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**Original Paper**

# Anti-Psoriasis Effects and Mechanisms of A-(8-Quinolinoxy) Zinc Phthalocyanine-Mediated Photodynamic Therapy

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**Key Words**

A-(8-quinolinoxy) zinc phthalocyanine • Psoriasis • Photodynamic therapy

**Abstract**

***Background/Aims:*** The aim of this study was to determine the anti-psoriasis effects of α-(8-quinolinoxy) zinc phthalocyanine (ZnPc-F7)-mediated photodynamic therapy (PDT) and to reveal its mechanisms. ***Methods:*** HaCaT cells were used to observe the influence of ZnPc-F7-PDT on cell proliferation *in vitro*. The *in vivo* anti-psoriasis effects of ZnPc-F7-PDT were evaluated using a mouse vagina model, a propranolol-induced cavy psoriasis model and an imiquimod (IMQ)-induced nude mouse psoriasis model. Flow cytometry was carried out to determine T lymphocyte levels. Western blotting was performed to determine protein expression, and a reverse transcription-polymerase chain reaction test was performed to determine mRNA expression. ***Results:*** The results showed that ZnPc-F7-PDT significantly inhibited the proliferation of HaCaT cells *in vitro*; when the light doses were fixed, changing the irradiation time or output power had little influence on the inhibition rate. ZnPc-F7-PDT significantly inhibited the hyperproliferation of mouse vaginal epithelium induced by diethylstilbestrol and improved propranolol- and IMQ-induced psoriasis-like symptoms. ZnPc-F7-PDT inhibited IMQ-induced splenomegaly and T lymphocyte abnormalities. ZnPc-F7-PDT did not appear to change T lymphocytes in the mouse vagina model. ZnPc-F7-PDT down-regulated the expression of proliferating cell nuclear antigen (PCNA), B-cell lymphoma-2 (Bcl-2), interleukin (IL)-17A mRNA and IL-17F mRNA, and up-regulated the expression of Bax. ***Conclusion:*** In conclusion, ZnPc-F7-PDT exhibited therapeutic effects in psoriasis both *in vitro* and *in vivo* and is a potential approach in the treatment of psoriasis. Potential mechanisms of these effects included the inhibition of hyperproliferation; regulation of PCNA, Bcl-2, Bax, IL-17A mRNA and IL-17F mRNA expression; and immune regulation.



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## Introduction

Psoriasis is a chronic inflammatory disease affecting approximately 2% of the global population [1]. It is characterized by hyperproliferation, incomplete terminal differentiation of the epidermis, elongation and dilatation of capillaries in the papillary dermis and migration of activated neutrophils and T lymphocytes into the dermis and the epidermis. The main clinical manifestations of psoriasis are erythroderma, silvery scales, extensive pustule lesions and associated arthritis [2]. Psoriatic lesions are generally visible, leading to considerable emotional and social disabilities for patients; severe psoriasis is associated with an increased risk of mortality. In addition, the combined costs of long-term therapy and reduced income caused by psoriasis decrease patients' quality of life [3]. Currently available treatments for psoriasis are topical agents, systemic agents and phototherapy [4]. However, there is still no cure for this skin disorder, and all existing therapies have distinct limitations. For example, Psoralen Ultraviolet A (PUVA) is a treatment which combines psoralen and exposure to Ultraviolet A (UVA). PUVA is considered an effective treatment for psoriasis [5]. However, side effects include nausea and headache from the ingested psoralen, skin erythema, burns, pigmentation and an increased risk of non-melanoma skin cancers from long-term UVA irradiation [6, 7]. Thus, the development of new therapeutic approaches is urgent and challenging.

Photodynamic therapy (PDT) is used to treat many types of disease such as cancers, psoriasis, nevus flammeus and maculopathy [8]. The history of generalized PDT started approximately 3500 years ago; ancient Egyptians and Indians used light-sensitive plants combined with sunlight to treat vitiligo [9]. However, modern PDT has only developed in the past 100 years [10]. Photofrin was the first photosensitizer approved worldwide for use in clinical treatment in 1993. In recent years, studies of PDT have focused on the treatment of non-tumor diseases such as psoriasis. Many photosensitizers have shown excellent therapeutic effects in treating psoriasis, and some are already undergoing clinical studies [11-16].

Side effects and uncertain therapeutic effects are considered to be the limitations of PDT in treating psoriasis [17, 18]. Therefore, a safer irradiation source and photosensitizers with definite efficacy and less toxicity are the main directions in PDT development. α-(8-Quinolinoxy) zinc phthalocyanine (ZnPc-F7) is a new type of metallophthalocyanine coordination compound with an explicit structure, good solubility, a strong effect and low toxicity. The specific excitation source of ZnPc-F7 is 670 nm light. This light can reach a deeper layer of skin and is safer than the UVA used in PUVA.

The aim of this study was to determine the anti-psoriasis effects and mechanisms of ZnPc-F7-PDT using Sinoporphyrin sodium (DVDMS, a new porphyrin derivative with confirmed anti-psoriasis effects which can be specifically excited by 630 nm light) as the positive control. The influence of ZnPc-F7-PDT on HaCaT cell proliferation *in vitro* was determined. The *in vivo* anti-psoriasis effects of ZnPc-F7-PDT were evaluated using a mouse vagina model, a propranolol-induced cavy psoriasis model and an imiquimod (IMQ)-induced nude mouse psoriasis model. Flow cytometry was carried out to determine T lymphocyte levels. Western blotting was performed to determine protein expression, and a reverse transcription-polymerase chain reaction test was performed to determine mRNA expression. The results showed that significant anti-psoriasis effects were observed both *in vitro* and *in vivo*, and the potential mechanisms of these effects included the inhibition of hyperproliferation; regulation of PCNA, Bcl-2, Bax, IL-17A mRNA and IL-17F mRNA expression; and immune regulation.

## Materials and Methods

*Animals*

ICR and Nu/Nu mice weighing 18–22 g were bought from Vital River Laboratories (Beijing, China). Dunkin Hartley cavies weighing 250–300 g were bought from Beijing Keyu Animal Breeding Center (Beijing,

China). All animals were housed in cages at constant temperature and humidity and a 12 h light/dark cycle. Animals had free access to food and water. The study was approved by the Institutional Animal Care and Use Committee of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. All operations were performed under the guidance of veterinarians according to international guidelines concerning the care and treatment of experimental animals.

*Instruments and reagents*

PDT-630 and PDT-670 semiconductor laser units were provided by Guilin Xingda Optoelectronic Medical Instrument Co., Ltd (Guangxi, China). ZnPc-F7 was provided by Beijing Guiqianjin Medical Technology Co., Ltd (Beijing, China); DVDMS was provided by Qing Long High Technology Co., Ltd. Antibodies were purchased from Sigma–Aldrich (USA). Primers for reverse transcription-polymerase chain reaction (RT-PCR) were synthesized by Invitrogen (USA). HaCaT cells were purchased from the Cell Resource Center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China).

*Experiment 1: Effects of ZnPc-F7-PDT on cell proliferation in vitro*

HaCaT cells were cultured in RPMI-1640 medium (HyClone, USA) containing 10% fetal bovine serum at 37°C with 5% $CO_2$. Cells ($2 \times 10^5$/mL) were inoculated in 96-well plates with a volume of 100 μL per well. ZnPc-F7 was added after 24 h, with final concentrations of 0.01, 0.03, 0.10, 0.30, 1.00 and 3.00 μg/mL, and after 6 h cultivation the cells were exposed to a semiconductor laser unit for 180 s with a 1.50 W output power, and the energy density was 5.37 J/cm$^2$. Cells treated with laser irradiation and ZnPc-F7 without PDT were included as controls. The MTT test was performed after additional 24 h cultivation. Absorbance values were measured using a microplate reader (ELX 800 UV; Dialab, Austria). The measurement wavelength was 545 nm, and the reference wavelength was 450 nm. To observe the influence of irradiation conditions, the spot diameter was set to 8 cm and the energy density was set to 5.37 J/cm$^2$. The output power and irradiation time were set to 1.50 W and 180 s, 1.00 W and 270 s, 0.50 W and 540 s or 0.30 W and 900 s, respectively.

*Experiment 2: Effects of ZnPc-F7-PDT on the hyperproliferation of mouse vaginal epithelium*

In total, 70 female ICR mice were included and 60 were injected intraperitoneally with 0.20 mL of diethylstilbestrol (2.00 mg/mL) once daily for 3 consecutive days. On the fourth day, the mice were divided into seven groups: control group (Con), model group (M), ZnPc-F7 without PDT group (F7, 1.20 mg/kg), DVDMS-PDT group (DP, DVDMS 2.00 mg/kg), low dose ZnPc-F7-PDT group (LFP, ZnPc-F7 0.30 mg/kg), middle dose ZnPc-F7-PDT group (MFP, ZnPc-F7 0.60 mg/kg) and high dose ZnPc-F7-PDT group (HFP, ZnPc-F7 1.20 mg/kg). Mice in the PDT groups were exposed to lasers (PDT-630 for DVDMS and PDT-670 for ZnPc-F7) 6 h after intravenous drug injection. The output power was 0.10 W and irradiation time was 300 s. A 48 h light-avoiding period was carried out followed by a routine light/dark cycle. The mice were injected intraperitoneally with 4 mg/kg colchicine (Urchem, China, No.61601582) on the seventh day after PDT. Peripheral blood was collected to determine T lymphocytes using flow cytometry (FCM, BD Biosciences, USA). $CD3^+$, $CD4^+$ and $CD8^+$ cells were marked by APC-Cy7-A, PE-Cy7-A and APC-A, respectively. The mice were then sacrificed with an overdose of 1% pentobarbital sodium. Vaginas were dissected and fixed in 10% formalin solution for histopathology. Hematoxylin–eosin (HE) staining was performed. A total of 300 basal keratinocytes were observed, and the mitotic rate was defined as the mitotic cell number in 100 basal keratinocytes.

*Experiment 3: Effects of ZnPc-F7-PDT on propranolol-induced psoriatic lesions in cavy*

Seventy of eighty Dunkin Hartley cavies (half male and female) were exposed to 5% propranolol liniment on both ears twice daily for 3 consecutive weeks. Three cavies were sacrificed with an overdose of 1% pentobarbital sodium at the end of each week and the degree of histopathology in the lesions was observed. On day 22, the cavies were divided into seven groups: control group (Con), model group (M), laser-control group (LC, irradiation only), ZnPc-F7 without PDT group (F7, 5% ZnPc-F7 liniment), DVDMS-PDT group (DP, DVDMS 0.75 mg/kg), low dose ZnPc-F7-PDT group (LFP, 1% ZnPc-F7 liniment) and high dose ZnPc-F7-PDT group (HFP, 5% ZnPc-F7 liniment). ZnPc-F7 liniment was prepared as follows: 0.95 mL polyoxyethylene castor oil was mixed with 25 mL N, N-dimethylacetamide, and 100 mg (for 1% liniment or 500 mg for 5% liniment) ZnPc-F7 was dissolved in 5 mL of the aforementioned mixture. Then, 500 mg

polyoxyethylene pyrrolidone 30, 0.25 mL azone and 0.25 mL propylene glycol were added sequentially, and 50% ethyl alcohol was added to adjust the total volume to 10 mL.

Twenty-four hours after drug administration, PDT was performed in the last three groups. The output power, spot diameter and irradiation time were set to 1.00 W, 6 cm and 400 s, respectively, and the energy density was 14.15 J/cm$^2$. A light-avoiding period was carried out for 48 h, and then a routine light/dark cycle was resumed. The second PDT was performed seven days later. All cavies were sacrificed with an overdose of 1% pentobarbital sodium with 7 days later, peripheral blood was collected to determine cytokines and reactive oxygen species using ELISA kits. Both ears were removed and fixed in 10% formalin solution for histopathology. A 3-D microscopic system (VHX-2000; Keyence, Japan) was used to observe and collect histopathology images. The thickness of the stratum spinosum was measured. Quantitative analysis of hyperkeratosis, abnormal stratum granulosum, irregular epidermal hyperplasia, clubbed papillary layer and hemangiectasis was performed using injury degree scores.

*Experiment 4: Effects of ZnPc-F7-PDT on IMQ-induced psoriatic lesions in Nu/Nu mice*

Seventy of eighty Nu/Nu mice (half male and female) were locally treated with 5% imiquimod (IMQ) cream on the dorsal skin once daily for 6 consecutive days. On the seventh day, these mice were divided into eight groups: control group (Con), model group (M), laser-control group (LC), ZnPc-F7 without PDT group (F7, ZnPc-F7 1.20 mg/kg), DVDMS-PDT group (DP, DVDMS 2.00 mg/kg), low dose ZnPc-F7-PDT (LFP, ZnPc-F7 0.30 mg/kg), middle dose ZnPc-F7-PDT (MFP, ZnPc-F7 0.60 mg/kg) and high dose ZnPc-F7-PDT (HFP, ZnPc-F7 1.20 mg/kg). All drugs were injected intravenously at the above doses. After 6 h, PDT was performed in the PDT groups. The output power, spot diameter and irradiation time were set to 0.10 W, 2 cm and 600 s, respectively, and the energy density was 19.10 J/cm$^2$. A light-avoiding period was continued for 48 h, and then the routine light/dark cycle was resumed. Peripheral blood was collected on the eighth day for determination of T lymphocytes using flow cytometry (FCM). The mice were then sacrificed with an overdose of 1% pentobarbital sodium. The dorsal skin was removed and divided into three parts. One part was fixed in 10% formalin solution for histopathology, and the other two parts were stored in liquid nitrogen for Western blotting (WB) and RT-PCR analysis. The sequences of primers for RT-PCR were as follows: (1) GAPDH (124 bp), sense: 5'- CCTCGTCCCGTAGACAAAATG-3', antisense: 5'-TGAAGGGGTCGTTG ATGGC-3'; (2) IL-17 A (262 bp), sense: 5'-CAGACTACCTCAACCGTTCCA-3', antisense: 5'-ACAATCGAGGCCACGCAGGTGCAGC-3'; (3) IL-17 F (161 bp), sense: 5'-TGCTACTGTTGATGTTGGGAC-3', antisense: 5'-AATGCCCTGGTTTTG GTTGAA-3'.

*Experiment 5: Mechanisms of ZnPc-F7-PDT in proliferation inhibition*

Western blotting was performed to observe the expression of proliferating cell nuclear antigen (PCNA), B-cell lymphoma-2 (Bcl-2) and Bax. The procedure was as follows: A piece of skin was cut into small pieces and washed with phosphate-buffered solution (PBS). Lysis buffer (ThermoFisher, USA) containing 1% phenyl methane sulfonyl fluoride (ThermoFisher) was added at 2 μL/mg. The mixture was incubated at 4°C for 30 min and was then centrifuged at 12, 000 *g* for 30 min. The supernatant was removed and placed in a centrifuge tube, and protein quantification was performed with a micro-spectrophotometer (Q5000; Quawell, USA). The protein concentration was adjusted to 15.00 μg/mL and was then mixed with loading buffer. The mixture was incubated at 98°C for 10 min and was loaded after cooling. The loading volume of samples was 10.00 μL, and 10.00 μL of prestained dual-color protein molecular weight marker (ThermoFisher) was used to indicate the locations of proteins. The voltage was adjusted from 80 V to 120 V when the samples moved into the separation gel from the spacer gel, and the transfer time was 90 min with a voltage of 120 V. Polyvinylidene fluoride membranes were used to transfer proteins with a voltage 120 V for 90 min. The membranes were then blocked for 1 h at room temperature. After washing with Tris-buffered saline containing Tween (3 times × 10 min), the membranes were incubated with primary antibodies (overnight at 4°C) and then secondary antibody (1 h at room temperature), and were detected using a chemiluminescence detector (Image Quant LAS 4000 mini; General Electric Company, USA). The gray values of the protein bands were measured with ImageJ software and were compared with that of β-actin.

*Experiment 6: Mechanisms of ZnPc-F7-PDT for alleviating the inflammatory reaction*

RT-PCR was used to observe the mRNA expression of interleukin (IL)-17 A and IL-17 F. The procedure was as follows: A piece of skin was cut into pieces and placed in an RNase-free tube. A 1000 μL volume of

TRIzol reagent (Invitrogen) and 200 μL chloroform were added successively. The mixture was held for 5 min at room temperature and then centrifuged for 15 min at 12, 000 *g* and 4°C. A 400 μL volume of supernatant was removed and placed in another RNase-free tube, and 400 μL isopropanol was added. The tube was held at room temperature for 10 min and was then centrifuged for 15 min at 12, 000 *g* and 4°C. The supernatant was removed, and 1000 μL precooled ethyl alcohol at a concentration of 95% was added. The tube was then centrifuged for 10 min at 12, 000 *g* and 4°C. The supernatant was removed, and 30 μL RNase-free water was added to dissolve the RNA. A micro-spectrophotometer was used to detect the concentration and purity of the RNA. RNA samples with OD260/OD280 values in the range of 1.8 to 2.2 were chosen for the next step. The cDNA was synthesized according to the RevertAid™ first-strand cDNA synthesis kit instructions (Fermentas, USA), and DNA was synthesized according to the Taq® green master mix kit instructions (Promega, Beijing, China). The volume of the reaction system was 25 μL. The gray values of the mRNA bands were measured with ImageJ software and were compared with that of GAPDH.

The levels of tumor necrosis factor-α (TNF-α), IL-1β, glutathione peroxidase (GSH-PX), superoxide dismutase (SOD) and malondialdehyde (MDA) in cavies' blood were determined with ELISA kits (Nanjing Jiancheng Bioengineering Institute, China; catalog numbers: TNF-α, 20151218; IL-1β, 20160106; GSH-PX, 20160803; SOD, 20160809; MDA, 20160910) according to the manufacturer's specifications.

*Statistics*

Data were expressed as the means ± SD and analyzed using the SPSS 17.0 software package. One-way ANOVA was used to compare inter-group differences. A value of $P < 0.05$ was considered statistically significant.

## Results

*ZnPc-F7-PDT inhibited the proliferation of HaCaT cells*

Laser irradiation (5.37 J/cm$^2$) without drugs did not inhibit the proliferation of HaCaT cells; however, ZnPc-F7 at the concentrations of 0.10, 0.30, 1.00 and 3.00 μg/mL showed dose-dependent inhibitory effects without PDT ($P < 0.05$, $P < 0.01$). The combined application of ZnPc-F7 and laser (ZnPc-F7-PDT) significantly improved the anti-proliferative effects at the concentrations of 0.10, 0.30 and 1.00 μg/mL ($P < 0.01$) (Fig. 1 A). Changing the output power and irradiation time had little influence on the inhibition rate when the spot diameter and energy density were fixed (Fig. 1 B).

*ZnPc-F7-PDT inhibited diethylstilbestrol-induced hyperproliferation of mouse vaginal epithelium*

Diethylstilbestrol induced hyperproliferation of mouse vaginal epithelium (Fig. 2 A). The mitotic rate in the model group was significantly higher ($P < 0.01$) than that in the control group; middle and high doses of ZnPc-F7-PDT as well as DVDMS-PDT significantly inhibited the hyperproliferation of mouse vaginal epithelium ($P < 0.05$, $P < 0.01$); ZnPc-F7 (1.2 mg/kg) without laser irradiation did not significantly reduce the mitotic rate (Fig. 2 B). Diethylstilbestrol did not significantly influence $CD3^+$, $CD4^+$ and $CD8^+$ or $CD4^+/CD8^+$ levels, and these levels were not significantly different in all groups (Fig. 2 C).

*ZnPc-F7-PDT improved propranolol-induced psoriatic lesions in cavies*

Local application of 5% propranolol for 3 weeks induced psoriatic lesions on the ears of cavies. The changes were obvious in appearance and histopathology (Fig. 3). Normal ears were smooth and covered with hairs. Following local application of 5% propranolol for 1 week, the ears were bare and silvery scales were observed. At the end of week 2, a greater number of silvery scales were observed and the ears were thickened. Sections of the blood vessels were expanded, and pain reactions that were present initially disappeared. At the end of week 3, ear thickness increased further; however, the silvery scales were smaller and fewer than before. Classic characteristics of psoriasis, including inflammatory cell infiltration, hyperkeratosis, parakeratosis, hemangiectasis, abnormal stratum granulosum, acanthosis,

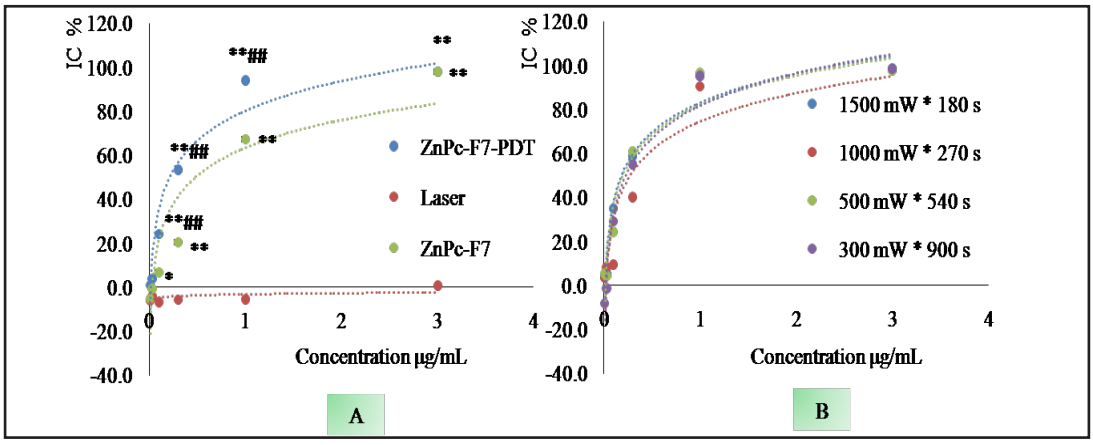


**Fig. 1.** Anti-proliferative effect of ZnPc-F7-PDT on HaCaT cells. Means ± SD; $^{*}$P< 0.05, $^{**}$P< 0.01, compared with the laser group; $^{\#\#}$P< 0.01, compared with the ZnPc-F7 group. A: Comparison of ZnPc-F7-PDT and laser or ZnPc-F7, respectively, n = 12. The concentration range of ZnPc-F7 was 0.01– 3.00 μg/mL; the spot diameter was fixed at 8 cm and the energy density was fixed at 5.37 J/cm$^2$. B: The influence of irradiation conditions, n = 4. To observe the influence of output power and irradiation time, a series of conditions were used as follows: 1500 mW × 180 s, 1000 mW × 270 s, 500 mW × 540 s, and 300 mW × 900 s.

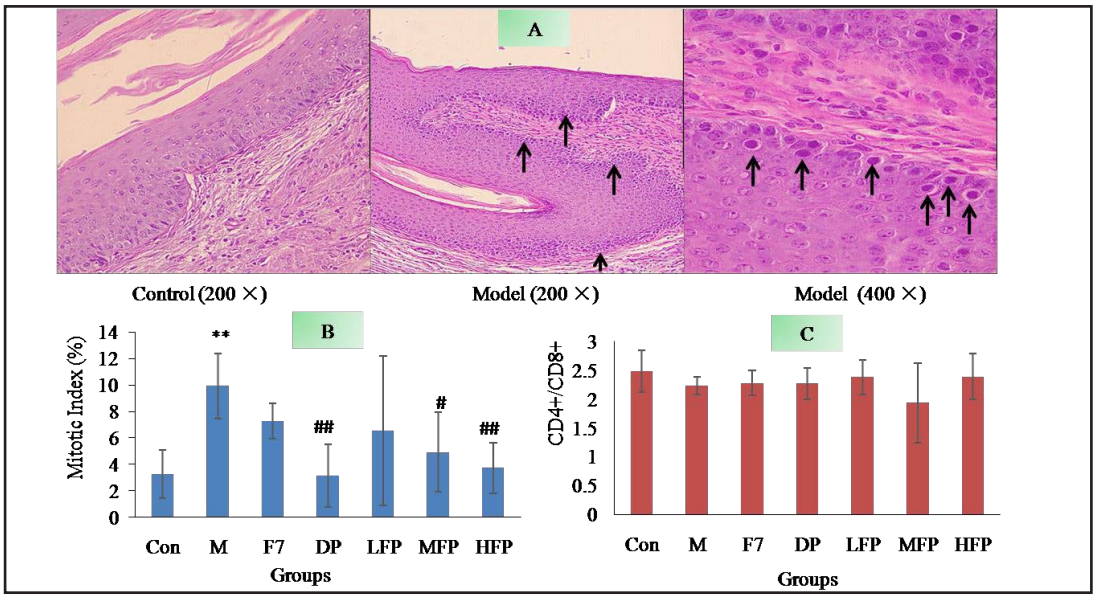


**Fig. 2.** Effects of ZnPc-F7-PDT on the hyperproliferation of mouse vaginal epithelium. n = 10, means ± SD. $^{**}$P<0.01, compared with the control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01, compared with the model group. Con: control group, M: model group, F7: ZnPc-F7 without PDT group, DP: DVDMS-PDT group, LFP: low dose ZnPc-F7-PDT group, MFP: middle dose ZnPc-F7-PDT group, HFP: high dose ZnPc-F7-PDT group. A: Mitotic cells of mouse vaginal epithelium, HE staining, ↑ indicates mitotic cells with characteristics of chromatin pyknosis and deep staining. B: Mitotic index of different groups, a total of 300 basal keratinocytes were observed, and the mitotic rate was defined as the mitotic cell number in 100 basal keratinocytes. C: CD4$^+$/CD8$^+$ level in different groups, diethylstilbestrol did not significantly influence CD4$^+$/CD8$^+$ levels, and the levels were not significantly different in all groups.

irregular epidermal hyperplasia and clubbed papillary layer, appeared successively. Munro microabscesses were uncommon. After 2 weeks' recovery, the ears showed no apparent differences compared with normal animals. However, the histopathology characteristics were still present, with the exception of inflammatory cell infiltration.

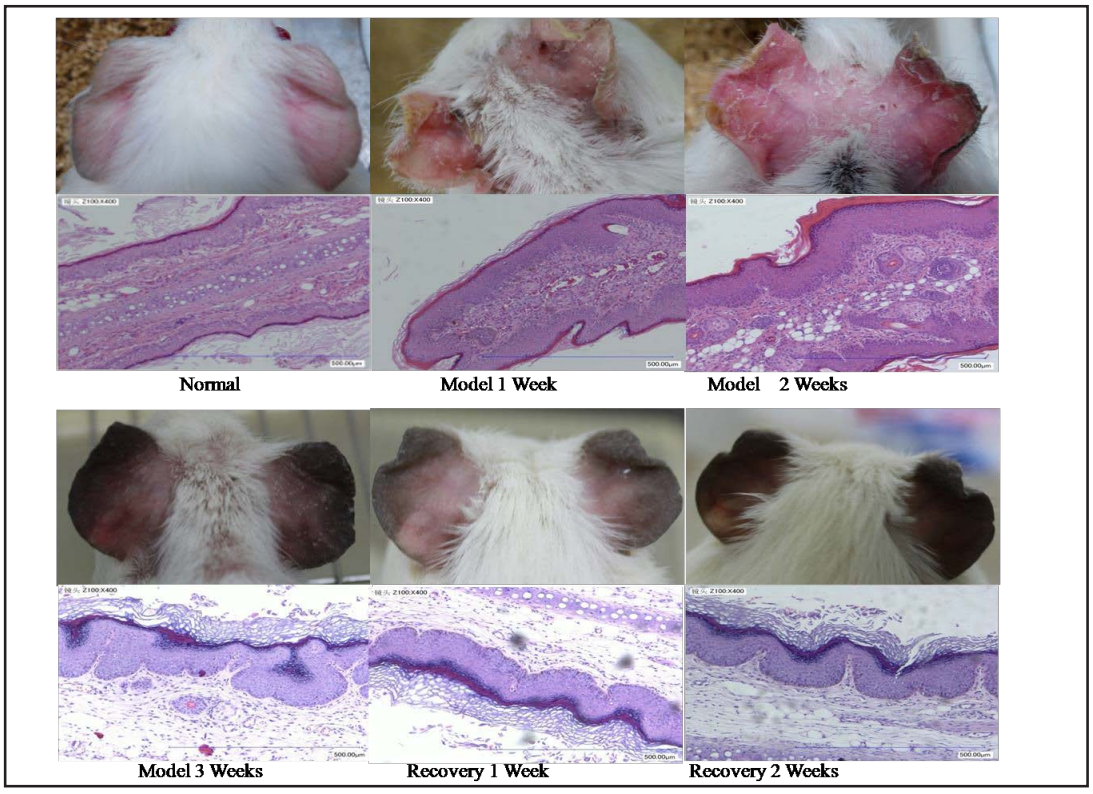


**Fig. 3.** Propranolol-induced psoriasis-like lesions on cavies' ears. A 3-D microscopic system was used to observe and collect histopathology images, magnification was Z100 × 400, HE staining. Normal ears of cavies were smooth and covered with hairs. Local application of 5% propranolol induced thickening, hemangiectasis and silvery scales. After two weeks' recovery, the ears were not different to normal animals. Histopathology constructions of normal ears were complete and clear. Classic characteristics of psoriasis, including inflammatory cell infiltration, hyperkeratosis, parakeratosis, hemangiectasis, abnormal stratum granulosum, acanthosis, irregular epidermal hyperplasia and clubbed papillary layer, appeared successively. These characteristics were still present, with the exception of inflammatory cell infiltration, after two weeks' recovery.

A 3-D microscope system was used to obtain histopathology images. Psoriatic characteristics, including abnormal stratum granulosum, irregular epidermal hyperplasia, clubbed papillary layer and hemangiectasis, were quantitatively analyzed under 400× views. Asymptomatic, mild, moderate and serious characteristics were scored as 0, 0.5, 1 and 1.5, respectively (Fig. 4 A). The total scores of all characteristics were calculated. The results showed that the scores of the model group were significantly higher than those of the control group ($P < 0.05$, $P < 0.01$); ZnPc-F7-PDT significantly reduced the total score, as did DVDMS-PDT ($P < 0.01$, $P < 0.05$). Laser and ZnPc-F7 alone did not significantly influence the psoriatic characteristics (Fig. 4 B).

The thickness of the stratum spinosum was measured under 100 × views. At least 16 points were measured for each sample, and the mean value of all points was considered to be the stratum spinosum thickness (Fig. 5 A). Stratum spinosum thickness in the model group was significantly higher than that in the control group ($P < 0.01$); a high dose of ZnPc-F7-PDT significantly reduced the stratum spinosum thickness ($P < 0.05$). Other groups did not exhibit significant changes in stratum spinosum thickness (Fig. 5 B).

*ZnPc-F7-PDT inhibited IMQ-induced psoriatic lesions in Nu/Nu mice*

IMQ induced psoriatic lesions, including parakeratosis, hyperkeratosis, abnormal stratum granulosum, acanthosis and irregular epidermal hyperplasia; IMQ also induced

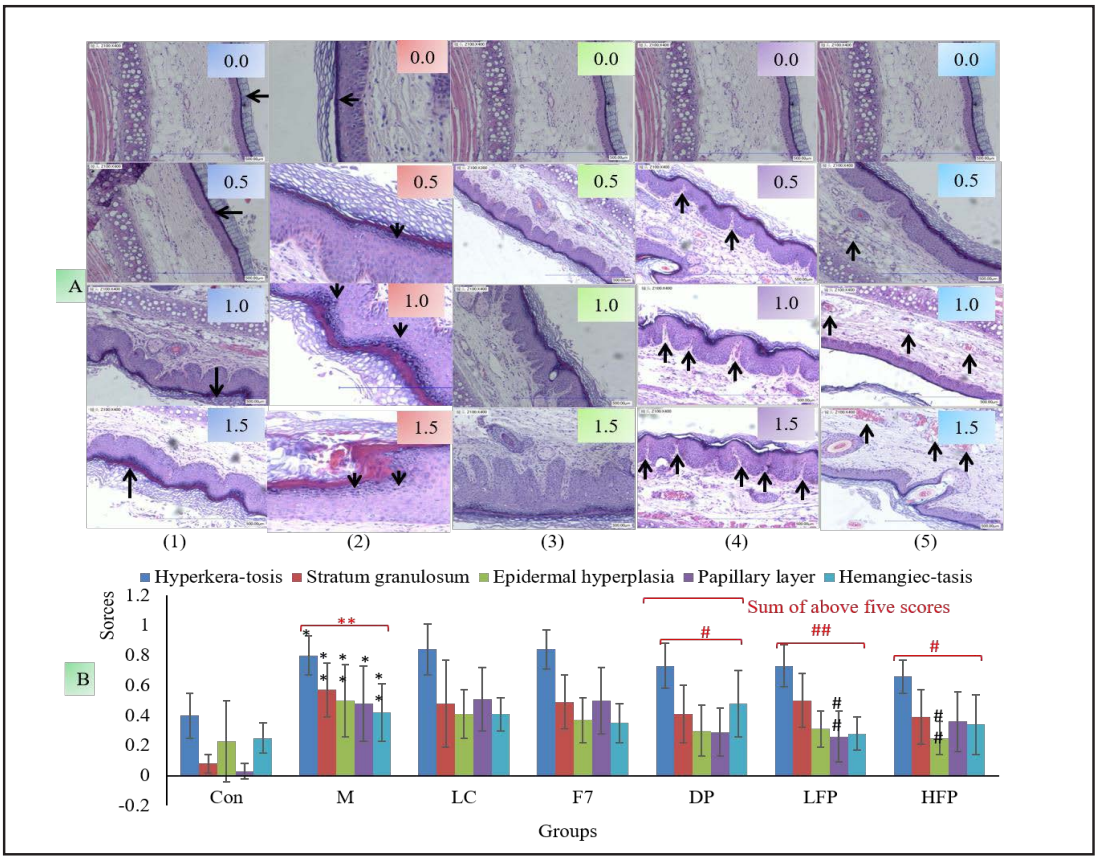


**Fig. 4.** Effects of ZnPc-F7-PDT on psoriatic characteristics scores induced by propranolol. n = 20 (two ears of each cavy), means ± SD. $^{**}P<0.01$, compared with the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the model group. Con: control group, M: model group, LC: laser-control group, F7: ZnPc-F7 without PDT group, DP: DVDMS-PDT group, LFP: low dose ZnPc-F7-PDT group, HFP: high dose ZnPc-F7-PDT group. A: Standard for evaluation, psoriatic characteristics, including hyperkeratosis (1), abnormal stratum granulosum (2), epidermal hyperplasia (3), clubbed papillary layer (4) and hemangiectasis (5), were quantitatively analyzed under Z100 × 400 views (HE staining, 3-D microscopic system as previously given). Asymptomatic, mild, moderate and serious characteristics of (1) – (5) were scored as 0, 0.5, 1 and 1.5, respectively. The total scores of all characteristics were calculated. B: Effects of ZnPC-F7-PDT on psoriatic characteristics. The scores of the model group were significantly higher than those of the control group; ZnPc-F7-PDT significantly reduced the total score, as did DVDMS-PDT. Laser and ZnPc-F7 alone did not significantly influence the psoriatic characteristics.

splenomegaly and liver injuries (Fig. 6 A). ZnPc-F7-PDT inhibited the psoriatic symptoms induced by IMQ, as did DVDMS-PDT (Fig. 6 B). The body weight and spleen coefficient of the model group were significantly different compared with the control group ($P < 0.05$, $P < 0.01$) (Fig. 6 C and D); middle and high doses of ZnPc-F7-PDT significantly inhibited the increase in spleen coefficient induced by IMQ ($P < 0.05$); the other groups exhibited no significant difference in the spleen coefficient compared with the model group; the coefficients of heart, liver, lung and kidney showed no significant differences among the groups (Fig. 6 D).

The FCW test showed that the $CD4^+$ level in the model group was decreased and the $CD8^+$ level was higher than that in the control group (Fig. 6 E), which induced abnormal $CD4^+/CD8^+$ levels (Fig. 6 F); ZnPc-F7-PDT had a tendency to correct the abnormal $CD4^+/CD8^+$ levels by increasing the $CD4^+$ level and decreasing the $CD8^+$ level: however, there were no significant differences compared with the model group (Fig. 6 E and F).

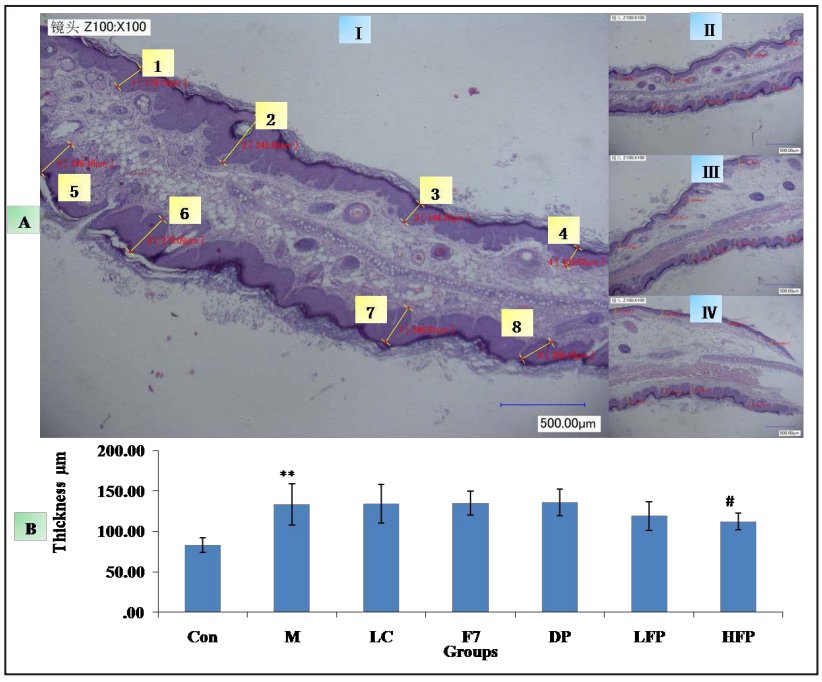


**Fig. 5.** Influence of ZnPc-F7-PDT on stratum spinosum thickness. A: Standard for evaluation, Z100 × 100 views (HE staining, 3-D microscopic system as above). The average stratum spinosum thickness (T) was calculated as follows: $T = [(t_{Ⅰ-1} + t_{Ⅰ-2} + \ldots + t_{Ⅰ-8})/8 + (t_{Ⅱ-1} + \ldots + t_{Ⅱ-8})/8 + (t_{Ⅲ-1} + \ldots + t_{Ⅲ-8})/8 + (t_{Ⅳ-1} + \ldots + t_{Ⅳ-8})/8]/4$. In which, Ⅰ, Ⅱ, Ⅲ and Ⅳ were different views of the same sample. B: Influence of ZnPc-F7-PDT, n = 20 (two ears of each cavy), Means ± SD. $^{**}P<0.01$, compared with the control group; $^{\#}P<0.05$, compared with the model group. Con: control group, M: model group, LC: laser-control group, F7: ZnPc-F7 without PDT group, DP: DVDMS-PDT group, LFP: low dose ZnPc-F7-PDT group, HFP: high dose ZnPc-F7-PDT group.

*ZnPc-F7-PDT influenced protein expression of PCNA, Bax and Bcl-2*

The WB results showed that IMQ induced the up-regulation of PCNA and Bcl-2 and the down-regulation of Bax ($P < 0.05$, $P < 0.01$) (Fig. 7 A). A high dose of ZnPc-F7-PDT significantly down-regulated the expression of PCNA and Bcl-2 and up-regulated the expression of Bax, as did DVDMS-PDT ($P < 0.05$, $P < 0.01$) (Fig. 7 A).

*ZnPc-F7-PDT influenced mRNA expression of IL-17 A and IL-17 F*

The results showed that IMQ significantly up-regulated the mRNA expression of IL-17A and IL-17F ($P < 0.01$); middle and high doses of ZnPc-F7-PDT as well as DVDMS-PDT significantly down-regulated the expression of IL-17A and IL-17F ($P < 0.05$, $P < 0.01$) (Fig. 7 B).

*Influence of ZnPc-F7-PDT on inflammatory factors and reactive oxygen species in cavies' blood*

No significant differences were observed in TNF-α, IL-1β, SOD and MDA levels between the groups. The GSH-PX content in the F7 group was significantly higher than that in the control and model group ($P < 0.05$); however, GSH-PX content in the LC group, DP group, LFP group and HFP group were not significantly different compared with the control group and model group (Fig. 8).

## Discussion

HaCaT cells are frequently used in anti-psoriasis research *in vitro* [19]. Byun et al. [20] observed cytokine expression in cultured HaCaT cells after intense pulse light (IPL) treatment or PDT utilizing 5-aminolevulinic acid and IPL at sublethal doses. The results showed that IL-10 protein increased after IPL treatment and PDT; TGF-β1 mRNA and protein increased after both IPL treatment and PDT. In the present study, HaCaT cells were used to determine

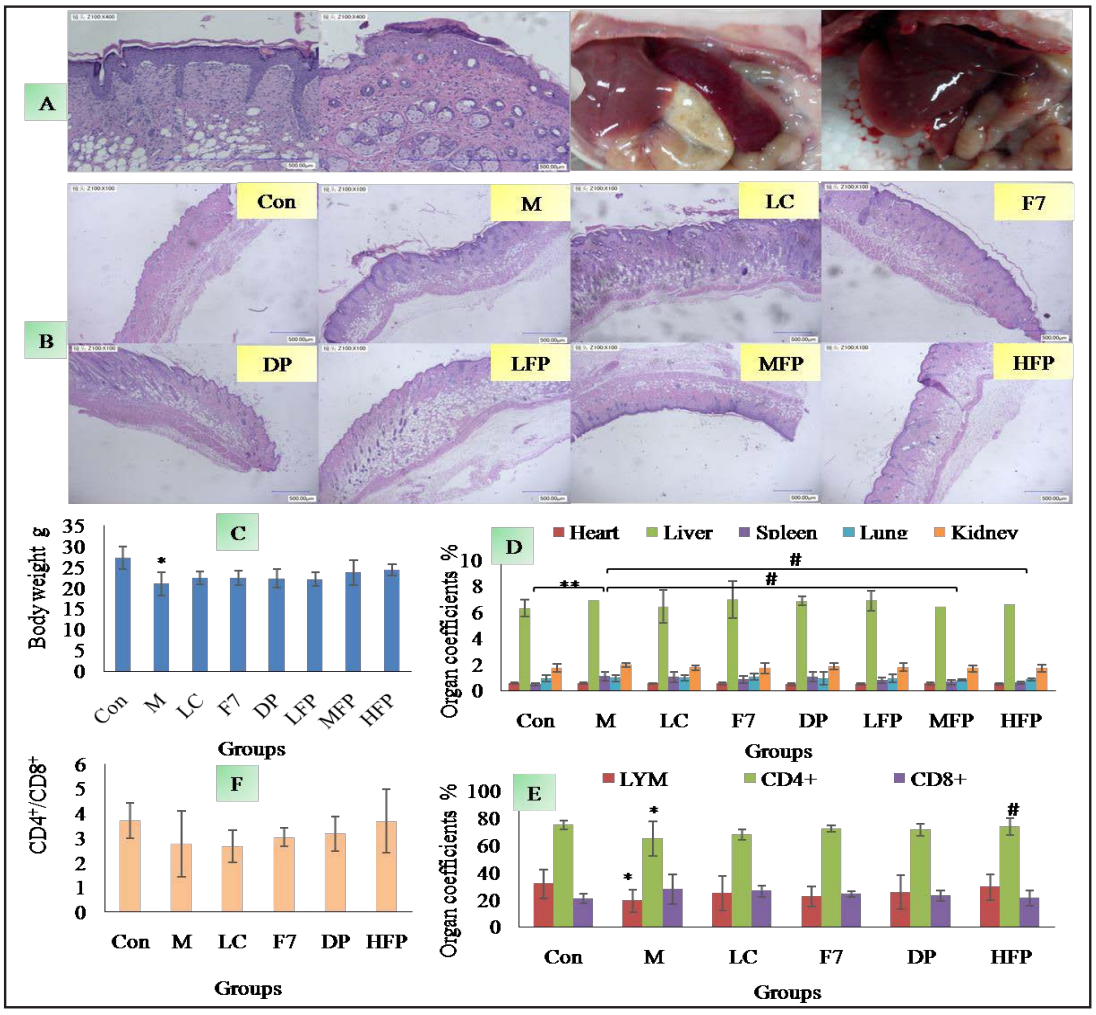


**Fig. 6.** Effects of ZnPc-F7-PDT on IMQ-induced psoriatic lesions in Nu/Nu mice. n = 10, means ± SD. $^{**}P<0.01$, compared with the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the model group. Con: control group, M: model group, LC: laser-control group, F7: ZnPc-F7 without PDT group, DP: DVDMS-PDT group, LFP: low dose ZnPc-F7-PDT group, MFP: middle dose ZnPc-F7-PDT group, HFP: high dose ZnPc-F7-PDT group. A: IMQ induced psoriatic lesions and organ changes including parakeratosis, hyperkeratosis, abnormal stratum granulosum, acanthosis and irregular epidermal hyperplasia; and induced splenomegaly and liver injuries, HE staining, Z100 × 100. B: Influence of ZnPc-F7-PDT on IMQ-induced psoriatic lesions; ZnPc-F7-PDT inhibited the psoriatic symptoms induced by IMQ, as did DVDMS-PDT. C: Body weight, the body weight of the model group was significantly different compared with the control group; however, body weights of all experimental groups were not significantly different to the control group or the model group. D: Organ coefficients, middle and high doses of ZnPc-F7-PDT significantly inhibited the increase in spleen coefficient induced by IMQ; the coefficients of heart, liver, lung and kidney showed no significant differences among the groups. E: Lymphocyte (LYM) levels, the $CD4^+$ level in the model group was decreased and the $CD8^+$ level was higher than the control group and high dose ZnPc-F7-PDT significantly increased the $CD4^+$ level compared to the model group. F: $CD4^+/CD8^+$ levels, there were no significant differences among the groups.

the influence of ZnPc-F7-PDT on cell proliferation *in vitro*. The results showed that ZnPc-F7-PDT significantly inhibited the proliferation of HaCaT cells; time and power of irradiation did not obviously influence the inhibition ratio when the energy density was fixed, which suggested that output power was as important as irradiation time.

Psoriasis animal models can be divided into five categories on the basis of the pathogenic mechanisms. (1) Biomedical models [21], which mainly include the mouse vagina model and

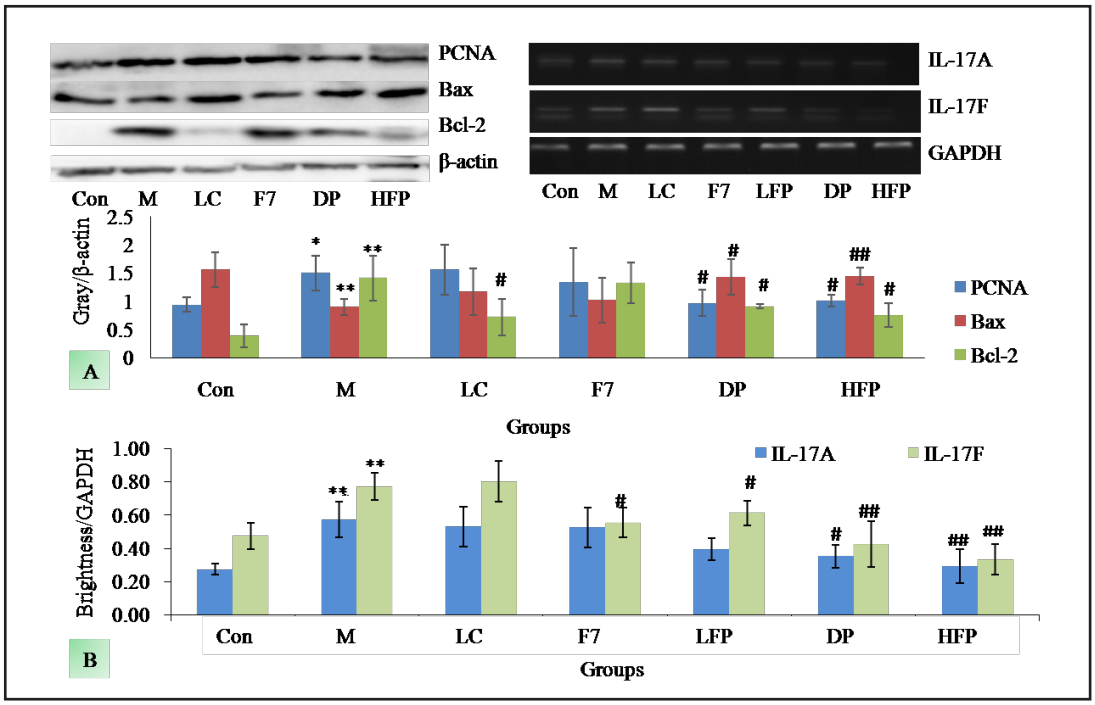


**Fig. 7.** Effects of ZnPc-F7-PDT on protein and mRNA expression in the IMQ-induced psoriatic model. n = 10, means ± SD. $^{**}P<0.05$, $^{**}P<0.01$, compared with the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the model group. Con: control group, M: model group, LC: laser-control group, F7: ZnPc-F7 without PDT group, DP: DVDMS-PDT group, LFP: low dose ZnPc-F7-PDT group, MFP: middle dose ZnPc-F7-PDT group, HFP: high dose ZnPc-F7-PDT group. A: Protein expression, IMQ induced the up-regulation of PCNA and Bcl-2 and the down-regulation of Bax. A high dose of ZnPc-F7-PDT significantly down-regulated the expression of PCNA and Bcl-2 and up-regulated the expression of Bax, as did DVDMS-PDT. B: mRNA expression, IMQ significantly up-regulated the mRNA expression of IL-17A and IL-17F; middle and high dose of ZnPc-F7-PDT as well as DVDMS-PDT significantly down-regulated the expression of IL-17A and IL-17F.

the mouse tail model simulating hyperkeratosis and parakeratosis of psoriasis, respectively. (2) Induced models, in which psoriatic lesions are induced by UV[22], agents or a deficiency of essential fatty acids [23]; propranolol and IMQ are the most commonly used agents. Propranolol is an inhibitor of the β-adrenergic receptor. This compound can block the β-adrenergic receptors of keratinocytes and reduce cAMP levels, which induces psoriatic lesions, such as hyperkeratosis, parakeratosis and acanthosis. IMQ is an agonist of the Toll-like receptor and induces psoriatic lesions and promotes the production of cytokines, such as interferon, tumor necrosis factor and interleukin [24]. (3) Xenograft models, in which psoriatic skin from patients is grafted to an SCID mouse to form psoriasis-like lesions. This model is limited by technical requirements and a source of psoriatic skin [25, 26]. (4) Transgenic models [27], which are often used in pathogenesis research rather than pharmacodynamics evaluation. In addition, there is no single feature that is truly unique to psoriasis. (5) Spontaneous mutation models [28], in which psoriasis-like symptoms occur in mice spontaneously.

There is no generally accepted animal model at present. In this study, the mouse vagina model, propranolol-induced cavy psoriasis model and IMQ-induced mouse psoriasis model were used to confirm the therapeutic actions of ZnPc-F7-PDT. Jin et al. [29] found that zinc phthalocyanine polymer conjugate, an amphiphilic photosensitizer synthesized using zinc phthalocyanine (ZnPc), mediated PDT and showed excellent anti-psoriasis effects in the cavy psoriasis model. ZnPc-F7 is a coordination compound of ZnPc. Our results showed that ZnPc-F7-PDT exhibited good effects on all three models, which suggested that ZnPc-F7-PDT

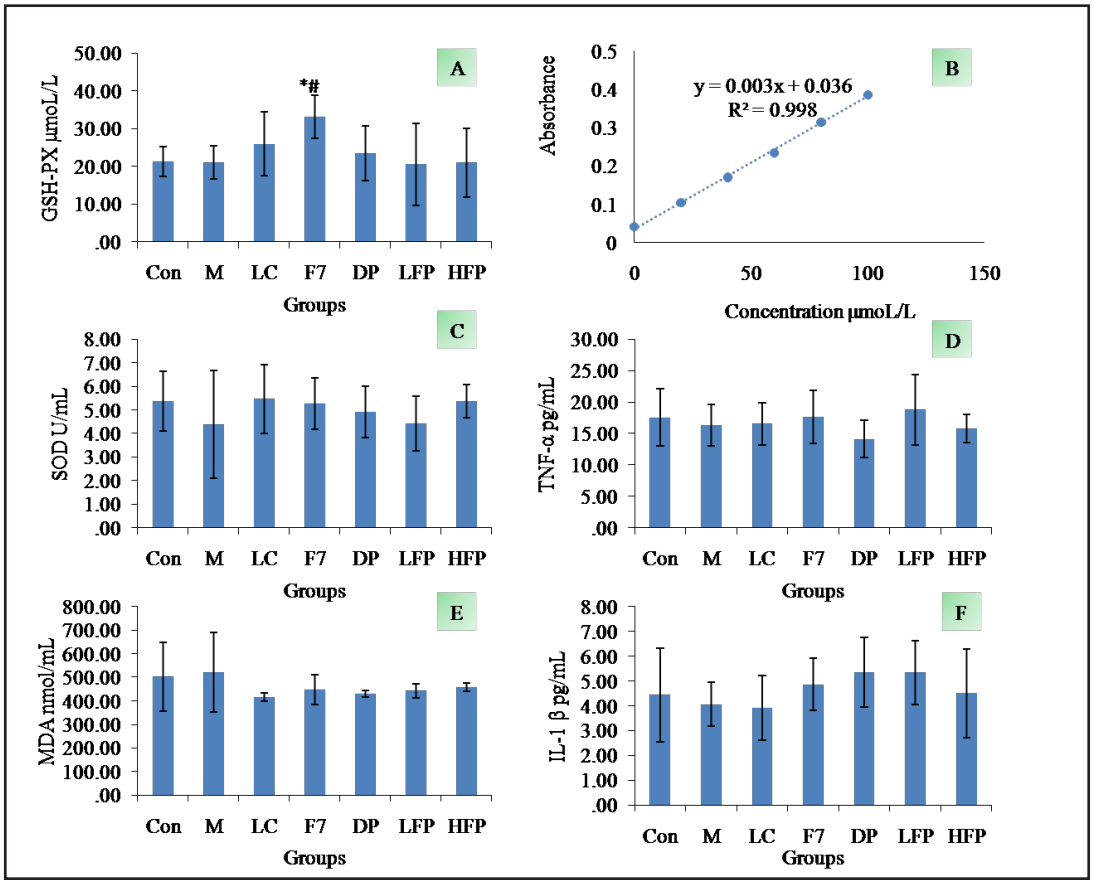


**Fig. 8.** Influence of ZnPc-F7-PDT on inflammatory factors and reactive oxygen species in cavies' blood. n = 10, Means ± SD. *P<0.05, compared with the control group; #P<0.05, compared with the model group. Con: control group, M: model group, LC: laser-control group, F7: ZnPc-F7 without PDT group, DP: DVDMS-PDT group, LFP: low dose ZnPc-F7-PDT group, HFP: high dose ZnPc-F7-PDT group. A: Contents of GSH-PX, the content of GSH-PX in F7 group was significantly higher than that in the control and model group; however, the contents of GSH-PX in other groups were not significantly different to the control group or model group. B: The absorbance–concentration curve for GSH-PX detection, the absorbance-concentration curve was a straight line with a slope of 0.003 and intercept of 0.036, the $R^2$ was 0.998 and represented a good fit. C: SOD activities, there was no significant difference in SOD levels between the groups. D: Contents of TNF-α, there was no significant difference in TNF-α levels between the groups. E: Contents of MDA, there was no significant difference in MDA levels between the groups. F: Contents of IL-1β, there was no significant difference in IL-1β levels between the groups.

is worth further study in the treatment of psoriasis. In previous reports, research focused on the therapeutic effects of PDT on the inhibition of hyperproliferation. For example, Takahashi et al. [12] found that ATX-S10 (Na)-PDT inhibited TPA-induced epidermis hyperproliferation in mice. All three models in the present study had the characteristics of hyperproliferation, which indicated that hyperproliferation inhibition was an important biological effect of ZnPc-F7-PDT.

Therefore, we studied the mechanisms of ZnPc-F7-PDT in the inhibition of hyperproliferation. PCNA is a protein existing in only proliferative cells and has close relations with DNA synthesis. The expression of PCNA is considered a good index of cell proliferation [30, 31]. In this study, we found that IMQ increased the expression of PCNA and that ZnPc-F7-PDT inhibited this increase. Bax and Bcl-2 are the most important proteins for apoptosis regulation in the Bcl family [32]. The WB results showed that IMQ up-regulated the expression of Bcl-2 and down-regulated the expression of Bax, which meant that IMQ

induced the hyperproliferation of mouse skin and inhibited its normal apoptosis. ZnPc-F7-PDT down-regulated Bcl-2 expression and up-regulated Bax expression, which appeared to be a potential mechanism for inhibition of psoriatic skin hyperproliferation.

Current knowledge of the immunopathogenesis of psoriasis is based on the crucial role of $CD4^+$ and $CD8^+$ lymphocytes. The expression of $CD4^+$ lymphocytes and $CD8^+$ lymphocytes was found to be significantly altered in psoriasis patients [33, 34]. In the present study, we found that IMQ induced an increase in the spleen coefficient and abnormalities in the $CD4^+$/$CD8^+$ ratio compared with the control. ZnPc-F7-PDT restored the spleen coefficient and the $CD4^+/CD8^+$ ratio to normal levels. However, in the mouse vagina model, ZnPc-F7-PDT did not change the $CD4^+/CD8^+$ level, which suggested that ZnPc-F7-PDT had immunomodulatory effects, but did not influence the normal immunity of animals and may be very useful in the treatment of psoriasis.

IL-17 is the defining cytokine in the Th17, Tc17, and γδ T cell populations, and play critical roles in mediating inflammation and autoimmunity. IL-17 plays important roles in the pathogenesis of psoriasis and IL-17 inhibitors are considered important agents in the management of psoriasis [35-38]. The RT-PCR results in our study showed that IMQ induced the up-regulation of IL-17 A mRNA and IL-17 F mRNA in mouse skin, whereas ZnPc-F7-PDT down-regulated their expression, which is another potential mechanism of ZnPc-F7-PDT in the treatment of psoriasis.

Oxidative damage plays an important role in the treatment of cancers using PDT, and reactive oxygen species (ROS), such as $^1O_2$, are an important pathway by which cells are killed by PDT[39]. Skin is a major target of oxidative stress, mainly due to ROS originating from the environment and metabolism. Increased or prolonged presence of free radicals can override ROS defense mechanisms and mediate numerous cellular responses that contribute to the development of a variety of skin disorders, including psoriasis [40]. Recent reports have proposed opposing views. Kim et al. [41] investigated IMQ-induced psoriatic dermatitis in association with Treg function both in elevated and lowered levels of ROS and found that IQM-induced psoriasis was attenuated at elevated levels of ROS and was aggravated at reduced levels of ROS. Furuhashi et al. [42] found that photo (chemo) therapy reduced circulating Th17 cells and restored circulating regulatory T cells in psoriasis. Our results showed that ZnPc-F7-PDT inhibited the expression of IL-17 mRNA and reduced the psoriatic lesions induced by IMQ. However, the influence of ZnPc-F7-PDT on Th 17 cells requires further study.

## Conclusion

ZnPc-F7-PDT is a potential treatment option for psoriasis. Inhibition of hyperproliferation, down-regulation of the expression of IL-17 mRNA and immune regulation effects are possible mechanisms. However, in our previous studies, ZnPc-F7-PDT did not significantly influence the formation of stratum granulosum in a mouse tail test. Thus, it is necessary to confirm the effects of ZnPc-F7-PDT in parakeratosis. Moreover, the roles of ZnPc-F7-PDT in signal transduction require further study.

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## Disclosure Statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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