International Immunopharmacology 78 (2020) 106058

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

The environmental hormone nonylphenol interferes with the therapeutic effects of G protein-coupled estrogen receptor specific agonist G-1 on murine allergic rhinitis

Yun-xiu Wang[a], Zhao-wei Gu[b,*], Li-ying Hao[c]

[a] Department of Human Resources, China Medical University Affiliated Shengjing Hospital, Shenyang City 110004, Liaoning Province, China
[b] Department of Otorhinolaryngology, China Medical University Affiliated Shengjing Hospital, Shenyang City 110004, Liaoning Province, China
[c] Department of Pharmaceutical Toxicology, School of Pharmacy, China Medical University, Shenyang City 110122, Liaoning Province, China

ARTICLE INFO

Keywords:
G protein-coupled estrogen receptor
Nonylphenol
G-1
Allergic rhinitis
Treg

ABSTRACT

The G protein-coupled estrogen receptor (GPER) specific agonist G-1 has therapeutic effects in patients with allergic diseases, but any role for G-1 as a therapy for inflammation associated with allergic rhinitis (AR) remains unclear. The structure of the environmental hormone nonylphenol (NP) is very similar to that of estrogen; it binds to the estrogen receptor to produce estrogen-like effects and thus may also bind to the membrane GPER. We explored whether NP administration would reduce the effects of G-1 on AR, the interactions between the two materials, and their mechanisms of action using a murine model of AR. Mice were randomly assigned into control, AR, G-1, and G-1 + NP groups (n = 10/group). AR nasal symptoms were scored. Eosinophils in nasal mucosa were counted after staining with hematoxylin and eosin. Serum ovalbumin (OVA)-specific IgE was determined by ELISA. The proportions of splenic Th1, Th2, and Treg cells were determined by flow cytometry. The expression of transcription factors unique to Th1, Th2, Treg cells and cytokine levels in nasal mucosa were evaluated by real-time PCR and cytometric bead arrays. AR nasal symptoms, including sneezing, nasal scratching, eosinophil infiltration of nasal mucosa, and serum IgE, were reduced in G-1 group. After injection, Th2 cells proportions, Th2-immune response-related cytokines (IL-4, IL-5, and IL-13), and a Th2 cell-specific transcription factor (GATA-3) were significantly decreased in G-1 group. Treg immune response was enhanced (as reflected by Treg cell, IL-10, and Foxp3 levels). The levels of all of these were significantly increased after adding NP, and the Treg immune response was significantly decreased. These results indicate that G-1 attenuated the nasal symptoms, serum OVA-specific IgE, and Th2 cell immune response, whereas it enhanced Treg immune response, in mice with AR. Adding NP weakened these therapeutic effects.

1. Introduction

There are three known estrogen receptors (ERs), namely, ERα, ERβ, G protein-coupled estrogen receptor (GPER) [1]. Binding of estrogen to ERα and ERβ is followed by transfer of the ER-estrogen complex (a ligand-gated transcription factor) to nucleus, where it affects downstream genes expression via the classic ER pathway. In addition to the well-known transcriptional regulation afforded by the estrogen nuclear receptor/estrogen complex, estrogen binds to cell membrane receptors to trigger fast signaling pathways termed non-gene pathways or membrane-defined signaling pathways [1]. The estrogen-binding sites on cell membranes are termed GPERs; these are expressed in the brain, cardiovascular system and the lung [2–4].

The GPER exhibits unique features, triggers various biological effects, and affects different signaling pathways; it independently triggers membrane signaling pathways. In recent years, increasing attention has been paid to the fast signaling pathways triggered by cell membrane contact. Recent studies have found that GPER plays an important role in immune system regulation [5–7], but few studies have explored GPER activity in patients with allergic diseases, particularly allergic rhinitis (AR). Some studies have reported a role for the GPER-specific receptor agonist G-1 in a mouse asthma model. G-1 increases Treg action by elevating IL-10 levels, thereby alleviating airway hyperresponsiveness and inflammation [4]. Here, we offer a new perspective on estrogen binding to a membrane receptor. We infer that the GPER receptor-specific agonist G-1 may alleviate the AR inflammatory response and thus play a therapeutic role.

The incidence of AR has been increasing in recent years, especially

* Corresponding author at: No. 36 Sanhao Street, Heping District, Shenyang, Liaoning Province, China.
E-mail address: gordon_222@aliyun.com (Z.-w. Gu).

https://doi.org/10.1016/j.intimp.2019.106058
Received 3 July 2019; Received in revised form 27 October 2019; Accepted 13 November 2019
1567-5769/ © 2019 Elsevier B.V. All rights reserved.

in preschool children [8]. However, any effects of external factors remain unclear. Environmental hormones, also termed environmental endocrine disruptors (EEDs), are a heterogeneous group of pollutants existing in the environment and food, which may interfere with the endocrine and reproductive systems of humans and other animals even in low doses [9]. The environmental hormone nonylphenol (NP) is commonly used in the manufacture of detergents, emulsifiers, and solvents, and can therefore accumulate in the environment [10,11].

NP is lipophilic and has a long half-life, so it can be bioaccumulated *in vivo* [12,13]. The structure of NP is very similar to that of estrogen. It binds to ERs and exerts weak estrogen-like effects, or it may competitively inhibit estrogen binding. Estrogen protects against myocardial ischemia/reperfusion (I/R) injury by inflammation reduction, supporting myocardial mitochondria, and decreasing myocardial apoptosis and the area of myocardial infarction [14]. The environmental hormone bisphenol A (BPA) may compromise the protective effects of estrogen on myocardial I/R injury [15]. It reduces the cardioprotective effects of estradiol by lowering the heart rate, promoting the release of lactate dehydrogenase and creatinine kinase, increasing infarct size, and destroying the myocardium [15]. This chemical is found in rivers, lakes, reservoirs, and even the atmosphere. The question of whether environmental hormones interfere with the therapeutic effects of the GPER receptor-specific agonist G-1 in AR models deserves further investigation.

2. Materials and methods

2.1. Animals

Eight week-old female BALB/c mice (Liaoning Changsheng Biotechnology Co., Ltd., China) were maintained on a free ovalbumin (OVA) diet. All experiments were approved by the Shengjing Hospital Ethics Committee. A total of 40 mice were randomly assigned to control group, AR group, G-1 group, or G-1 + NP group (n = 10/group).

2.2. Experimental protocols

The AR group, G-1 group, and G-1 + NP group were sensitized intraperitoneally by 100 μL of PBS containing 2 mg $Al(OH)_3$ (Sigma-Aldrich, USA) and 100 μg of OVA (Sigma-Aldrich) on days 0, 7, and 14. The control group received 100 μL of PBS alone. From day 0 to day 14, the mice in G-1 + NP group were subcutaneously injected with 0.5 mg/kg/day of NP (Sigma-Aldrich) in corn oil, while the other three groups received only corn oil. After sensitization, AR group, G-1 group, and G-1 + NP group were challenged with 100 μg of OVA in 20 μL of PBS via nasal route from day 21 to day 27. The control group received PBS nose drops. G-1 group and G-1 + NP group received subcutaneous injections of 0.5 mg/kg/day of G-1 (Cayman Chemical Co., USA) in dimethyl sulfoxide (DMSO) from day 21 to day 27. The control group and AR group received subcutaneous injections of only DMSO according to the same schedule (Fig. 1).

2.3. AR nasal symptom scores

The numbers of sneezes and nasal rubbing motions were counted for 15 min by four observers blinded to the group allocations after the final intranasal challenge.

2.4. Histopathology

On day 27, all animals were euthanized 2 h after the final intranasal challenge. Five animals in each group were decapitated and their heads were placed in 4% paraformaldehyde. Subsequently, decalcification was performed with 10% EDTA. The paraffin-embedded samples were cut into slices with a thickness of 4 μm. Each section was stained with hematoxylin and eosin to observe eosinophils.

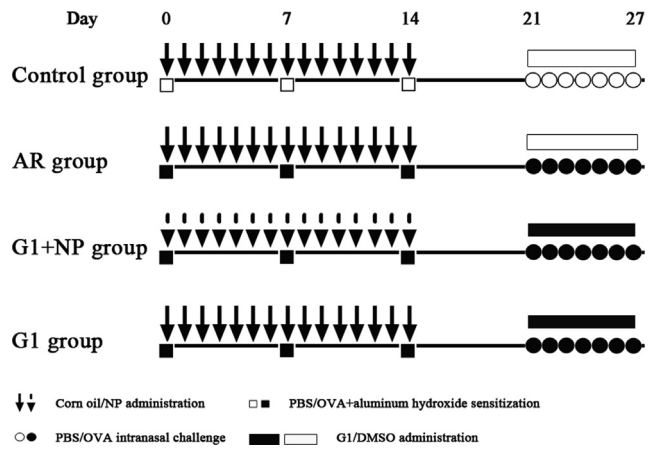

Fig. 1. Experimental protocol featuring ovalbumin (OVA) sensitization and later challenge to trigger the development of allergic rhinitis (AR) in mice (A). Briefly, on days 0, 7, and 14, mice in the AR, G1, and G1 + NP groups were sensitized with 100 μL of aluminum hydroxide solution (2 mg) and an OVA solution (100 μg) via intraperitoneal injection; control mice received PBS alone. Mice in the G-1 + NP group were subcutaneously injected with 0.5 mg/kg/day NP in corn oil from days 0 to 14; the other three groups received only corn oil. The AR, G-1, and G-1 + NP groups were intranasally challenged with 100 μg of OVA in 20 μL of PBS from days 21 to 27. The control group was exposed to PBS alone. Mice from the G-1 and G-1 + NP groups were subcutaneously injected with 0.5 mg/kg/day G-1 in dimethyl sulfoxide (DMSO) from days 21 to 27; the control and AR groups received only DMSO via the same route on the same days. PBS, phosphate-buffered saline; NP, nonylphenol.

2.5. Flow cytometry

Splenic mononuclear cells were incubated with phorbol myristate acetate (Sigma-Aldrich), ionomycin (Sigma-Aldrich), and GolgiStop (BD Biosciences, Franklin Lakes, NJ) at 37 °C for 6 h. For Th1 and Th2 cell subsets, cells were first incubated with APC-Cy7-CD3 and FITC-CD4, followed by APC-IFN-γ and PE-IL-4 (BD Biosciences) after fixation and permeabilization. For Treg cell subsets, cells were first incubated with FITC-CD4, followed by PE-Foxp3 and APC-Helios (BD Biosciences) intracellular staining. Th1, Th2, and Treg cells were performed on a FACS Aria III flow cytometer (BD Biosciences).

2.6. Cytokine measurement

The nasal mucosa was collected, crushed, and centrifuged. The supernatants were used to measure IFN-γ, IL-4, IL-5, IL-10, IL-13 using CBA Flex Set (BD Biosciences). All samples were performed on a FACS Aria III flow cytometer (BD Biosciences). The data were processed with FACSDiva and BD CBA software (ver. 4.2, BD Biosciences).

2.7. Determination of serum OVA-specific IgE levels

Mouse sera were thawed from −80 °C prior to analysis. OVA-specific IgE levels were determined by an enzyme-linked immunosorbent assay (ELISA) (BioLegend, USA) in accordance with the manufacturer's instructions.

2.8. Real-time PCR

For total RNA collection, RNA from the nasal mucosa was extracted with TRIzol (Invitrogen, USA) and was used to produce cDNA with a PrimeScript RT kit (Takara, China). The cDNAs were amplified with a Roche LightCycler 480 II System (Basel, Switzerland). The target gene sequences, GPER, T-bet, GATA-3, and Foxp3, were normalized relative to β-actin expression. The primer sequences were as follows: β-actin forward 5′-GCA GAA GGA GAT TAC TGC TCT-3′, reverse 5′-GCT GAT

CCA CAT CTG CTG GAA-3′; T-bet forward 5′-TAC AAC AGC CAG CCA AAC AG-3′, reverse 5′-CAC CCT TCA AAC CCT TCC TC-3′; Gata3 forward 5′-TAC CAC CTA TCC GCC CTA TG-3′, reverse 5′-GCC TCG ACT TAC ATC CGA AC-3′; Foxp3 forward 5′- GCC AAG CAG AAA GAT GAC AG-3′, reverse 5′-TTC CAG ATG TTG TGG GTG AG-3′; and GPER forward 5′-GAG GTG TTC AAC CTG CAC-3′ GA, reverse 5′-GTA GCG GTC GAA GCT CAT CC-3′.

2.9. Statistical analysis

SPSS software (ver.23.0; IBM Corp., USA) was used for statistical analysis. All data are expressed as the mean ± SEM. According to the data structure, a variety of statistical methods were used to evaluate the data. The *t* test was used to compare data with a normal distribution between the two groups, and the Mann-Whitney test was used to test data with a non-normal distribution between the two groups. For comparisons among multiple groups of data, a one-way ANOVA was used to compare the data with homogeneous variance, while the data with non-homogeneous variance were compared using the Kruskal-Wallis test. Graphics were drawn using GraphPad Prism (GraphPad Software, USA). $P < 0.05$ was taken to indicate statistical significance.

3. Results

3.1. GPER expression in nasal mucosa and splenic tissue of BALB/c mice

Some studies have shown that GPER is expressed in human eosinophils, lungs, and other tissues [16]. Another study assessed the roles of GPER in lung tissues of BALB/c and C57BL/6 mice [4], but there have been no previous reports confirming GPER expression in nasal mucosa and spleen of BALB/c mice. We first evaluated GPER mRNA in nasal mucosa and spleen of control BALB/c mice and AR BALB/c mice by real-time PCR.

GPER was expressed in both nasal mucosa and spleen of BALB/c mice. Comparing with normal BALB/c mice, the expression of GPER in AR BALB/c mice was significantly decreased. As shown in Fig. 2, GPER levels in nasal mucosa were 1.01 ± 0.18 and 0.70 ± 0.12 in control and AR groups, respectively ($P < 0.05$). GPER levels in the spleen were 1.01 ± 0.14 and 0.53 ± 0.11 in control and AR groups, respectively ($P < 0.05$).

3.2. Effects of G-1 on nasal symptoms

We first evaluated the effects of G-1 on AR symptoms. The number of sneezes in AR group was significantly higher than control group (8.80 ± 1.32 vs. 2.30 ± 0.82/15 min, respectively, $P < 0.05$); the number in G-1 group was significantly lower than AR group (4.80 ± 1.14 vs. 8.80 ± 1.32/15 min, respectively, $P < 0.05$). The amount of nasal scratching was consistent with these data (6.30 ± 1.49, 4.40 ± 1.07, and 2.10 ± 0.74/15 min in AR, G-1, and control groups, respectively). Sneezing and nose scratching both decreased significantly after G-1 treatment (both $P < 0.05$) (Fig. 3). G-1 effectively alleviated the nasal symptoms of AR.

3.3. Effects of G-1 on eosinophil infiltration of the nasal mucosa

As eosinophils play key roles in AR inflammation, we evaluated whether G-1 would affect mucosal infiltration by such cells. Eosinophil infiltration was significantly greater in AR group (26.30 ± 2.09 cells/high-power field [HPF]) than control group (2.51 ± 0.67 cells/HPF, $P < 0.05$). G-1 significantly reduced infiltration compared to that in OVA-induced mice (19.75 ± 2.05 vs. 26.3 ± 2.09 cells/HPF, $P < 0.05$) (Fig. 4). Thus, the GPER-specific agonist G-1 decreased eosinophil infiltration.

3.4. Effects of G-1 on serum OVA-specific IgE levels

As AR is a predominantly IgE-mediated immune response, we evaluated whether G-1 treatment can affect OVA-specific IgE levels. As shown in Fig. 5A, the IgE level was significantly increased in AR group compared to control group (254.64 ± 10.77 pg/mL vs. 32.77 ± 4.65 pg/mL, respectively; $P < 0.05$), but significantly decreased in the G-1 group (135.21 ± 5.97 pg/mL). Thus, G-1 can reduce serum levels of OVA-specific IgE.

3.5. Effects of G-1 on the Th1 and Th2 immune responses of AR mice

The principal pathogenesis of AR is an imbalance in the Th1/Th2 cell-mediated immune responses. In the AR model, a series of immune responses initiated by Th2 cells trigger local inflammation. The principal pathological changes are nasal mucosal edema and eosinophil and mast cell infiltration, which in turn cause nasal obstruction, itching, and sneezing. We used flow cytometry to measure Th1 and Th2 cells proportions in single-cell suspensions of spleen tissue to evaluate the effects of G-1 on the Th1/2 immune responses. Th1 and Th2 cells proportions were 0.77% ± 0.05% and 2.89% ± 0.19% in G-1 group, 0.82% ± 0.05% and 3.66% ± 0.22% in AR group, and 0.72% ± 0.11% and 1.90% ± 0.19% in controls, respectively. As shown in Fig. 6, the proportion of Th2 cells was significantly decreased by G-1 ($P < 0.05$), whereas Th1 cells proportion did not change significantly. Thus, G-1 significantly attenuated the Th2 immune response in AR mice.

The Th1/Th2 immune response balance controls the expression levels of cytokines IFN-γ, IL-4, IL-5, and IL-13. To evaluate the effects of G-1 in this context, we used the CBA assay to measure above cytokine levels in nasal mucosa tissue supernatant. IFN-γ, IL-4, IL-5, and IL-13

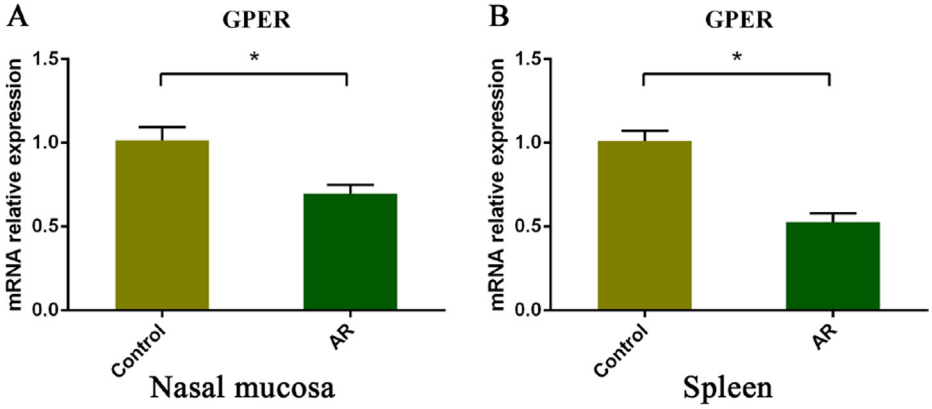

Fig. 2. The levels of mRNA were measured by real-time PCR in the nasal mucosa (A) and spleen (B). *$P < 0.05$.

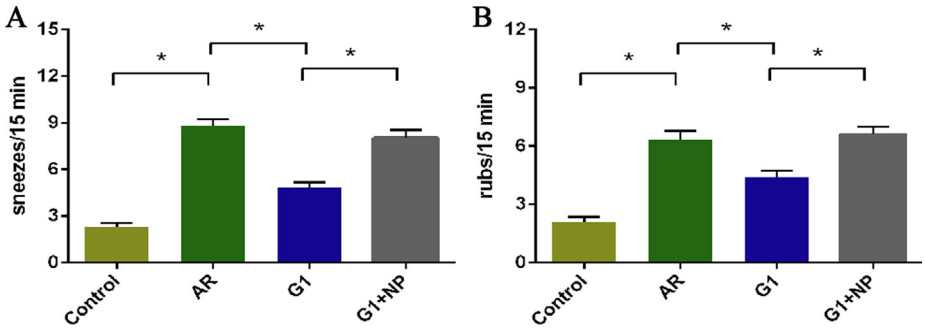

Fig. 3. Nasal symptom scores. (A) Sneezes and (B) nasal rubs in the four groups during a 15-min period after the final intranasal OVA challenge. *$P < 0.05$.

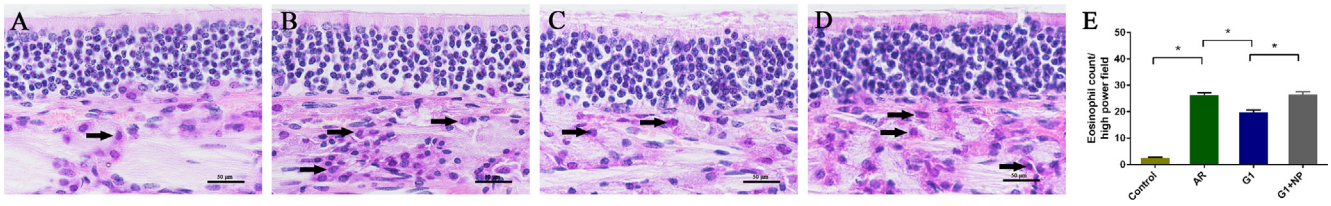

Fig. 4. Histopathology of the nasal mucosa. The extent of eosinophil infiltration in the four groups (black arrow, original magnification 400×). (A) Control, (B) AR, (C) G1, and (D) G1 + NP groups. (E) Eosinophil counts in the nasal mucosa. *$P < 0.05$.

were 3.30 ± 0.24, 6.36 ± 0.29, 5.27 ± 0.32, and 54.35 ± 0.41 pg/mL in AR group; 2.90 ± 0.40, 4.03 ± 0.86, 4.48 ± 0.22, and 3.90 ± 0.10 pg/mL in G-1 group; and 2.95 ± 0.29, 3.78 ± 0.55, 4.30 ± 0.34, and 3.45 ± 0.20 pg/mL in controls, respectively (Fig. 5B–E). In summary, the concentrations of Th2 immune response-related cytokines were significantly reduced by G-1.

To confirm that G-1 inhibited the Th2 immune response system, we evaluated its effects on Th2 and Th1 immune response-specific transcription factors. We used real-time PCR to measure Th1 cells and Th2 cells specific transcription factor T-bet and GATA-3 expression, respectively. T-bet levels were 1.00 ± 0.05, 1.03 ± 0.15, and 0.94 ± 0.05, and GATA-3 levels were 1.00 ± 0.04, 1.59 ± 0.13, and 1.26 ± 0.09 in control, non-G-1 treated AR, and G-1 treated AR groups, respectively (Fig. 7A and B). These data are in line with the observed changes in the Th1/Th2 balance described above; the GATA-3 level significantly decreased in G-1 group ($P < 0.05$) but the T-bet level did not change significantly ($P > 0.05$).

3.6. Effects of G-1 on Treg immune responses

To evaluate the effects of G-1 on Treg cellular response, we measured changes in Treg cell proportions, the levels of IL-10 (a major cytokine of Treg cells), and the effects of G-1 on expression of a Treg-specific transcription factor. The Treg cell levels were 14.86% ± 1.11% and 12.46% ± 0.67% in controls and non-G-1-treated AR group, respectively, $P < 0.05$. The level in G-1-treated AR mice was 14.14% ± 1.07%, significantly higher than that in non-G-1-treated AR mice ($P < 0.05$). Thus, G-1 significantly improved the Treg response of AR mice. Fig. 8 details how Treg cell proportions were derived via flow cytometry and the proportions in the three groups.

Treg cells have an immunosuppressive effect by virtue of cytokine IL-10 secretion. We used the CBA assay to measure IL-10 levels in nasal mucosae of the three groups. The levels were 11.92 ± 1.98, 6.93 ± 0.52, and 8.67 ± 0.42 pg/mL in control, non-G-1 treated AR, and G-1 treated groups, respectively (Fig. 5F), significantly lower in AR group (without G-1) than control group ($P < 0.05$). However, the levels did not differ significantly between AR group given G-1 and controls. Thus, G-1 significantly mitigated the decrease in IL-10 level. We also assayed the expression levels of mRNA encoding the Treg-specific transcription factor Foxp3 in nasal mucosae; the relative values were 1.00 ± 0.09, 0.73 ± 0.05, and 0.88 ± 0.03 in control, non-G-1-treated AR, and G-1-treated AR groups, respectively. Thus, Foxp3 expression was significantly lower in AR group than controls but was rescued by G-1 ($P < 0.05$). The transcription factor levels are shown in Fig. 7C. The data are consistent with the trends in Treg cell and IL-10 levels; G-1 countered inhibition of the Treg immune response.

In summary, the GPER-specific agonist G-1 countered inhibition of the Treg immune response in the AR model as shown by the levels of IL-10 (a major cytokine of Treg cells) and Foxp3 (a Treg cell-specific transcription factor). Thus, G-1 is a useful treatment for AR, consistent with the initial experiments showing that G-1 effectively alleviated the Th2 immune response in AR mice.

3.7. Influence of NP on the therapeutic effects of G-1

There were 2.89% ± 0.19% and 4.09% ± 0.37% Th2 cells, and 14.14% ± 1.07% and 10.97% ± 1.07% Treg cells in the G-1 and G-1 + NP groups, respectively. Thus, NP compromised the effects of G-1 on Th2 and Treg cell numbers (Figs. 6 and 8). The levels of IL-4, IL-5, IL-10, and IL-13 expressed by Th2 and Treg cells were 4.03 ± 0.86, 4.48 ± 0.22, 8.67 ± 0.42, and 3.90 ± 0.10 pg/mL in G-1 group and 6.33 ± 0.84, 5.60 ± 0.25, 7.13 ± 0.33, and 4.87 ± 0.22 pg/mL in G-1 + NP group, respectively (Fig. 5C–F). Significant differences were apparent between the two groups (all $P < 0.05$). NP significantly compromised the effects of G-1 on cytokine expression by Th2 and Treg cells of the AR model. GATA-3 mRNA and Foxp3 mRNA were 1.26 ± 0.09 and 0.88 ± 0.03 in G-1 group and 2.67 ± 0.65 and 0.71 ± 0.06 in G-1 + NP group (Fig. 7B and C). In addition, serum OVA-specific IgE levels are affected by NP. OVA-specific IgE were 135.21 ± 5.97 pg/mL in G-1 group and 260.87 ± 13.65 pg/mL in G-1 + NP group (Fig. 5A).

In conclusion, NP significantly weakened the therapeutic effects of G-1 in the AR murine model; NP and G-1 may bind competitively to GPER.

4. Discussion

G-1 inhibits cancer cell growth, migration [17], and is cardioprotective [18]. We found that G-1 not only attenuated the symptoms of AR mice, it also significantly inhibited the Th2 cell-associated inflammatory response, as revealed by changes in the levels of specific transcription factors and cell proportions. IL-4, IL-5, and IL-13 were

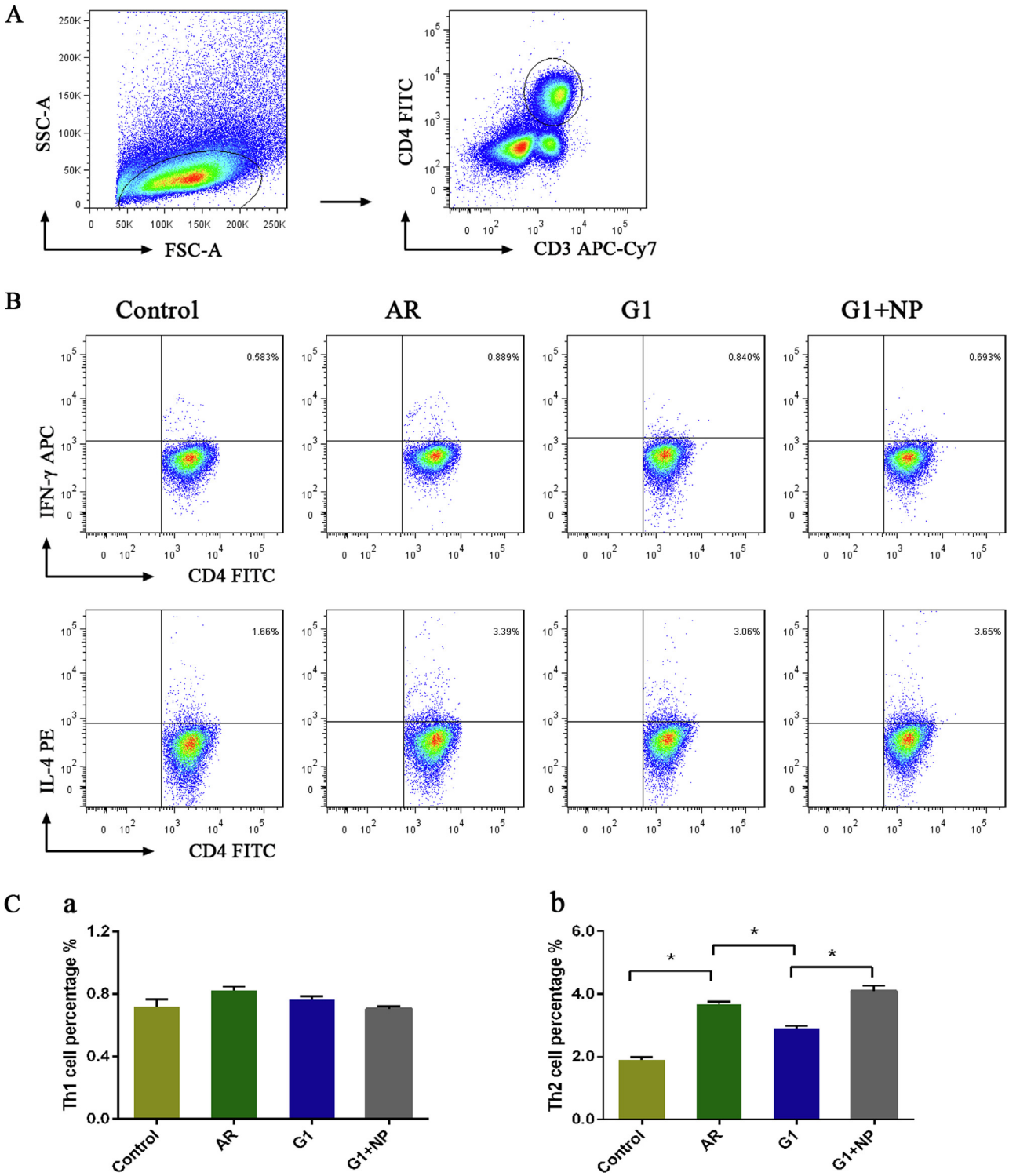

Fig. 5. Th1 and Th2 cell proportions as revealed by flow cytometry of $CD3^{+}CD4^{+}$ T cells (A). Representative Th1 and Th2 staining data for all groups (B); the statistics are shown in (C). $*P < 0.05$.

significantly decreased, but that of IFN-γ did not change. G-1 significantly increased Treg cells proportion and the specific cytokine IL-10 and Foxp3 transcription factor. These results are the first to comprehensively confirm the therapeutic effects of G-1 on AR. The data are consistent with those of Prabhushankar [4], confirming our preliminary hypothesis. NP is found in drinking water, food, and air, and may be absorbed by the skin and transmitted to a fetus or an infant via placental absorption and breastfeeding [19,20]. NP is lipophilic and easily bioaccumulated in adipose tissue, and especially after long-term contact, it may regulate immune cell actions *in vivo* [21].

We detected GPER expression in nasal mucosa and spleen of Balb/c mice, which was decreased in both nasal mucosa and spleen of AR Balb/c mice. These results are consistent with the therapeutic effect of G-1 on AR. Both GPER and G-1 have positive effects in treatment of allergic disease. Additional detailed studies of GPER are required, including the determination of its specific localization in eosinophils,

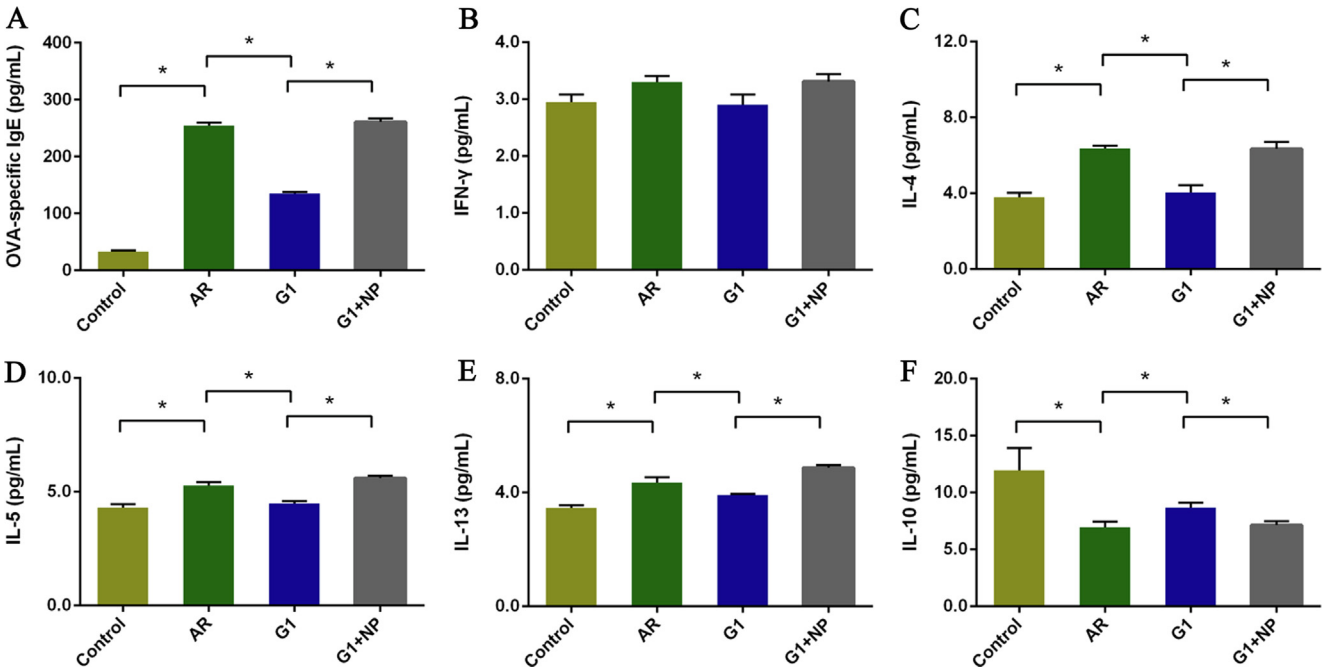

Fig. 6. Serum OVA-specific immunoglobulin levels (A). Cytokine levels as measured using the cytometric bead array (CBA) assay in nasal mucosae. The levels of IFN-γ and of IL-4, IL-5, IL-13, and IL-10 were measured (B–F, respectively). *$P < 0.05$.

mast cells, and Th subgroups.

We previously [22] found that NP affected the immune response of significant T cell subsets of AR mice, particularly the classic Th2 response to AR, and significantly inhibited the Treg immune response. Here, we report for the first time that the GPER-specific agonist G-1 plays the important therapeutic role in AR murine model. We confirmed that NP modulated the G-1-mediated effects on Th2 and Treg cells. It affected the proportions of such cells; the levels of cytokines; and the levels of specific transcription factors. Therefore, we speculate that NP may completely or partially block the therapeutic effects of G-1.

NP is very similar to estrogen in structure, and binds to the estrogen receptor to have a weak estrogenic effect [23]. Thus, NP may block the therapeutic effects of G-1 via competitive binding to GPER, but the details require further study. Similarly, the environmental hormone BPA interferes with the myocardial protective effects of estrogen in a guinea pig model of ischemia-reperfusion injury [15].

When EEDs bind to the estrogen receptor, cellular apoptosis and oxidation are triggered by both genomic and non-genomic signals. In skull osteoblasts, NP inhibits estrogen action by increasing proteins ERα and ERβ expression [24]. GPER mediates the effects of NP on myocardial contraction and iCa-l level. Thus, GPER involvement may explain non-monotonic effects of NP [25]. In a previous study, a BPA/estradiol combination enhanced signal transduction through ERβ receptor-mediated pathway more so than through ERα pathway, perhaps associated with more rapid development of arrhythmia [26]. However, the mechanism by which NP compromises the effects of G-1 in the AR mouse model remains unclear. The environmental hormone NP may compete with G-1 binding to GPER, thus compromising G-1 action. Although the levels of environmental hormones in the environment are much lower than the experimental concentrations in this study, many endocrine disruptors are present in the environment and these agents may interact with each other. However, the synergistic effects of various environmental hormones remain unclear. More studies on estrogen mixtures are required. The potential health hazards of mixtures are difficult to assess, as many chemical mechanisms may be involved [27]. It is important to avoid the harmful effects of environmental hormones and provide appropriate treatment. However, this study has some limitations. Further study of the localization of GPER and evaluation of the transcription factor levels in Th subgroups is required.

In conclusion, we found that the GPER-specific agonist G-1 attenuated Th2 immune response and enhanced Treg response in AR mice. NP compromised the therapeutic effects of G-1. These findings increase our understanding of etiology and mechanism of action of AR, and will be useful when developing targeted therapies and interventions for AR.

5. Authors' contributions

YW, ZG, and LH conceived and designed the experiments. ZG and

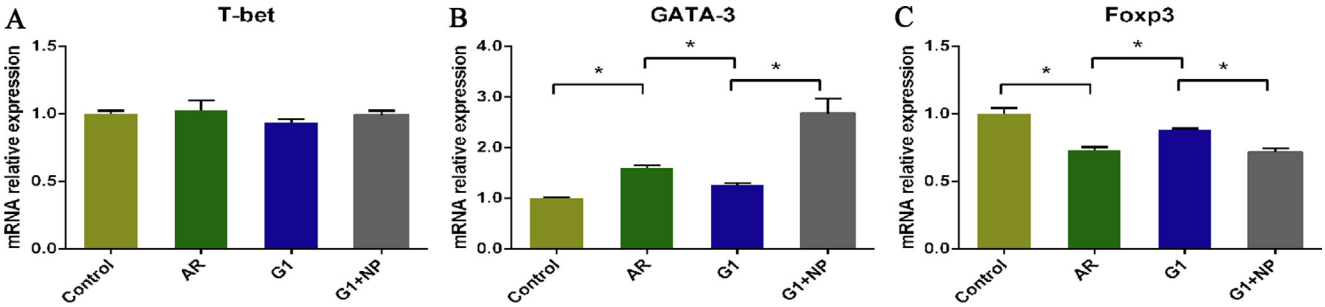

Fig. 7. Transcription factor expression levels measured via real-time polymerase chain reaction in nasal mucosae. The levels of mRNAs encoding T-bet, GATA-3, and Foxp3 were measured (A–C, respectively). *$P < 0.05$.

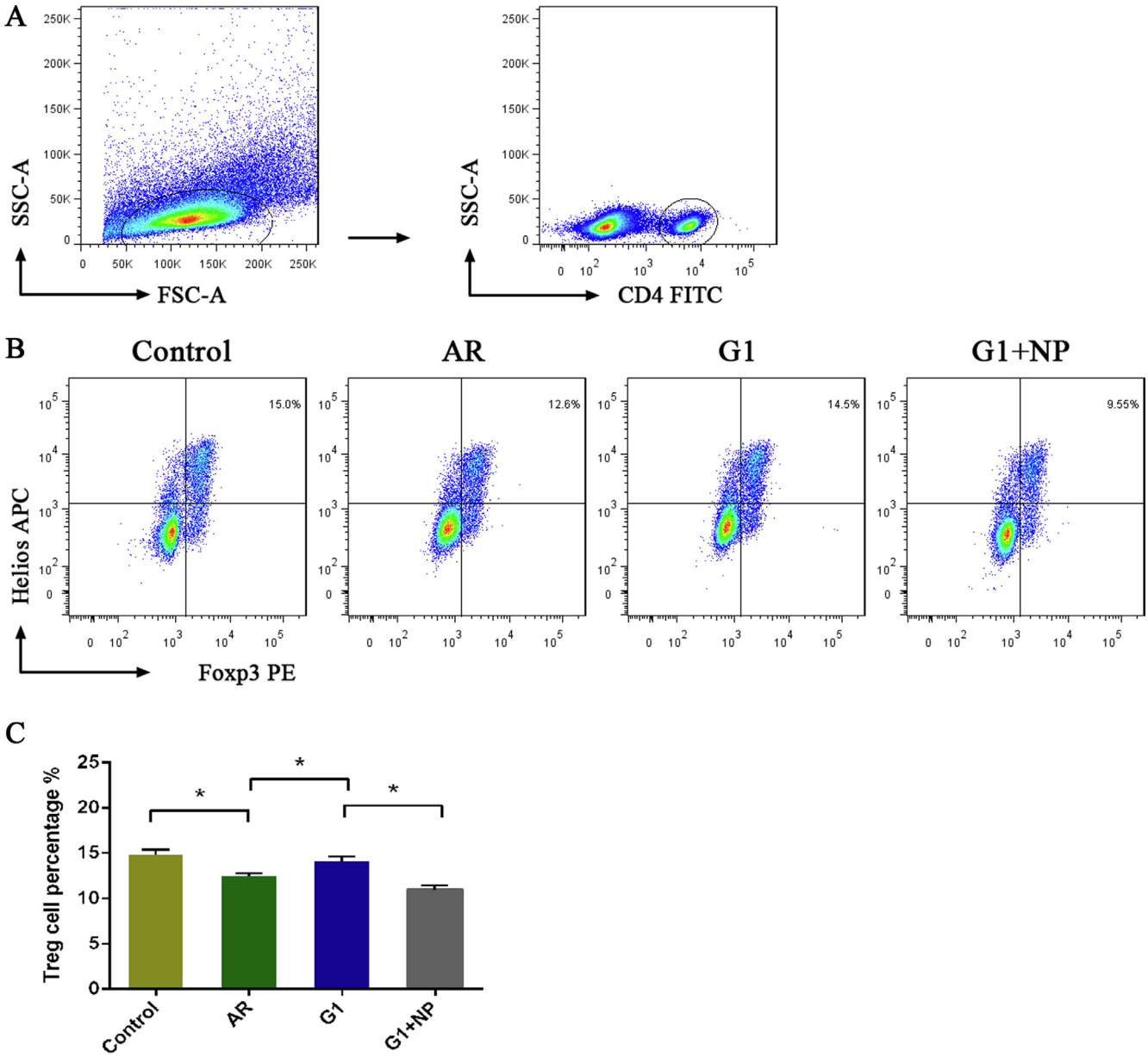

Fig. 8. $CD4^+$ $Helios^+Foxp3^+$ T cell levels in all groups of mice as assessed by flow cytometry. $CD4^+$ T cell subgroup (A). Representative staining of $CD4^+Helios^+Foxp3^+$ T cells of each group (B); the statistics are shown in (C). $*P < 0.05$.

YW performed the experiments. ZG and YW wrote the paper. The authors have read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (Grant No. 21677030) and the Liaoning Province Ministry of Education Scientific Study Project (Grant No. JC2019010).

References

[1] S.C. Hewitt, W. Winuthayanon, K.S. Korach, What's new in estrogen receptor action in the female reproductive tract, J. Mol. Endocrinol. 56 (2) (2016) R55–R71.
[2] R.D. Feldman, R. Gros, Unraveling the mechanisms underlying the rapid vascular effects of steroids: sorting out the receptors and the pathways, Br. J. Pharmacol. 163 (6) (2011) 1163–1169.
[3] R. Gros, Q. Ding, B. Liu, J. Chorazyczewski, R.D. Feldman, Aldosterone mediates its rapid effects in vascular endothelial cells through GPER activation, Am. J. Physiol. Cell Physiol. 304 (6) (2013) C532–C540.
[4] M. Itoga, Y. Konno, Y. Moritoki, Y. Saito, W. Ito, M. Tamaki, et al., G-Protein-coupled estrogen receptor agonist suppresses airway inflammation in a mouse model of asthma through IL-10, PLoS One 10 (3) (2015) e0123210.
[5] V. Pelekanou, M. Kampa, F. Kiagiadaki, A. Deli, P. Theodoropoulos, G. Agrogiannis, et al., Estrogen anti-inflammatory activity on human monocytes is mediated through cross-talk between estrogen receptor ERα36 and GPR30/GPER1, Leukoc. Biol. 99 (2) (2016) 333–347.
[6] E. Blasko, C.A. Haskell, S. Leung, G. Gualtieri, M. Halks-Miller, M. Mahmoudi, et al., Beneficial role of the GPR30 agonist G-1 in an animal model of multiple sclerosis, J. Neuroimmunol. 214 (1–2) (2009) 67–77.
[7] I. Cabas, M.C. Rodenas, E. Abellán, J. Meseguer, V. Mulero, A. García-Ayala, Estrogen signaling through the G protein-coupled estrogen receptor regulates granulocyte activation in fish, J. Immunol. 191 (9) (2013) 4628–4639.
[8] J. Bousquet, N. Khaltaev, A.A. Cruz, J. Denburg, W.J. Fokkens, A. Togias, et al., Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen), Allergy 63 (Suppl. 86) (2008) 8–160.

[9] J. Knez, Endocrine-disrupting chemicals and male reproductive health, Reprod. BioMed. Online 26 (5) (2013) 440–448.
[10] R. Jubendradass, S.C. D'Cruz, S.J. Rani, P.P. Mathur, Nonylphenol induces apoptosis via mitochondria- and Fas-L-mediated pathways in the liver of adult male rat, Regul. Toxicol. Pharm. 62 (3) (2012) 405–411.
[11] C.J. Hao, X.J. Cheng, H.F. Xia, X. Ma, The endocrine disruptor 4-nonylphenol promotes adipocyte differentiation and induces obesity in mice, Cell. Physiol. Biochem. 30 (2) (2012) 382–394.
[12] M.J. Lopez-Espinosa, C. Freire, J.P. Arrebola, N. Navea, J. Taoufiki, M.F. Fernandez, et al., Nonylphenol and octylphenol in adipose tissue of women in Southern Spain, Chemosphere 76 (6) (2009) 847–852.
[13] J.L. Suen, C.H. Hung, H.S. Yu, S.K. Huang, Alkylphenols—potential modulators of the allergic response, Kaohsiung J. Med. Sci. 28 (7 Suppl) (2012) S43–S48.
[14] A.M. Terrell, P.R. Crisostomo, T.A. Markel, M. Wang, A.M. Abarbanell, J.L. Herrmann, et al., Postischemic infusion of 17-beta-estradiol protects myocardial function and viability, J. Surg. Res. 146 (2) (2008) 218–224.
[15] N. Mao, Q. Gao, H. Hu, T. Zhu, L. Hao, BPA disrupts the cardioprotection by 17β-oestradiol against ischemia/reperfusion injury in isolated guinea pig hearts, Steroids 146 (2019) 50–56.
[16] M. Tamaki, Y. Konno, Y. Kobayashi, M. Takeda, M. Itoga, Y. Moritoki, et al., Expression and functional roles of G-protein-coupled estrogen receptor (GPER) in human eosinophils, Immunol. Lett. 160 (1) (2014) 72–78.
[17] S. Liang, Z. Chen, G. Jiang, Y. Zhou, Q. Liu, Q. Su, et al., Activation of GPER suppresses migration and angiogenesis of triple negative breast cancer via inhibition of NF-κB/IL-6 signals, Cancer Lett. 386 (2017) 12–23.
[18] A.M. Deschamps, E. Murphy, Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats, Am. J. Physiol. Heart Circ. Physiol. 297 (5) (2009) H1806–H1813.
[19] K. Guenther, V. Heinke, B. Thiele, E. Kleist, H. Prast, T. Raecker, Endocrine disrupting nonylphenols are ubiquitous in food, Environ. Sci. Technol. 36 (8) (2002) 1676–1680.
[20] Y.F. Huang, P.W. Wang, L.W. Huang, W. Yang, C.J. Yu, S.H. Yang, et al., Nonylphenol in pregnant women and their matching fetuses: placental transfer and potential risks of infants, Environ. Res. 134 (2014) 143–148.
[21] J.L. Suen, S.H. Hsu, C.H. Hung, Y.S. Chao, C.L. Lee, C.Y. Lin, et al., A common environmental pollutant, 4-nonylphenol, promotes allergic lung inflammation in a murine model of asthma, Allergy 68 (6) (2013) 780–787.
[22] Y.X. Wang, Z.W. Gu, Z.W. Cao, L.Y. Hao, Nonylphenol can aggravate allergic rhinitis in a murine model by regulating important Th cell subtypes and their associated cytokines, Int. Immunopharmacol. 70 (2019) 260–267.
[23] M. Verderame, E. Limatola, R. Scudiero, Estrogenic contamination by manure fertilizer in organic farming: a case study with the lizard Podarcis sicula, Ecotoxicology 25 (1) (2016) 105–114.
[24] M.G. Sabbieti, D. Agas, F. Palermo, G. Mosconi, G. Santoni, C. Amantini, et al., 4-nonylphenol triggers apoptosis and affects 17-β-estradiol receptors in calvarial osteoblasts, Toxicology 290 (2–3) (2011) 334–341.
[25] Q. Gao, S. Liu, F. Guo, S. Liu, X. Yu, H. Hu, et al., Nonylphenol affects myocardial contractility and L-type Ca(2+) channel currents in a non-monotonic manner via G protein-coupled receptor 30, Toxicology 334 (2015) 122–129.
[26] S.M. Belcher, Y. Chen, S. Yan, H.S. Wang, Rapid estrogen receptor-mediated mechanisms determine the sexually dimorphic sensitivity of ventricular myocytes to 17β-estradiol and the environmental endocrine disruptor bisphenol A, Endocrinology 153 (2) (2012) 712–720.
[27] R. Viñas, C.S. Watson, Mixtures of xenoestrogens disrupt estradiol-induced nongenomic signaling and downstream functions in pituitary cells, Environ. Health 12 (2013) 26.