



Obaculactone exerts a novel ameliorating effect on contact dermatitis through regulating T lymphocyte



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ARTICLE INFO

Article history:

Received 6 April 2015

Received in revised form 6 May 2015

Accepted 9 May 2015

Available online 21 May 2015

Keywords:

Obaculactone

Allergic contact dermatitis

T lymphocyte

Cell cycle

Apoptosis

ABSTRACT

It is still deficient that an immunosuppressant with a negligible toxicity for patients suffering from contact hypersensitivity. In the present study, we identified a natural occurring compound named obaculactone that effectively alleviated the macroscopic and microscopic appearances of the contact hypersensitivity, while it scarcely possessed toxic effect on mice at 5–20 mg/kg. The mRNAs of IL-2, IL-17a, TNF- α and IFN- γ expressed in lymph nodes of mice with dermatitis were also decreased by obaculactone in a dose-dependent manner. Moreover, the hypersensitivity couldn't be adoptively transferred from obaculactone-treated donor mice to normal mice. *In vitro* study, proliferation arrest in activated hapten-specific T cells and anti-CD3/CD28 stimulated T cells were observed in obaculactone-treated groups. In addition, the enhanced expressions of CD25 and CD69 in activated T cells were reduced by obaculactone. Meanwhile, obaculactone caused G0/G1 phase arrest and up-regulated the levels of cleaved-caspases and cleaved-PARP inducing apoptosis in activated T cells. Taken together, suppressing cell growth in activated T lymphocytes may contribute to the novel ameliorating effect for obaculactone against the contact hypersensitivity.

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1. Introduction

Contact hypersensitivity (CHS) is an extensively used animal model for T cell-mediated allergic contact dermatitis [1–4]. It is a delayed-type hypersensitivity response elicited by application of low molecular weight chemicals in the skin previously sensitized with the same hapten [5]. Cell proliferation and activation in T lymphocytes exert a pivotal role in this acute cutaneous inflammatory reaction [6–8]. Apart from the contact hypersensitivity, T cells also play a vital role in many other autoimmune-related diseases such as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis [9–11]. Dexamethasone is one of the clinical corticosteroids, which is usually used to counteract dermatitis [12,13]. However, side effects, including weight loss, stomach upset, vomiting, insomnia and headache, may be induced, if dexamethasone is given for a few days [12]. Therefore, there is an urgent need for novel effective natural agents with low toxicity to minimize the risk from side effects.

After screening of multiple compounds, we found that obaculactone (7, 16-dioxo-7, 16-dideoxylimondiol) had a potent immunomodulatory activity. Obaculactone is a triterpenoid dilactone extracted from citrus fruit [14]. In this study, we investigated the effect of obaculactone on

picryl chloride (PCI)-induced contact hypersensitivity and explored its regulatory role on T lymphocytes. The underlying mechanisms that involved proliferation, activation and apoptosis in T lymphocytes were investigated in this study.

2. Materials and methods

2.1. Mice

Specific pathogen-free, six- to eight-week-old female BALB/c mice were purchased from the Experimental Animal Center of Yangzhou University (Yangzhou, China). They were maintained with free access to pellet food and water in plastic cages at 21 ± 2 °C and kept on a 12 h light–dark cycle. All the animal experiments were approved by Nanjing University Animal Care and Use Committee (NJU-ACUC) and made to minimize suffering (sacrificed under anesthesia) and to reduce the number of animals used. Animal welfare and experimental procedures were carried out strictly in accordance with the Guide for the Care and Use of Laboratory Animals (The Ministry of Science and Technology of China, 2006) and the related ethical regulations of our university.

2.2. Cells and reagents

Dexamethasone was from Sanhome Pharmaceutical Co., Ltd. (Nanjing, China). Obaculactone (7,16-dioxo-7,16-dideoxylimondiol) and picryl

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chloride (PCI) were purchased from Sigma (St. Louis, MO). BALB/c mice CD3⁺ T cells from lymph nodes were purified using the Pan T cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) via magnetic cell separation with more than 98% purity. Purified anti-mouse CD28 (37.51) and purified anti-mouse CD3 (145-2C11) were purchased from BD PharMingen (San Diego, CA). Annexin V-FITC/PI kit was purchased from BD Biosciences (San Jose, CA). Anti-actin, anti-cleaved caspase 3, anti-PARP, anti-cleaved caspase 9 and anti-cleaved caspase 12 were from Cell Signaling Technology (Boston, MA). TRIzol reagent and DNase I were purchased from Invitrogen Life technologies. Toluidine blue staining assay was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Other reagents and solvents are all from Sigma (St. Louis, MO).

2.3. Picryl chloride (PCI)-induced contact hypersensitivity

On the first day (day 0), female BALB/c mice were shaved and sensitized by painting 0.1 mL of 1% PCI in absolute ethanol on their naked abdomen skin. Five days after sensitization (day 5), these mice were challenged on their left ears with 30 μ L of 1% PCI in olive oil. Ears' swelling was evaluated by the difference in thickness between the left and the right, as measured with a digimatic micrometer 20 h after challenge. Mice in normal group were normally sensitized and challenged with olive oil without PCI. Obaculactone (5, 10, 20 mg/kg, intragastrically) or dexamethasone (1 mg/kg, intramuscularly) was administered once a day from day 0 to day 5. Mice in the normal group and model group were given saline instead of drugs.

2.4. Histologic analysis

Histological assessment was performed as described before [4]. Formalin-fixed, paraffin-embedded ear tissue was sectioned at 5 μ m in thickness, and the sections were stained with hematoxylin and eosin. The histological scores were evaluated from 0 to 4 to assess edema, congestion, and inflammatory cell infiltration. Final data are the average scores of each group, and the higher score indicates more serious inflammation.

2.5. Quantification of mRNA expression by means of real-time PCR

Lymph node cells were isolated and collected from mice. In some cases these cells were purified to T lymphocytes by mouse T-cell enrichment columns (R&D Systems, Minneapolis, MN). Total RNA was homogenized and extracted with TRIzol reagent. Then total RNA was treated by DNase I and subjected to quantitative PCR, which was performed with the BioRad CFX96 Touch™ Real-Time PCR Detection System (BioRad, CA) using iQTM SYBR® Green Supermix (BioRad, CA), and threshold cycle numbers were obtained using BioRad CFX Manager software. The primer sequences used in this study were as follows: actin, forward 5'-ACATCTGCTGGAAGGTGGAC and reverse 5'-GGTACCACCATGTACCCAGG; IL-2, forward 5'-CTACAGCGGAAGCACAGC, reverse 5'-TCCTCAGAAAGTCCACCA; IFN- γ , forward 5'-CTTCTTCAGCAACAGCAAGGCGAAAA, reverse 5'-CCCCCA GATACAACCCCGCAATCA; TNF- α , forward 5'-CATCTTCTCAAAATTCGAGTGACAA, reverse 5'-TGGGAGTAGACAAGGTACAACCC; IL-17a, forward TCGAGAAGATGCTGCTGGT, reverse CTCTGTTTAGGCTGCC TGGC. Amplification included 1 stage of 5 min at 94 °C followed by 40 cycles of a 3-step loop: 5 s at 94 °C, 10 s at 58 °C, and 10 s at 72 °C. Relative message RNA (mRNA) expression was calculated as a ratio to actin.

2.6. Adoptive transfer of contact hypersensitivity

This work was performed as described before [15]. As described above, donor BALB/c mice were immunized with PCI. The donor mice were administered with saline alone or 20 mg/kg obaculactone once

at the time of challenge. After a six hour challenge, single-cell suspensions were made from the draining regional lymph nodes (axillary, inguinal and brachial), then adjusted cell number to 2.5×10^8 cells/mL in PBS. To adoptive transfer of contact hypersensitivity, the suspensions (200 μ L) were intravenously injected into each recipient mouse, followed by challenge in the ear with 30 μ L of 1% PCI. Ear's swelling of the recipient mice was measured after 16, 20 and 24 h since challenge.

2.7. Hapten-specific T cell proliferation

This work was performed as described before [16]. Cells were incubated in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 μ g/mL of streptomycin and 100 U/mL of penicillin under a humidified 5% (v/v) CO₂ atmosphere at 37 °C. Splenocytes, isolated from sensitized BALB/c mice, were treated with 25 mg/mL mitomycin C and 1.0 mM TNBS (water-soluble analog of picryl chloride), then cells were incubated at 37 °C for 30 min. After being washed 3 times in HBSS (containing 0.6% glycylglycine), these trinitrophenylated splenocytes were used as stimulator cells. Meanwhile, lymph node cells (the adherent cells had been removed) from the above described sensitized mice were used as responder cells. The 4×10^5 stimulator cells and 2×10^5 responder cells were co-cultured for 72 h. Then cell proliferation was examined using MTT assay.

2.8. Measurement of cell proliferation

Mouse lymphocytes were isolated from lymph nodes. T cells were purified via magnetic cell separation according to the standard procedures. Purified cells (purity was higher than 97%) were stimulated with 2 μ g/mL anti-CD3/CD28 and incubated in 96 well-plate at a density of 5×10^5 /mL cells per well in 0.2 mL RPMI 1640 medium supplemented with or without various concentrations of obaculactone for 72 h. The cell growth was measured by modified MTT assay. The stimulation index (SI) was calculated as follows: stimulation index = OD_{stimulated cells} / OD_{nonstimulated cells}.

2.9. Flow cytometric analysis

T lymphocytes were from lymph nodes of 4 days sensitized BALB/c mice. Cells were cultured at 37 °C and 5% CO₂ in the presence of 2 μ g/mL anti-CD3/CD28 and obaculactone (0.1, 1, 10, and 100 μ M). Then cells were harvested and washed twice with cold phosphate-buffered saline (PBS). After being incubated with 2 μ L anti-CD25 antibody or 2 μ L anti-CD69 antibody for 30 min on ice, cells were evaluated with flow cytometry.

2.10. Measurement of cell cycle

Cells were seeded in 12-well plates at a density of 1×10^6 cells/well in RPMI 1640 medium and treated with obaculactone (0.1, 1, 10 and 100 μ M) for 48 h in the presence of anti-CD3/CD28 (2 μ g/mL). Cell cycle was examined as we previously reported [4]. Briefly, cells were harvested and fixed in 75% ethanol at 4 °C overnight, and then stained with 50 μ g/mL of PI containing 1% Triton X-100 and 100 μ g/mL of RNase A in the dark at room temperature for 45 min. DNA contents of stained cells were analyzed with Modfit software (Becton Dickinson, San Jose, CA, USA).

2.11. Cell apoptosis assay

Cells were seeded in 12-well plates at a density of 1×10^6 cells/well in RPMI 1640 medium and treated with obaculactone (0.1, 1, 10 and 100 μ M) for 48 h in the presence of anti-CD3/CD28 (2 μ g/mL). The purified mouse T cells were harvested and washed twice with cold PBS, then suspended them in $1 \times$ binding buffer

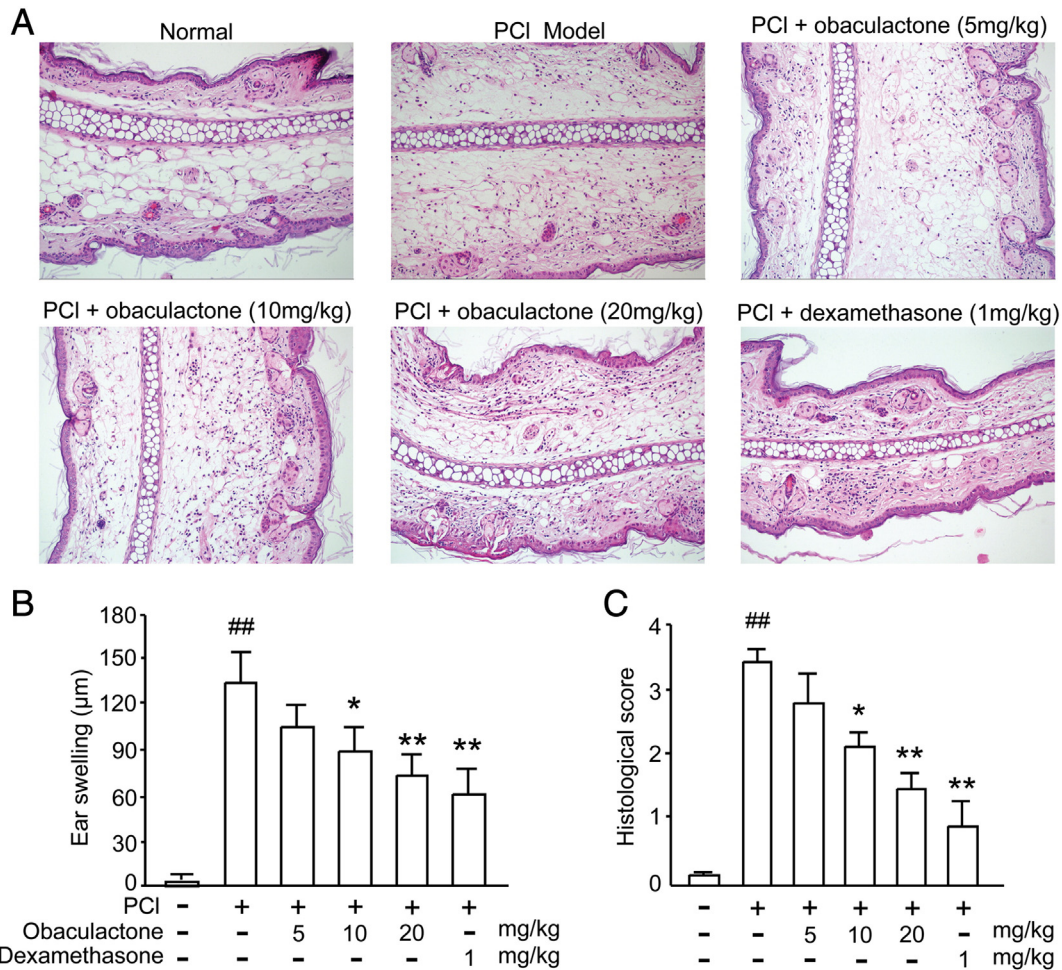


Fig. 1. Effects of obaculactone on the microscopic and macroscopic appearances of mice ears with contact hypersensitivity. Samples were taken as described in the Materials and methods section. (A) Hematoxylin and eosin stain (original magnification 200 $\times$). (B) The detection of ear swelling. (C) Histopathological scores. The data indicated the mean $\pm$ SEM. of experimental animals ($n = 8$). One-way ANOVA revealed a significant difference at $P < 0.01$. $^*P < 0.05$, $^{**}P < 0.01$, vs. Control; $^{##}P < 0.01$, vs. normal (Dunnett's test).

(0.5 mL). Incubation of cells with 2 μ L Annexin V-FITC and 2 μ L PI in the dark at room temperature for 20 min, then flow cytometric analysis was carried out. Data were analyzed using Cell Quest software.

2.12. Western blot

Cells were seeded in 6-well plates at a density of 1×10^7 cells/well in RPMI 1640 medium and treated with obaculactone (0.1, 1, 10 and

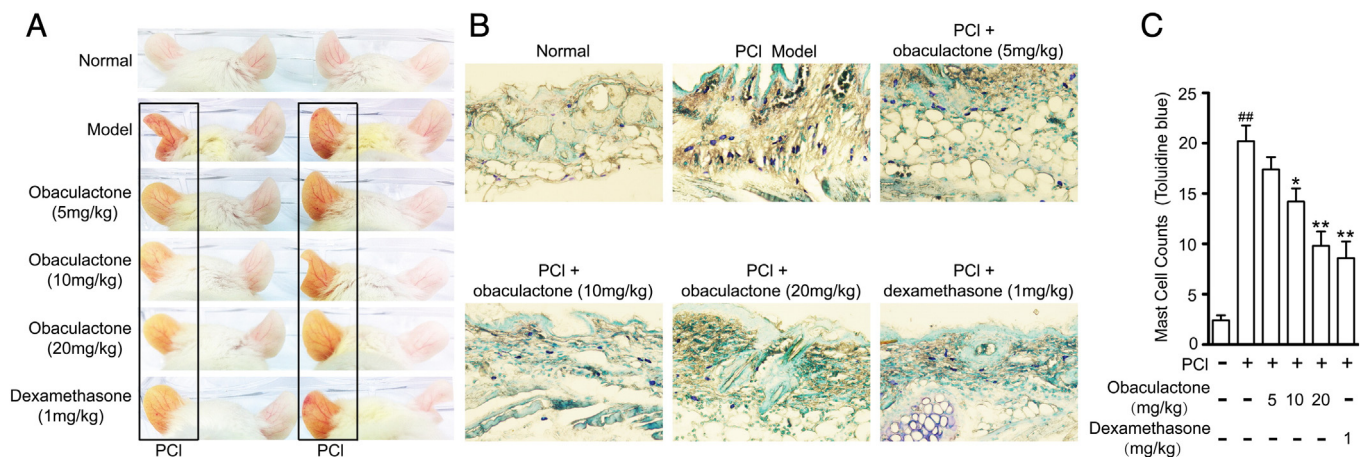


Fig. 2. Lesions and symptoms of mice with contact hypersensitivity and the mast cells staining in the ears tissue. (A) After being anesthetized, photos of mice ears were taken by a high-resolution camera. (B) The mast cells in ears tissue were stained by toluidine blue assay. (C) Quantification of the positive mast cells in each group, the data indicated the mean $\pm$ SEM. of per experimental group ($n = 8$). One-way ANOVA revealed a significant difference at $P < 0.01$. $^*P < 0.05$, $^{**}P < 0.01$, vs. Control; $^{##}P < 0.01$, vs. normal (Dunnett's test).

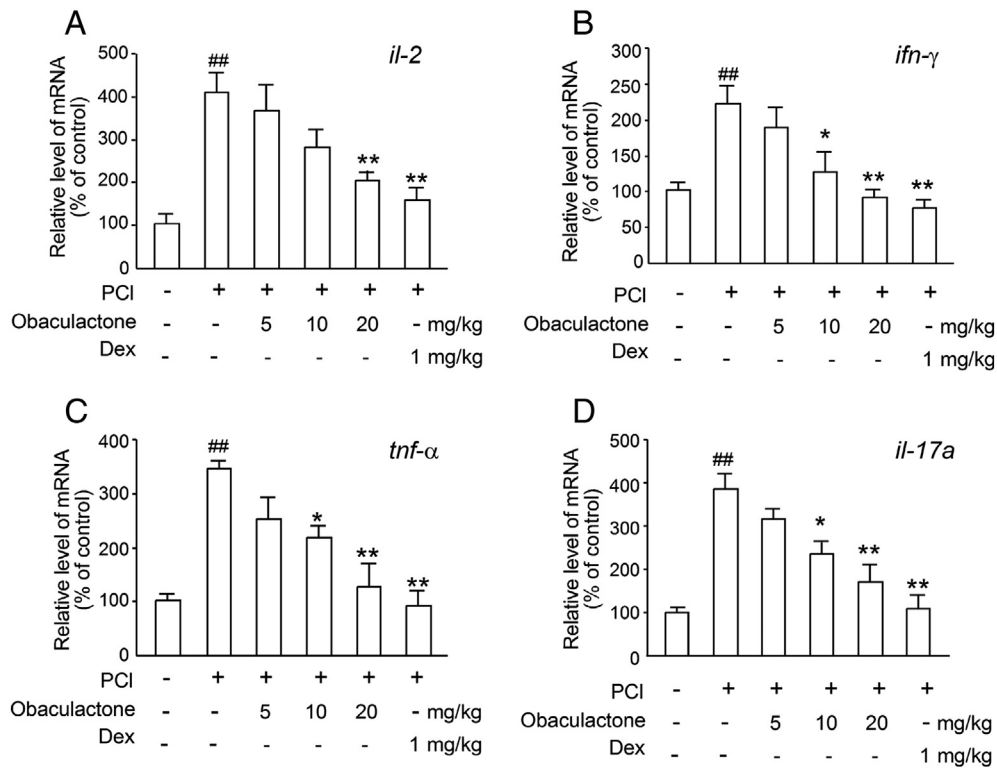


Fig. 3. Effects of obaculactone on the inflammatory cytokines mRNA expression in lymph nodes from mice with contact hypersensitivity. Messenger RNA levels of IL-2 (A), IFN- γ (B), TNF- α (C) and IL-17a (D) were determined by real-time PCR, respectively. One-way ANOVA revealed a significant difference at $P < 0.01$. * $P < 0.05$, ** $P < 0.01$, vs. control; ^{##} $P < 0.01$, vs. normal (Dunnett's test).

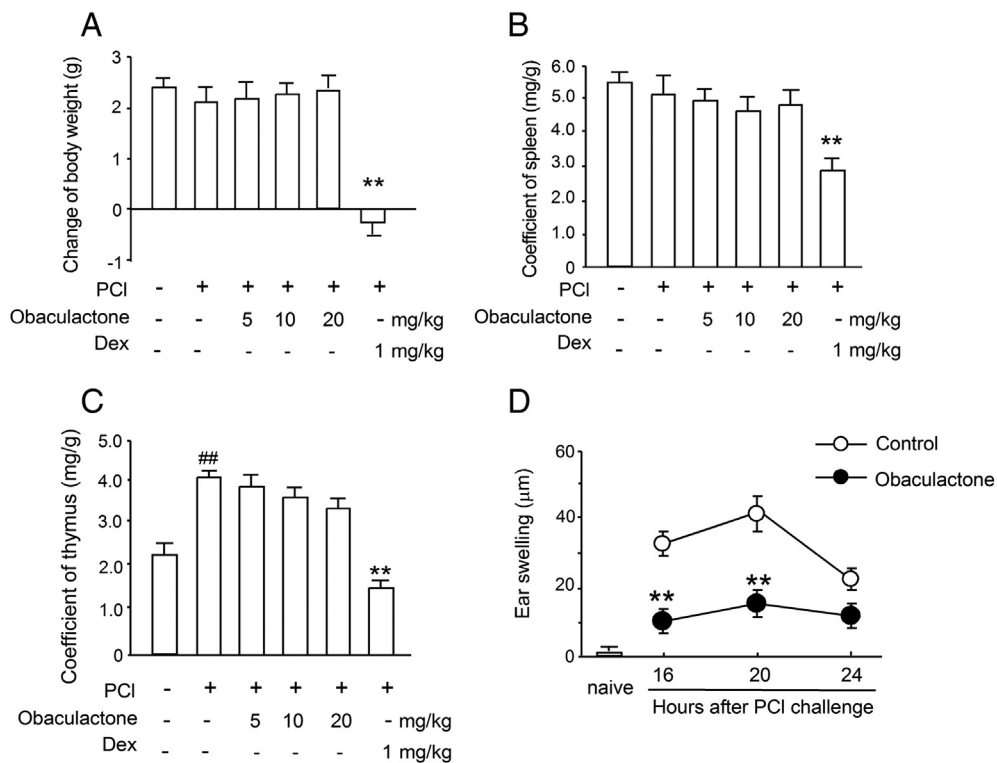


Fig. 4. Safety of obaculactone *in vivo* and its inhibitory effect on the ability of T lymphocytes to transfer contact hypersensitivity. Obaculactone (intragastrically) or dexamethasone (intramuscularly) were administered once a day for 6 days after sensitization. Each figure indicates the mean $\pm$ SEM. of 12 mice. Body weight (A), spleen coefficient (B) and thymus coefficient (C). Mice were orally administered obaculactone at challenge. Six hours later, 5×10^7 single-cell suspensions from the draining regional lymph nodes were collected and injected intravenously into each recipient mouse (D). One-way ANOVA revealed a significant difference at $P < 0.01$. * $P < 0.05$, ** $P < 0.01$, vs. control; ^{##} $P < 0.01$, vs. normal (Dunnett's test).

100 μ M) for 24 h in the presence of anti-CD3/CD28 (2 μ g/mL). Proteins from cultured cells were separated by SDS-PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes (Millipore, Bedford, MA). After being blocked in 5% nonfat milk at room temperature for 1 h, membranes were incubated with aimed primary antibodies at 4 °C overnight, and then with a horseradish-coupled secondary antibody at room temperature for 1.5 h. Final detection was performed using a LumiGLO chemiluminescent substrate system (KPL, Guildford, UK).

2.13. Mast cell histological analysis counts

The mast cell staining was performed by toluidine blue assay according to the manufacturer's instructions. The positive cells were assessed by counting five random fields per experimental group.

2.14. Statistical analysis

All results were expressed as mean $\pm$ S.E.M. of three independent experiments with each experiment including triplicate sets *in vitro*, or of ten animals per group *in vivo*. Comparisons between groups were evaluated using one-way analysis of variance (ANOVA) followed by

Dunnett's test. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Obaculactone improved contact hypersensitivity in mice

In order to elicit the allergic contact dermatitis, mice were sensitized with PCI on day 0 and challenged with the PCI on day 5. Histopathological analysis revealed that the ear sections of vehicle-treated mice still showed marked vascular congestion, moderate edema and severe inflammatory cell infiltration in the dermis and subcutaneous tissue. Compared with the vehicle group, these pathological reactions were much ameliorated after obaculactone treatment (Fig. 1A & C). In addition, the ear swelling also was dose-dependently relieved by administration of obaculactone to mice with the contact hypersensitivity (Fig. 1B).

As shown in Fig. 2A, the vasodilation and ear swelling were obviously observed in PCI-challenged ears, while these lesions and symptoms of contact dermatitis were ameliorated by obaculactone in a dose-dependent manner. Mast cell infiltration correlates with the severity of contact dermatitis, administration of obaculactone to mice could decrease the number of mast cell in ear tissue (Fig. 2B & C).

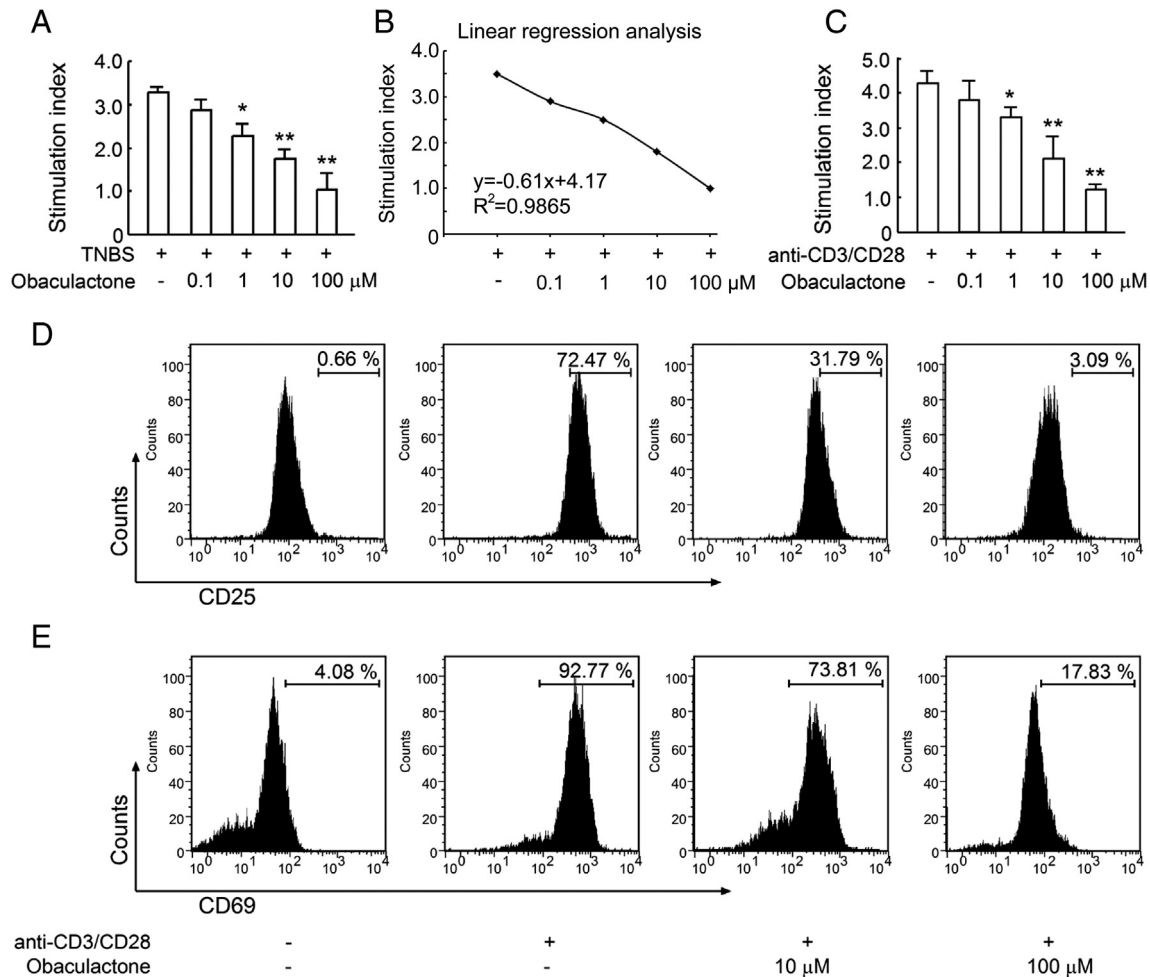


Fig. 5. Effect of obaculactone on T lymphocyte proliferation and activation. (A) The lymph node cells from sensitized mice were activated in an antigen-specific manner and proliferated *in vitro* by trinitrophenylated splenocytes. The stimulated hapten-specific T lymphocytes were treated with obaculactone (0.1, 1, 10, and 100 μ M). (B) Using linear regression to analyze the concentration-dependent effect of obaculactone on the hapten-specific lymphocyte proliferation. (C) Purified CD3⁺ T cells were incubated for 72 h at 37 °C under 5% CO₂ in the presence of anti-CD3/CD28 (2 μ g/mL), with or without 0.1–100 μ M obaculactone. Cell proliferation was measured by MTT. (D–E) Obaculactone inhibited T cells activation. CD3⁺ T cells were stimulated by the anti-CD3/CD28 (2 μ g/mL), and the expression of CD69 and CD25 was determined by flow cytometry. Results represent mean $\pm$ SEM. of three independent experiments. One-way ANOVA revealed a significant difference at *P* < 0.01. **P* < 0.05, ***P* < 0.01, vs. control; ****P* < 0.01, vs. normal (Dunnett's test).

3.2. Obaculactone reduced several cytokines expression in lymph nodes from mice with contact hypersensitivity

To analyze the anti-inflammatory effect of obaculactone against the contact hypersensitivity, mRNA expressions of IL-2, TNF- α , IL-17a and IFN- γ in lymph nodes were measured in parallel. As shown in Fig. 3, the enhanced mRNA levels of these inflammatory cytokines were dose-dependently reduced by obaculactone. IL-4 is a primary cytokine secreted by Th2 cells. However, obaculactone scarcely affected the mRNA expression of IL-4 in this study (data not shown).

3.3. Obaculactone could significantly block the adoptive transfer of contact hypersensitivity while it had a negligible toxicity for mice

Compared with the normal-control group, there were just very few changes in body weight, spleen coefficient and immune organ coefficients upon administration of 5–20 mg/kg obaculactone to mice (Fig. 4A–C). However, dexamethasone caused a significant reduction

in body weight, spleen coefficient and immune organ coefficient (Fig. 4A–C), although it showed a strong inhibition on ear swelling (Fig. 1).

To appraise whether T cells are the targets with which obaculactone could interfere, we applied the adoptive transfer experiment in our study. Lymph node cells from the vehicle-control group caused the contact hypersensitivity in recipient mice. Until 20 h later, the responses come up to the maximal level (Fig. 4D). However, lymph node cells from the obaculactone-treated mice were almost unable to induce contact hypersensitivity.

3.4. Obaculactone inhibited T lymphocytes activation and proliferation

Cell proliferation of hapten-specific T lymphocytes was examined, reflecting through stimulation index (SI). The SI was dose-dependently decreased by obaculactone (Fig. 5A and B). In addition, the SI also indicated that the amplification of anti-CD3/

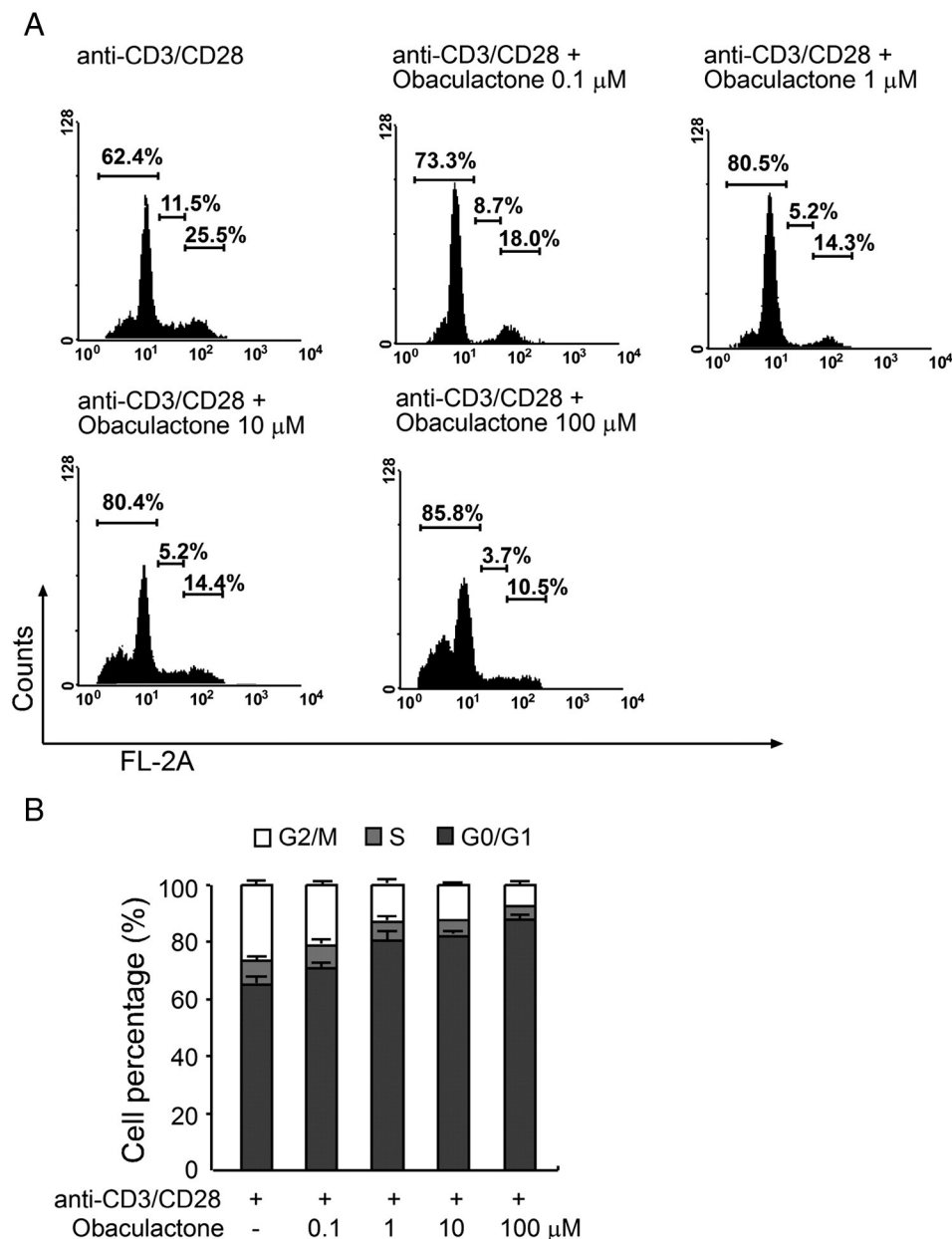


Fig. 6. Effects of obaculactone on cell cycle. (A) The purified T cells from lymph node were treated with obaculactone (0.1, 1, 10, and 100 μ M) for 48 h in the presence of anti-CD3/CD28 (2 μ g/mL), then cells were stained with PI for cell cycle distribution analysis with flow cytometry. (B) Data shown here were one of the three different experiments with similar results.

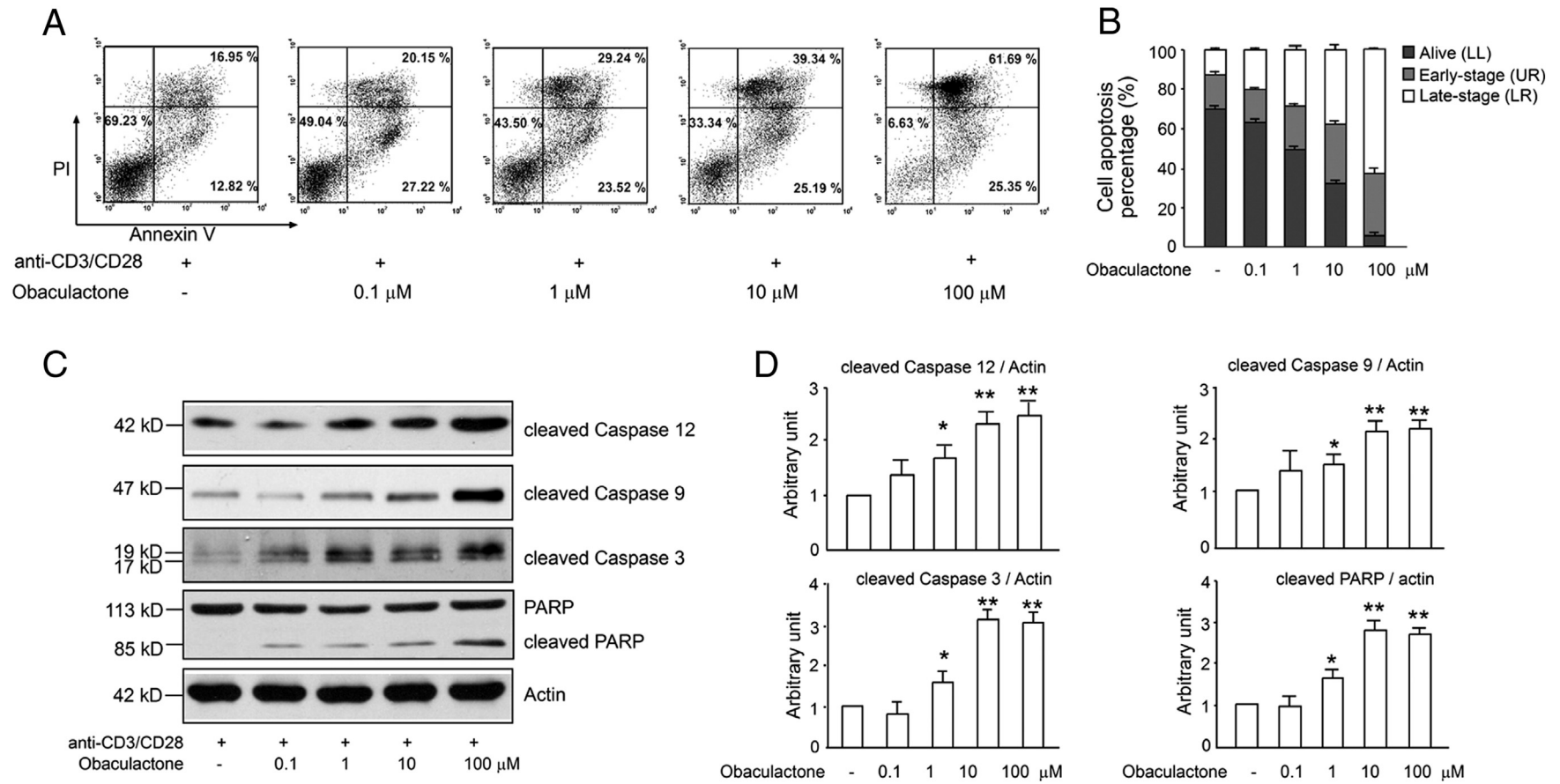


Fig. 7. Apoptosis of activated T lymphocytes induced by obaculactone. Cells were seeded in a 12-well plate and incubated with 0, 0.1, 1, 10 and 100 μM obaculactone for 48 h at the presence of anti-CD3/CD28 (2 μg/mL). (A) The apoptosis of cells was determined by Annexin V/PI staining. (B) Annexin V⁺/PI⁻ and Annexin V⁺/PI⁺ cells of three independent experiments were shown in column statistics. Representative Western blot bands (C) and data summary (D) of cleaved-caspase 3, 9, 12 and PARP in activated T cells after treated by obaculactone for 24 h. Data are expressed as a histogram of mean ± SEM. of three independent experiments. One-way ANOVA revealed a significant difference at $P < 0.01$. * $P < 0.05$, ** $P < 0.01$, vs. control (Dunnett's test).

CD28 activated T cells was obviously suppressed by obaculactone in a dose-dependent manner (Fig. 5C).

The enhanced surfaces, CD25 and CD69, are the hallmarks for anti-CD3/CD28 activated T cells. Flow cytometric analysis showed that the percentages of CD25⁺ T cells and CD69⁺ T cells were markedly reduced by obaculactone in a dose-dependent manner (Fig. 5D & E), suggesting that T cell activation was inhibited by obaculactone.

3.5. Obaculactone caused cell cycle arrest at G0/G1 phase in activated T lymphocytes

T cells were initially at the G0/G1 phase after isolated from lymph nodes. Stimulation of T cells with anti-CD3/CD28 resulting in the cell cycle progression comes into the S phase and G2/M phase. Obaculactone showed an efficient inhibition against this progression (Fig. 6).

3.6. Obaculactone induced apoptosis in activated T lymphocytes

Flow cytometric analysis with Annexin V and PI labeling was used to detect apoptosis in cells. Our results demonstrated that the anti-CD3/CD28 activated T cells underwent apoptosis in a dose-dependent manner when exposed to obaculactone (Fig. 7A & B).

Western blot was used to determine the potential mechanism of apoptosis in anti-CD3/CD28 activated T cells induced by obaculactone. After being incubated with various concentrations of obaculactone (0, 0.1, 1, 10, 100 μM) in the presence of anti-CD3/CD28 (2 μg/mL) for 48 h, the cleaved-caspases 3, 9, and 12 and cleaved-PARP in T cells were found to be dose-dependently increased by obaculactone (Fig. 7C & D).

4. Discussion

In this study, we examined the therapeutic effect of obaculactone on PCI-induced contact hypersensitivity. The results demonstrated that obaculactone could remarkably ameliorate PCI-induced ear swelling, and the pathological responses were much relieved as well (Figs. 1 and 2). IFN-γ and IL-2 are produced by type-1 T cells, which play crucial roles in PCI-induced contact hypersensitivity [15,17]. IL-2 is able to enhance the activation and proliferation of T cells [18], while IFN-γ is considered to stimulate macrophages [19]. In addition, the other important pro-inflammatory cytokines, such as TNF-α and IL-17a, are also involved in hypersensitivity responses [20–22]. Our data indicated that obaculactone could reduce the mRNA levels of IL-2, IFN-γ, TNF-α and IL-17a (Fig. 3). Importantly, the organ weight and body weight showed no marked changes compared with the normal controls (Fig. 4A–C) while mice after routine administration of dexamethasone therapy showed obvious immune organ atrophy and body weight loss (Fig. 4A–C), suggesting that obaculactone is a safe candidate immunosuppressant for the contact hypersensitivity.

Continuously, we confirmed that T cells were the major target of obaculactone in the therapy of the contact hypersensitivity. In addition, the contact hypersensitivity could be successfully transferred from saline-treated mice to naive BALB/c mice, whereas this adoptive transferred pathological reaction was blocked by obaculactone (Fig. 4D), revealing that obaculactone did play its therapeutic role through intervention with T lymphocytes.

T cell activation and proliferation were induced by an antigen involved in the inflammatory process of the contact hypersensitivity [23]. The proliferation of hapten-specific lymphocytes was decreased by obaculactone in a concentration-dependent manner (Fig. 5A & B), indicating that limitation of T cell amplification contributed to the therapeutic effect of obaculactone against the contact hypersensitivity. This conclusion was further confirmed by the inhibitory effect of obaculactone on the amplification of purified T lymphocytes (Fig. 5C).

CD69 plays a very important role in regulating immune functions, which was recognized as an early activation marker for stimulated T lymphocytes [24]. Expression of surface CD25 also is a hallmark of anti-CD3/CD28 activated T cells [4]. In this study, obaculactone inhibited the activation of T cells, since both of the CD25 and CD69 were down-regulated following the obaculactone treatment (Fig. 5D & E). In addition, further investigation suggested that obaculactone could cause G0/G1 phase arrest in activated T cells (Fig. 6).

Apoptosis is recognized as an important mechanism in counteraction of the excessive immune responses [25,26]. In our study, obaculactone triggered the apoptosis in activated T cells in a dose-dependent manner, which was proved by the increased apoptotic rate and enhanced pro-apoptotic proteins expression (Fig. 7). Cleaved-PARP serves as a marker of cells undergoing apoptosis, and the activated forms of Caspases 3, 9 and 12 and cleaved-caspases 3, 9 and 12 are all the key executioners of apoptosis [16]. Our results indicated that up-regulation of apoptosis through both intrinsic mitochondrion-dependent and endoplasmic reticulum stress-mediated apoptotic pathways in excessive activated T lymphocytes may contribute to the therapeutic effect of obaculactone against the contact hypersensitivity. Besides our reported effects of obaculactone on T lymphocytes, researchers have investigated the inhibitory roles of obaculactone in IgE production from human B cells [27] and NO production from lipopolysaccharide-activated RAW264.7 macrophages [28].

In summary, we explored a safe and effective ameliorating effect of obaculactone on PCI-induced contact hypersensitivity. The mechanism may involve multiple effects against the activated T cells: inhibition of inflammatory cytokines, suppression of cell activation and proliferation, causing cell cycle arrest and induction of apoptosis in activated T cells. Taken together, targeting activated T cells from multiple aspects by obaculactone may contribute to a novel therapy for T-cell-mediated autoimmune diseases.

Conflict of interest statement

The authors have declared no competing interests.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 81373466, 91313303, 91229109), the Natural Science Foundation of Jiangsu Province (BK20131282) and the Jiangsu Province Clinical Science and Technology Project (Clinical Research Center, BL2012008).

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