did not affect TLR4 protein expression in both MRL/lpr and C57 mice (P<0.05, **Figure 7D**).

Discussion

The etiology and pathogenesis of SLE are still unclear, which may be the results of combined effects of genetics and environmental factors [31]. However, genes are not controllable in the process of disease occurrence and development, while the environmental factor can be prevented. Therefore, paying attention to the influence of environmental factors on the occurrence and development of SLE and its mechanism is beneficial to control and delay the disease progression.

BPA is an endocrine disruptor characterized by estrogen-like and anti-androgen effects. Although the United States and Europe have recommended the lowest observed adverse effect level (LOAEL) of BPA (50 μg/kg bw/day), some studies showed that BPA exposure with even below LOAEL still cause adverse affect on human health, which showed an U-shaped curve relationship sometimes [32, 33]. Because

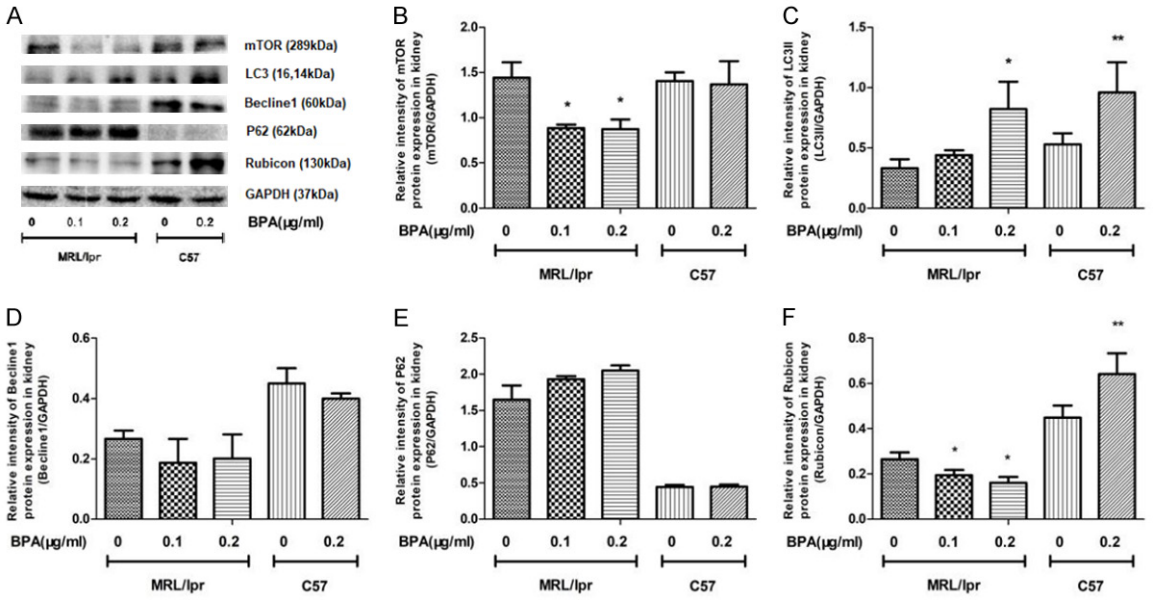

Figure 6. Effect of BPA exposure on renal mTOR and autophagy-related protein expression in MRL/lpr and C57 mice. MRL/lpr and C57 female mice were exposed to ethanol or 0.1 μg/mL or 0.2 μg/mL BPA through free access of drinking water for six weeks. A. Expression levels of relative protein by Western blotting. B. Quantitative analysis of renal mTOR protein levels. C. Quantitative analysis of renal LC3 protein levels. D. Quantitative analysis of renal Becline1 protein levels. E. Quantitative analysis of renal P62 protein levels. F. Quantitative analysis of renal Rubicon protein levels. Data are shown as mean ± SD (n=4) and were analyzed by one-way analysis of variance (ANOVA). *, P<0.05 vs. MRL/lpr control. ** P<0.05 vs. C57 control.

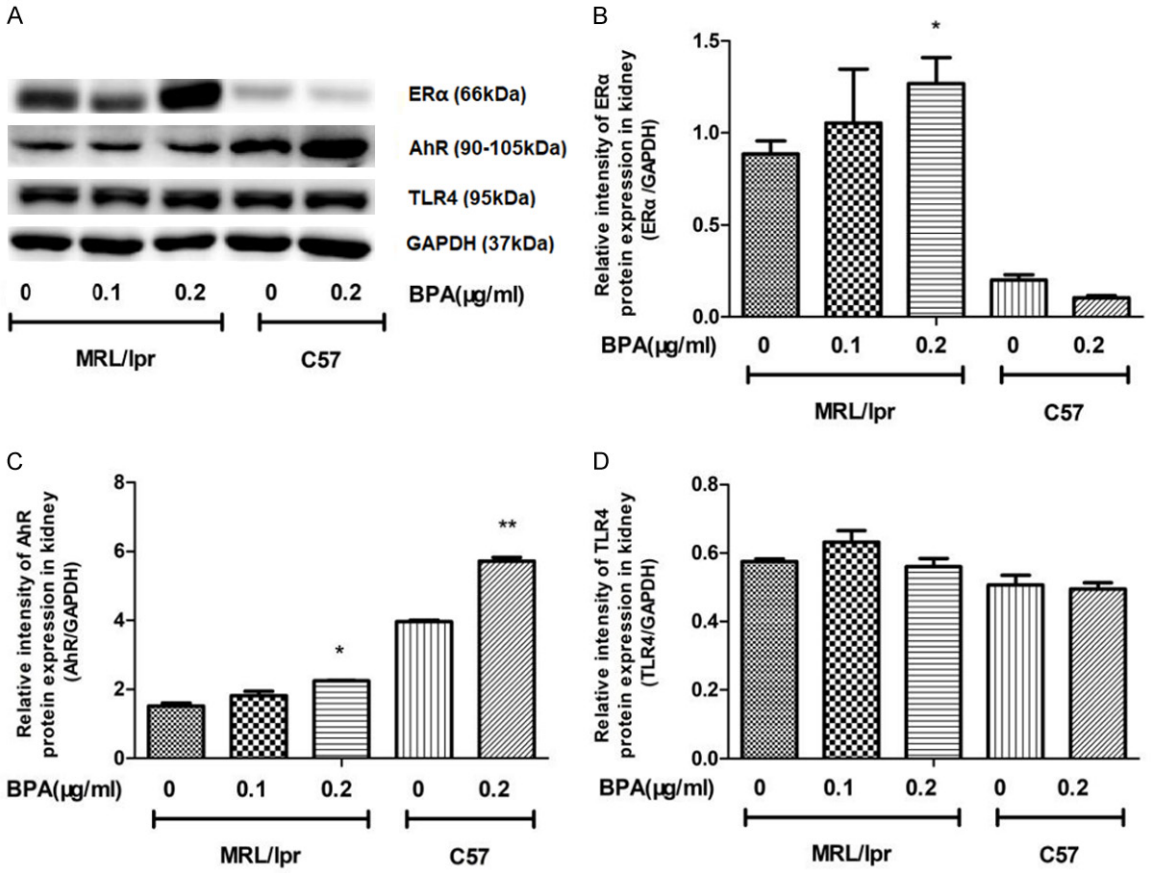

Figure 7. Effect of BPA exposure on renal ERα, AhR, TLR4 protein expression in MRL/lpr and C57 mice. MRL/lpr and C57 female mice were exposed to ethanol or 0.1 μg/mL or 0.2 μg/mL BPA through free access of drinking water for six weeks. A. Expression levels of relative protein by Western blotting. B. Quantitative analysis of renal ERα protein levels. C. Quantitative analysis of renal AhR protein levels. D. Quantitative analysis of renal TLR4 protein levels. Data are shown as mean ± SD (n=4) and were analyzed by one-way analysis of variance (ANOVA). *, P<0.05 vs. MRL/lpr control. ** P<0.05 vs. C57 control.

the adverse effects of BPA are closely related to its exposure dose, time, duration and species. Therefore, we designed BPA exposure doses lower than 50 μg/kg/day through free drinking water, which is closer to the daily BPA exposure dose and mode. The results showed that the average water intake of MRL/lpr mice was about 5.09 mL/d, and the actual BPA exposure dose was about 16.15 μg/kg/d and 32.3 μg/kg/d respectively in 0.1 and 0.2 μg/mL BPA exposure groups. The average water intake was 4.46 mL/d, and the actual BPA exposure dose was about 48.48 μg/kg/d in 0.2 μg/mL BPA exposed C57 mice. Under the doses of BPA exposure mentioned above, body weight, the amount of food and water intake and the coefficient of kidney were not affected in each group of mice.

The increasing levels of serum anti-dsDNA antibody accompanied with heavy albuminuria are important clinical manifestations of LN. We found that BPA exposure at 16-32 μg/kg/d doses for 6 weeks increased serum anti-dsDNA antibody levels and urinary protein in MRL/lpr mice, and aggravated pathological kidney injury characterized by glomerular leukocyte infiltration, microthrombosis, interstitial inflammatory cells increased, atrophy and sclerosis of glomeruli, and crescents formation, which showed a dose-effect relationship. Nuñez P et al. found that prenatal BPA exposure at 10 or 100 μg/kg/day to mice predisposed to type 2 diabetes caused replacement of the flat squamous parietal cells of Bowman capsule as tall cuboidal epithelium in the kidney of female offspring, and crescents formation in higher dose group [34]. A recent study found that BPA exposure leaded to impaired renal function and expansion of glomerular mesangial matrix, meanwhile, and increased serum dsDNA could be detected. In addition, a study confirmed that BPA treatment caused podocyte injury in a vitro experiment [35]. Adult male rats orally treated with 10 mg/kg/d BPA for 14 days developed glomerular inflammation and congestion, loss of glomerular epithelial cells with widened urinary space, widened tubules and erosion of their epithelial cells [35, 36]. Our study found that exposure to nearly 50 μg/kg/d BPA for 6 weeks did not have significant effects on urinary protein and pathological changes in kidney tissue of C57 mice, which implied that the same BPA exposure dose had more damage to the kidneys of immunodeficient mice. Therefore, the renal toxicity induced by low doses of BPA exposure cannot be ignored, especially in immunocompromised individual.

Although the exact mechanism of BPA causing kidney injury is not fully understood, studies have reported that BPA causes oxidative stress by increasing the levels of NO, MDA, and decreasing the levels of GSH and SOD, Meanwhile, BPA caused disrupted mitochondrial membrane potential, promoted the expression of mitochondrial fission related protein p-DRP1, and affected SIRT3, which was the key regulator of mitochondrial homeostasis [36, 37]. A recent study showed that oral administration of BPA at 250 mg/kg increased the levels of serum uric acid, creatinine, and urea nitrogen in wistar male rats, and confirmed that renal toxicity of BPA was associated with inflammation (affecting TNF-α, IL-6, IL-1β) and oxidative stress (Nrf2/HO-1 pathways involved) [38]. Our results showed that BPA exposure increased the expression of NF-κB protein in the kidney of both MRL/lpr and C57 mice, which implied that kidney damage induced by BPA exposure was associated with activation of inflammatory response. Meanwhile, the levels of Nrf2 protein were increased to initiate an antioxidant mechanism in the kidney of C57 mice, while the surprising findings were the decrease of Nrf2 protein in MRL/lpr mice. We speculate that the defect of antioxidant ability may be one of the reasons for the further aggravation of kidney injury induced by BPA expsore in lupus prone MRL/lpr mice.

There is increasing evidences suggesting the relationship between autophagy and lupus nephritis [39]. Autophagy is considered as playing a protective role on podocytes through

inducing endoplasmic reticulum stress of podocytes and inhibiting inflammatory response [40, 41]. In this study, we found that BPA exposure partially activated autophagy proteins (LC3II was increased) due to decreased mTOR protein expression, but the autophagy signaling pathway lacked the regulation of Becline1 and the effective reduction of autophagosome (P62 protein remained unchanged). Rubicon is iconic index in LC3-associated phagocytosis (LAP). Similar to the change of Nrf2 protein, the level of Rubicon expression decreased in renal tissue of MRL/lpr mice, while it increased in C57 mice, and there was a significantly higher Rubicon protein expression in 0.2 μg/mL BPA exposed C57 mice than that in C57 control group. These results suggested that the functions of autophagy and LAP were defective in lupus-prone mice, thus autoantibodies could not be effectively eliminated in MRL/lpr mice, which was aggravated by BPA exposure, and resulted in the progress of kidney injury. Our findings supported that the environmental risk factors for lupus nephritis can regulate the autophagy process, which was consistent with Wang L's report [39]. Because low doses of BPA exposure could activate autophagy (increased LC3 protein), and increased antioxidant ability (Nrf2 protein) in C57 mice, and none of these occurred in MRL/lpr mice in this study. Our study indicates that lower doses of BPA exposure may have more serious adverse health effects in people with autoimmune diseases than in health population. At present, increasing evidences also indicate that inflammation, oxidative stress and autophagy are involved in the development of SLE and LN [21, 24, 39].

The toxicological mechanism of BPA is related to estrogen receptors (ERs) and arylhydrocarbon receptor (AhR) [42]. In this study, the pathways of BPA toxicity may be different in MRL/lpr and C57 mice. In MRL/lpr renal tissue, BPA could increase the levels of both ERa and AhR protein. But only renal AhR protein increased in BPA exposed C57 mice. All these implied that increased inflammatory response induced by BPA in MRL/lpr mice was associated with activating ERα and AhR pathway, while BPA mainly played a renal toxic role through AhR pathway in C57 mice. TLR4 protein expression was not significantly affected by BPA in both MRL/lpr and C57 mice, which implied that increased inflammation induced by BPA was not related to TLR4. Our study also supports that the nephrotoxic effect of BPA is via mutiple pathways.

In conclusion, our results showed that lower doses of BPA increased both serum anti-dsDNA level and urinary protein concentration in MRL/lpr mice, which was consistent with aggravating renal pathological injury. The progression of lupus nephritis induced by BPA exposure was associated with up-regulation of NF-κB protein, down-regulation of Nrf2 protein and activating an incomplete autophagy. Our findings imply that aggravated lupus nephritis induced by BPA exposure involves in activating inflammation, weakened antioxidant ability and abnormal autophagy. Our study indicates that exposure to lower doses of BPA may have more serious adverse health effects in population with immune deficiency than in health population.

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Disclosure of conflict of interest

None.

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References

[1] Yen EY and Singh RR. Brief report: lupus-an unrecognized leading cause of death in young females: a population-based study using nationwide death certificates, 2000-2015. Arthritis Rheumatol 2018; 70: 1251-1255.

[2] Maroz N and Segal MS. Lupus nephritis and end-stage kidney disease. Am J Med Sci 2013; 346: 319-323.

[3] Miranda-Hernandez D, Cruz-Reyes C, Angeles U, Jara LJ and Saavedra MA. Prognostic factors for treatment response in patients with lupus nephritis. Reumatol Clin 2014; 10: 164-169.

[4] Mok CC, Kwok RC and Yip PS. Effect of renal disease on the standardized mortality ratio and life expectancy of patients with systemic lupus erythematosus. Arthritis Rheum 2013; 65: 2154-2160.
[5] Sprangers B, Monahan M and Appel GB. Diagnosis and treatment of lupus nephritis flares-an update. Nat Rev Nephrol 2012; 8: 709-717.
[6] Hard GC and Khan KN. A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. Toxicol Pathol 2004; 32: 171-180.
[7] Bonventre JV, Vaidya VS, Schmouder R, Feig P and Dieterle F. Next-generation biomarkers for detecting kidney toxicity. Nat Biotechnol 2010; 28: 436-440.
[8] Shankar A and Teppala S. Urinary bisphenol A and hypertension in a multiethnic sample of US adults. J Environ Public Health 2012; 2012: 481641.
[9] Covaci A, Den Hond E, Geens T, Govarts E, Koppen G, Frederiksen H, Knudsen LE, Morck TA, Gutleb AC, Guignard C, Cocco E, Horvat M, Heath E, Kosjek T, Mazej D, Tratnik JS, Castano A, Esteban M, Cutanda F, Ramos JJ, Berglund M, Larsson K, Jonsson BA, Biot P, Casteleyn L, Joas R, Joas A, Bloemen L, Sepai O, Exley K, Schoeters G, Angerer J, Kolossa-Gehring M, Fiddicke U, Aerts D and Koch HM. Urinary BPA measurements in children and mothers from six European member states: overall results and determinants of exposure. Environ Res 2015; 141: 77-85.
[10] Rochester JR. Bisphenol A and human health: a review of the literature. Reprod Toxicol 2013; 42: 132-155.
[11] Martella A, Silvestri C, Maradonna F, Gioacchini G, Allara M, Radaelli G, Overby DR, Di Marzo V and Carnevali O. Bisphenol A induces fatty liver by an endocannabinoid-mediated positive feedback loop. Endocrinology 2016; 157: 1751-1763.
[12] Dong YD, Zhai LL, Zhang L, Jia LH and Wang XF. Bisphenol A impairs mitochondrial function in spleens of mice via oxidative stress. Mol Cell Toxicol 2013; 9: 401-406.
[13] Li MA, Bi YF, Qi L, Wang TG, Xu M, Huang Y, Xu Y, Chen YH, Lu JL, Wang WQ and Ning G. Exposure to bisphenol A is associated with low-grade albuminuria in Chinese adults. Kidney Int 2012; 81: 1131-1139.
[14] Trasande L, Attina TM and Trachtman H. Bisphenol A exposure is associated with low-grade urinary albumin excretion in children of the united states. Kidney Int 2013; 83: 741-748.
[15] Hu J, Wang Y, Xiang X, Peng C, Gao R, Goswami R, Zhou H, Zhang Y, Zhen Q, Cheng Q, Yang S and Li Q. Serum bisphenol A as a predictor of chronic kidney disease progression in primary hypertension: a 6-year prospective study. J Hypertens 2016; 34: 332-337.
[16] Gowder SJ. Nephrotoxicity of bisphenol A (BPA)--an updated review. Curr Mol Pharmacol 2013; 6: 163-172.
[17] Kobroob A, Peerapanyasut W, Chattipakorn N and Wongmekiat O. Damaging effects of bisphenol A on the kidney and the protection by melatonin: emerging evidences from in vivo and in vitro studies. Oxid Med Cell Longev 2018; 2018: 3082438.
[18] Anders HJ and Schaefer L. Beyond tissue injury-damage-associated molecular patterns, toll-like receptors, and inflammasomes also drive regeneration and fibrosis.J Am Soc Nephrol 2014; 25: 1387-1400.
[19] Zheng L, Sinniah R and Hsu SI. In situ glomerular expression of activated NF-kappaB in human lupus nephritis and other non-proliferative proteinuric glomerulopathy. Virchows Arch 2006; 448: 172-183.
[20] Zheng L, Sinniah R and Hsu SI. Pathogenic role of NF-kappaB activation in tubulointerstitial inflammatory lesions in human lupus nephritis. J Histochem Cytochem 2008; 56: 517-529.
[21] Jhou JP, Chen SJ, Huang HY, Lin WW, Huang DY and Tzeng SJ. Upregulation of FcgammaRIIB by resveratrol via NF-kappaB activation reduces B-cell numbers and ameliorates lupus. Exp Mol Med 2017; 49: e381.
[22] Hu Q, Yang C, Wang Q, Zeng H and Qin W. Demethylzeylasteral (T-96) treatment ameliorates mice lupus nephritis accompanied by inhibiting activation of NF-kappaB pathway. PLoS One 2015; 10: e0133724.
[23] Yang J, Yang X, Zou H and Li M. Oxidative stress and Treg and Th17 dysfunction in systemic lupus erythematosus. Oxid Med Cell Longev 2016; 2016: 2526174.
[24] Jiang T, Tian F, Zheng H, Whitman SA, Lin Y, Zhang Z, Zhang N and Zhang DD. Nrf2 suppresses lupus nephritis through inhibition of oxidative injury and the NF-kappaB-mediated inflammatory response. Kidney Int 2014; 85: 333-343.
[25] Yoh K, Itoh K, Enomoto A, Hirayama A, Yamaguchi N, Kobayashi M, Morito N, Koyama A, Yamamoto M and Takahashi S. Nrf2-deficient female mice develop lupus-like autoimmune nephritis. Kidney Int 2001; 60: 1343-1353.
[26] Green DR, Oguin TH and Martinez J. The clearance of dying cells: table for two. Cell Death Differ 2016; 23: 915-926.

[27] Mistry P and Kaplan MJ. Cell death in the pathogenesis of systemic lupus erythematosus and lupus nephritis. Clin Immunol 2017; 185: 59-73.
[28] Rodriguez-Manzanet R, Sanjuan MA, Wu HY, Quintana FJ, Xiao S, Anderson AC, Weiner HL, Green DR and Kuchroo VK. T and B cell hyperactivity and autoimmunity associated with niche-specific defects in apoptotic body clearance in TIM-4-deficient mice. Proc Natl Acad Sci U S A 2010; 107: 8706-8711.
[29] Martinez J, Cunha LD, Park S, Yang M, Lu Q, Orchard R, Li QZ, Yan M, Janke L, Guy C, Linkermann A, Virgin HW and Green DR. Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. Nature 2016; 533: 115-119.
[30] Martinez J, Malireddi RK, Lu Q, Cunha LD, Pelletier S, Gingras S, Orchard R, Guan JL, Tan H, Peng J, Kanneganti TD, Virgin HW and Green DR. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nat Cell Biol 2015; 17: 893-906.
[31] Crispin JC, Kyttaris VC, Terhorst C and Tsokos GC. T cells as therapeutic targets in SLE. Nat Rev Rheumatol 2010; 6: 317-325.
[32] Ge LC, Chen ZJ, Liu HY, Zhang KS, Liu H, Huang HB, Zhang G, Wong CK, Giesy JP, Du J and Wang HS. Involvement of activating ERK1/2 through G protein coupled receptor 30 and estrogen receptor alpha/beta in low doses of bisphenol A promoting growth of Sertoli TM4 cells. Toxicol Lett 2014; 226: 81-89.
[33] Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr, Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT and Myers JP. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev 2012; 33: 378-455.
[34] Nunez P, Fernandez T, Garcia-Arevalo M, Alonso-Magdalena P, Nadal A, Perillan C and Arguelles J. Effects of bisphenol A treatment during pregnancy on kidney development in mice: a stereological and histopathological study. J Dev Orig Health Dis 2018; 9: 208-214.
[35] Tong S, Yang S, Li T, Gao R, Hu J, Luo T, Qing H, Zhen Q, Hu R, Li X, Yang Y, Peng C and Li Q. Role of neutrophil extracellular traps in chronic kidney injury induced by bisphenol-A. J Endocrinol 2019; [Epub ahead of print].
[36] Ola-Davies OE and Olukole SG. Gallic acid protects against bisphenol A-induced alterations in the cardio-renal system of Wistar rats through the antioxidant defense mechanism. Biomed Pharmacother 2018; 107: 1786-1794.
[37] Peerapanyasut W, Kobroob A, Palee S, Chattipakorn N and Wongmekiat O. Activation of sirtuin 3 and maintenance of mitochondrial integrity by N-acetylcysteine protects against bisphenol A-induced kidney and liver toxicity in rats. Int J Mol Sci 2019; 20.
[38] Alekhya Sita GJ, Gowthami M, Srikanth G, Krishna MM, Rama Sireesha K, Sajjarao M, Nagarjuna K, Nagarjuna M, Chinnaboina GK, Mishra A and SreeHarsha N. Protective role of luteolin against bisphenol A-induced renal toxicity through suppressing oxidative stress, inflammation, and upregulating Nrf2/ARE/HO-1 pathway. IUBMB Life 2019; 71: 1041-1047.
[39] Wang L and Law HK. The role of autophagy in lupus nephritis. Int J Mol Sci 2015; 16: 25154-25167.
[40] Zhou XJ, Klionsky DJ and Zhang H. Podocytes and autophagy: a potential therapeutic target in lupus nephritis. Autophagy 2019; 15: 908-912.
[41] Qi YY, Zhou XJ, Cheng FJ, Hou P, Ren YL, Wang SX, Zhao MH, Yang L, Martinez J and Zhang H. Increased autophagy is cytoprotective against podocyte injury induced by antibody and interferon-alpha in lupus nephritis. Ann Rheum Dis 2018; 77: 1799-1809.
[42] Rogers JA, Metz L and Yong VW. Review: endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. Mol Immunol 2013; 53: 421-430.

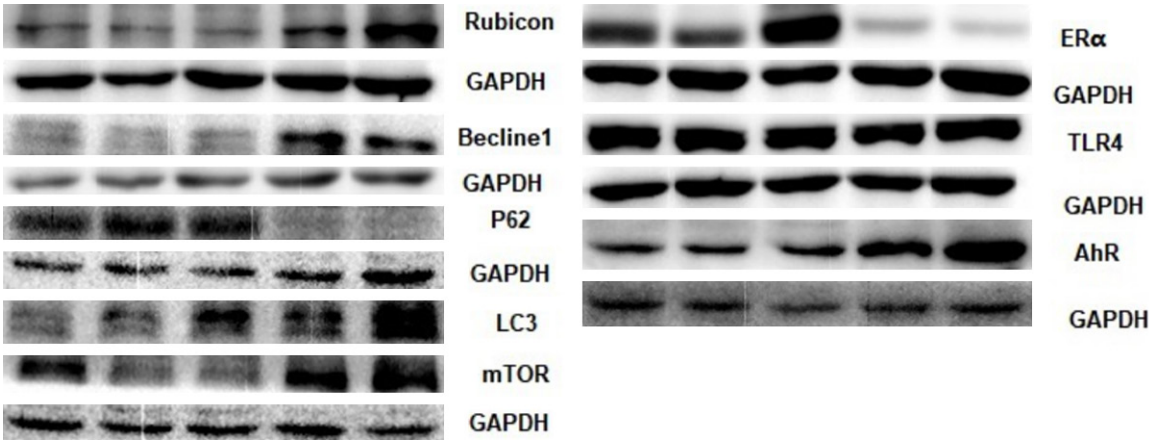

Figure S1. The protein band and the corresponding internal reference (GAPDH) by Western blot.

Table S1. Mean ± SD of each protein. n=4, repeated it three times

	NF-κB	Nrf2	mTOR	Rubicon	P62
MRL/lpr	0.564±0.063	0.841±0.116	1.444±0.167	0.266±0.029	1.650±0.194
MRL/lpr+0.1BPA	0.653±0.069	0.623±0.089	0.888±0.036	0.195±0.023	1.932±0.042
MRL/lpr+0.2BPA	0.87±0.092	0.58±0.042	0.873±0.106	0.161±0.025	2.050±0.073
C57	0.443±0.02	0.628±0.054	1.408±0.094	0.449±0.053	0.444±0.031
C57+0.2BPA	0.643±0.05	0.85±0.068	1.365±0.258	0.642±0.091	0.449±0.028
	Becline1	LC3	ER	TLR4	AhR
MRL/lpr	0.266±0.028	0.331±0.076	0.887±0.071	0.575±0.008	1.507±0.097
MRL/lpr+0.1BPA	0.187±0.079	0.440±0.040	1.053±0.295	0.631±0.034	1.817±0.128
MRL/lpr+0.2BPA	0.201±0.08	0.821±0.226	1.27±0.141	0.560±0.024	2.252±0.01
C57	0.450±0.050	0.530±0.089	0.201±0.028	0.507±0.027	3.955±0.057
C57+0.2BPA	0.400±0.017	0.961±0.248	0.105±0.012	0.495±0.018	5.723±0.108