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Original Article

Ozonated autohemotherapy elevates PPAR-γ expression in CD4$^+$ T cells and serum HDL-C levels, a potential immunomodulatory mechanism for treatment of psoriasis

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Abstract: Psoriasis is widely accepted as a metabolic syndrome with significantly abnormal lipid metabolism and high level of blood lipids that induce a persistent low level of inflammatory condition in patients. T cell mediated immune response plays a critical role in the occurrence and persistence of psoriasis lesions. Hyperlipidemia and associated inflammatory reaction are believed to be the major risk factors for the onset and recurrence of psoriasis. Peroxisome proliferator activated receptor-gamma (PPAR-γ) is known to effectively regulate the blood lipid level and inhibit inflammatory reaction. In this study, we examined the efficacy of ozonated autohemotherapy (OAHT) treatment on psoriatic patients by evaluating the Psoriasis Area and Severity Index (PASI) score and blood lipid level. In addition, PPAR-γ expression level and the correlation of PASI scores or blood lipid level with the PPAR-γ expression were also assessed to determine the psoriasis-associate targets of OAHT. We found that OAHT significantly decreased patients' PASI scores and increased blood HDL-C level. Furthermore, we found that PPAR-γ expression in CD4$^+$ T cells from psoriasis patients was significantly lower than healthy controls, and OAHT treatment increased the expression of PPAR-γ. In conclusion, OAHT attenuates the psoriatic severity in patients and increased blood HDL-C level, which may be associated with increased PPAR-γ expression. Our data suggests that OAHT is an effective treatment in psoriasis and deserves further evaluations in clinical applications.

Keywords: Ozonated autohemotherapy, psoriasis, PPAR-γ, hyperlipidemia, CD4$^+$ T cells

Introduction

Psoriasis is a T-cell-mediated inflammatory cutaneous disease that is triggered by the combination of genetic, immune, obesity, and many other environmental factors. Hyperlipidemia and the associated inflammatory reaction are identified as the major risk factors contributing to the onset and recurrence of psoriasis [1]. White fat, extensively existing in the human body and containing adipocytes and immune cells [macrophages and dendritic cells (DC_s)], can produce inflammatory and anti-inflammatory markers such as C-reactive protein (CRP), adiponectin, leptin, resistin, and lipoprotein-related phospholipase A2 (Lp-PLA2) to accelerate the occurrence and recurrence of psoriasis [2]. Previous studies showed that high-density lipoprotein cholesterol (HDL-C) inhibited antigen or toxins to interact with antigen-presenting cell and leukocyte adhesion to endothelial cells. In addition, apolipoprotein (a) (Apo(a)), lipoprotein (a) (Lp(a)), and low-density lipoprotein cholesterol (LDL-C) particles promoted the inflammatory response in patients with psoriasis [3]. These evidences indicate that the inflammatory response induced by hyperlipidemia is a key trigger in psoriasis. Admittedly, peroxisome proliferator activated receptor-gamma (PPAR-γ) is known to regulate the expression of enzymes involved in lipid metabolism and gene transcription, as well as the blood lipid levels and adipocyte differentiation [4, 5]. Thus, targeting the regulation of PPAR-γ expression to modulate

inflammatory response and regulate blood lipid levels is a potential novel therapeutic strategy for psoriasis.

Ozone has been widely used in dermatology clinics, including for infectious skin diseases, allergic diseases, wound healing and ulcer recovery. The underlying mechanism refers to antimicrobial effect, immune regulation, antioxidant defense, and apparent regulation [6]. The clinical application forms of ozone are also constantly enriched and improved. Although local ozone-therapy strategies are various, the systemic ozone application has ozonated autohemotherapy (OAHT) only. Studies have found that ozone induces and activates the body's antioxidant enzyme system to produce superoxide dismutase (SOD), which removes free radicals produced during the inflammatory reaction and interfere with the inflammatory factors produced in the progress of diseases [7]. In animal experiments, low-dose ozone treatment increases the level of blood lipid and inhibits oxidative stress to alleviate the progress of atherosclerotic diseases [8]. However, the regulation mechanism of ozone on blood lipid level has not been fully elucidated.

In this study, we observed the therapeutic effects of short term OAHT on psoriasis. We found that OAHT treatment improves psoriasis by upregulating the expression of PPAR-γ. PPAR-γ expression and serum HDL-C levels to alleviate inflammatory response in psoriasis patients, indicating OAHT treatment is a potential novel therapeutic method for psoriasis.

Materials and methods

Enrolled patients

This study was approved by the IRB committee of the Third Xiangya Hospital, Central South University, China. A total of 30 patients who were diagnosed with histopathology examination as psoriasis vulgaris and 30 sex- and age-matched healthy volunteers were enrolled in this study. Among these enrolled individuals, 12 psoriatic patients were confirmed with hyperlipidemia and selected in this study trial after signing informed consent. Psoriasis disease activity was assessed by Psoriasis Area and Severity Index (PASI) score. The PASI scores of all patients were lower than 12 and their conditions were stable for half a year at least. The inclusion criteria included the following: age of 18 to 60 years old and diagnosed with psoriasis vulgaris by pathologic examinations and with hyperlipidemia [total cholesterol (TC) ≥ 6.2 mmol/L; triglycerides (TG) ≥ 2.3 mmol/L; LDL-C ≥ 4.1 mmol/L; and HDL-C < 1.0 mmol/L]. The exclusion criteria included the following: allergic to ozone; severe cardiovascular disease; local vessel intolerable treatment; abnormal coagulation; and treatment with systemic or local topical corticosteroid, immune inhibitors [including conventional systemic therapies (MTX, CsA, etc) and biologics], or vitamin D3 derivative within the past 2 weeks.

Medical ozonated major autohemotherapy

All subjects were treated with OAHT, as follows: 100 to 150 mL of venous blood was mixed with medical pure oxygen and ozone (20 μg/mL) (Humares ozone therapy device, Germany), then transported back into the body, once every other day for consecutive 10 times in a course of treatment, the mean duration of each treatment was about 30 minutes.

Blood lipid test

All participants accepted blood lipid tests before and after treatment. Venous blood was collected in the morning before treatment after a night of fasting and the next day 24 hours after treatment. Serum was used for determination of TC, TG, HDL-C, and LDL-C according to protocols provided by the manufacturer of Hitachi 7060 automatic biochemical analyzer.

Evaluation of clinical photographs and reflectance confocal microscope images of skin lesions

All subjects received OAHT treatment only, without any other treatments during the trial. Clinical photographs, PASI scores, and reflectance confocal microscope (RCM) images were collected by the same professional physicians to evaluate disease severity. The PASI score contains skin lesion area, erythema color, erythema infiltration depth, and scale thickness according to the literature [9]. Each subject was assessed by RCM images from three different skin lesion sites. The scanned total thickness of skin was 51 layers ×3.05 μm vertically for each scan. Under RCM, epidermal thickness and infiltrated inflammatory cells were also evaluated before and after treatment.

Total CD4⁺ T cells isolation

Peripheral blood mononuclear cells (PBMCs) were separated from the whole blood of all patients before and after treatment using density gradient centrifugation (GE Healthcare, Switzerland). $CD4^+$ T cells were collected using CD4 beads (Miltenyi, Germany) per manufacturer's instructions; the purity was generally greater than 95%. The isolated $CD4^+$ T cells were used for subsequent experiments.

RNA isolation and quantitative PCR (qPCR)

Total RNA was extracted from $CD4^+$ T cells using Trizol reagent according to the manufacturer's instructions (Invitrogen, USA). The mRNA was reverse-transcribed with the PrimeScript®RT reagent kit (TaKaRa Biotech Co., China) using 1 μg of total RNA. The reaction mixture in RT-PCR contained 2 μL of cDNA, 10 μL of SYBR Premix Ex Taq™ (TaKaRa Biotech Co., China), and 400 nM sense and antisense primers to a total volume of 20 μL. qPCR was performed on a LightCycler®96 (Roche, Switzerland) thermocycler. The quantity of gene expression was calculated using comparative cycle thres-hold (CT) methods and normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers are listed in Table S1.

Western blotting

$CD4^+$ T cells were lysated and proteins were extracted by Nuclear Extraction Reagent (Boster, China). Total proteins from the whole cell lyates were quantified by the Bradford assay (HyClone-Pierce, USA) followed by 10% vertical dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, USA). The PVDF membrane was blocked in 5% skim milk for 1 hour at room temperature and then incubated with an antibody against PPAR-γ (Abcam, China) for 12 to 16 hours at 4°C, followed by mouse anti-rabbit immunoglobulin G (IgG) antibody (H&L) (GenScript, USA) for 1 hour at room temperature. Proteins were detected with an ECL western blot detection kit (Thermo Scientific, USA). Band intensity was quantified using an ImageQuant™ LAS 4000 mini (GE-Healthcare). Quantification of PPAR-γ was normalized to GAPDH by band densitometry.

Statistical analysis

All diagrams and graphs of reporting cumulative data were made using GraphPad Prism 6.0. The data are represented as the mean ± standard deviation (SD) or standard error of the mean (SEM). Distributions of the means were analyzed with non-parametric tests (SPSS 18.0, USA). Differences in individual treatments were analyzed by unpaired or paired *t* test. Statistical significance ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$) was assessed using a two-tailed unpaired Student *t* test for comparisons between two groups and one-way analysis of variance (ANOVA) with relevant post-hoc tests for multiple comparisons.

Results

OAHT reduces psoriasis severities

We discovered that OAHT treatment leads to the remission of erythema, scales, and itching in psoriatic patients with hyperlipidemia. To confirm the curative effect of ozone therapy in hyperlipidemia psoriasis patients, we evaluated the effect of OAHT treatment on the disease severity of 12 hyperlipidemia psoriatic patients. Skin lesions were assessed by clinical photographs and RCM images. Notably, OHAT treatment improved the psoriatic lesions clinically (**Figure 1A**), along with significant attenuation of inflammatory erythema and scales, as evaluated with RCM images (**Figure 1B**). In addition, we found the thickness of epidermis and epidermal infiltrating inflammatory cells were markedly decreased. The thickness of the epidermis was quantified by RCM with a count of the vertical swept layers (**Figure 1C**). Correspondingly, OAHT treatment markedly decreased psoriasis severity after 10 treatments as assessed by the PASI score (**Figure 1D**).

OAHT significantly decreases blood lipid levels

Based on previous experience, the therapeutic potential of OAHT was appreciated in patients with severe peripheral arterial disease, coronary disease, cholesterol embolism, severe dyslipidemia, Madelung disease, and sudden deafness of vascular origin [20]. In this study, we evaluated whether OAHT treatment improves psoriasis through regulating serum lipid level in hyperlipidemia psoriasis patients. Results showed that OAHT reduced the serum level of TG and TC (**Table 1**; **Figure 2A** and **2B**) significantly, and increased the level of HDL-C (**Table 1** and **Figure 2C**) in most patients. However, OAHT treatment had no significant

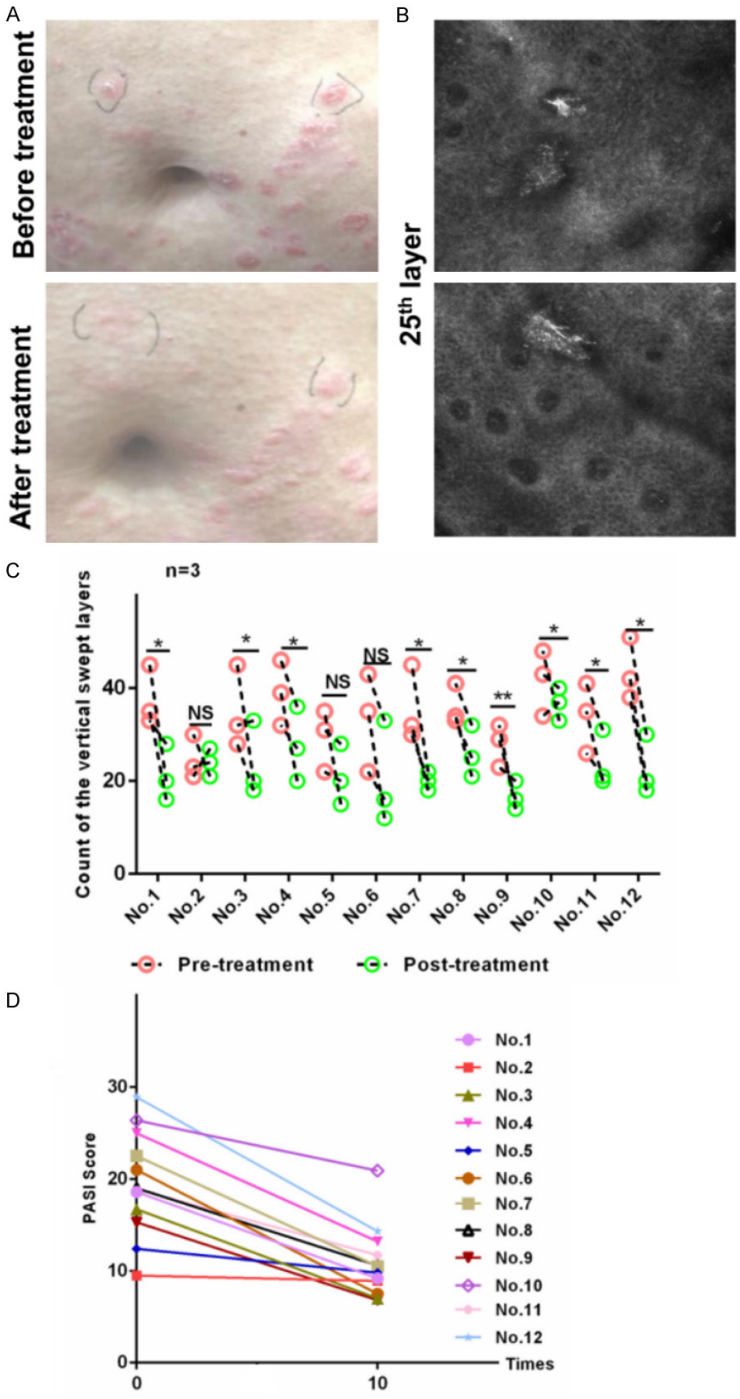

Figure 1. OAHT improves the condition of psoriatic skin lesions. The impact of OAHT on psoriatic lesion was evaluated by (A) clinical photographs of a psoriatic skin lesion before and after treatment with OAHT; (B) The RCM images showing the 25th scanning layer before and after treatment; (C) Average statistical values of vertical scan depth of three lesions with quantitative RCM images for an assessment of thickness changes of the epidermis before and after treatment; and (D) PASI scores for all participants. n=3 represented that three peripheral lesions were scanned for each RCM examination). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, no statistical significance.

effect on the level of LDL-C (**Table 1** and **Figure 2D**). These data suggested ozone treatment reduced psoriasis severities by decreasing the generation of circulating lipid in serum. The mechanism of OAHT treatment on lipid metabolism deserves further investigation.

OAHT elevates PPAR-γ expression in psoriatic CD4⁺ T cells to relieve psoriasis

PPAR-γ activation downregulates the synthesis of inflammatory cytokines in T cells [10]. By quantitative RT-qPCR and western blotting, we found that PPAR-γ expression in CD4⁺ T cells from peripheral blood in psoriasis patients is decreased than healthy controls (**Figure 3A-C**). Notably, we observed a significant correlation between the PPAR-γ expression in CD4⁺ T cells and PASI scores in serum in patients (**Figure 3D**). The relevance between PPAR-γ gene expression level and blood lipid level was also assessed, and we found that it was negatively correlated with circulating TG level in serum (**Figure 3E**), whereas it had no significant relationship with TC and LDL-C levels (**Figure 3F** and **3G**). Visually, these data suggest a positively relevant trend of HDL-C without statistical significance (**Figure 3H**).

Based on the aforementioned studies, we examined PPAR-γ expression in peripheral blood CD4⁺ T cells from hyperlipidemia psoriasis patients pre- and post-OAHT treatment to investigate the mechanism of ozone action on the level of serum lipid in psoriasis. As assessed by qPCR and western blot techniques, OAHT treatment increased PPAR-γ ex-

Table 1. The blood lipid level before and after OAHT

Number	Date	TG (mmol/l)	TC (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)
No.1	2019/05/22	2.48	4.33	1.08	2.38
	2019/06/12	1.92	4.19	1.35	1.95
No.2	2019/04/26	1.90	4.93	1.04	2.76
	2019/05/17	1.32	5.38	1.59	2.56
No.3	2019/03/24	2.06	7.56	1.41	3.12
	2019/04/14	1.25	4.08	1.57	2.62
No.4	2019/03/15	2.52	7.18	0.9	3.13
	2019/04/05	1.66	4.79	0.87	2.91
No.5	2018/12/27	1.56	6.06	1.24	2.09
	2019/01/17	1.38	4.42	1.33	1.96
No.6	2018/12/14	2.98	7.66	0.96	3.47
	2019/01/04	1.62	4.05	1.18	2.16
No.7	2018/11/09	1.72	5.01	1.09	3.44
	2018/11/30	1.32	5.19	1.12	3.29
No.8	2018/07/11	1.89	4.38	1.32	2.37
	2018/08/01	1.77	4.02	1.40	2.33
No.9	2018/06/05	1.66	7.66	0.95	3.64
	2018/06/26	1.35	6.15	1.08	3.17
No.10	2018/03/03	2.98	5.08	0.89	2.15
	2018/03/24	2.05	5.22	1.34	2.36
No.11	2017/11/07	2.32	4.35	1.28	2.01
	2017/11/28	1.89	4.39	1.29	2.09
No.12	2017/10/14	2.52	4.14	0.96	1.78
	2017/11/05	2.56	4.08	1.27	1.89
Comments	Reference values	< 1.7	2.85~5.69	1.16~1.42	< 2.59

Note: the number with red background is beyond reference values; the number with blue background is below reference values.

pression in $CD4^+$ T cells from patients when compared with the same cells prior treatment (**Figure 4A-C**). Simultaneously, we performed RT-PCR to detect the relative expression levels of key cytokines [tumor necrosis factor-alpha (TNF-α), IL-6, transforming growth factor-β (TGF-β), IL-17a, and IL-23] in the pathogenesis of psoriasis. Altogether, the levels of TNF-α, IL-6, IL-17a and IL-23 in $CD4^+$ T cells from patients were decreased by OAHT treatment, whereas OAHT treatment has no effect on the expression of TGF-β (**Figure 4D**). These results suggest that OAHT treatment improve psoriasis through decreasing psoriasis-related inflammatory factors in the systemic circulation. According to these data, we concluded that OAHT increased PPAR-γ expression in psoriatic $CD4^+$ T cells to reduce inflammation and relieve psoriatic severities.

Discussion

It is well known that abnormal activated T cells play a key role in the immunopathogenesis and chronic inflammatory response of psoriasis [11]. The interaction of T cells and keratinocytes, also called T cells-mediated inflammatory persistence loop, can trigger the pervasive chronic inflammatory changes in psoriatic lesions. Intralesional activated T cells produce cytokines such as TNF-α to stimulate primed basal stem skin cell, keratinocytes, proliferating and perpetuating the disease [12, 13]. Previous studies have suggested that intralesional existed plasmacytoid dendritic cells (pDCs) produce IL-23, IL-12, IL-6, and TNF-α, which activate and induce helper T (Th) cells toward Th17, Th1, and Th22 cells. The polarization of these Th cells are defined by the production of IL-17a, IFN-γ, and IL-22, respectively [14,

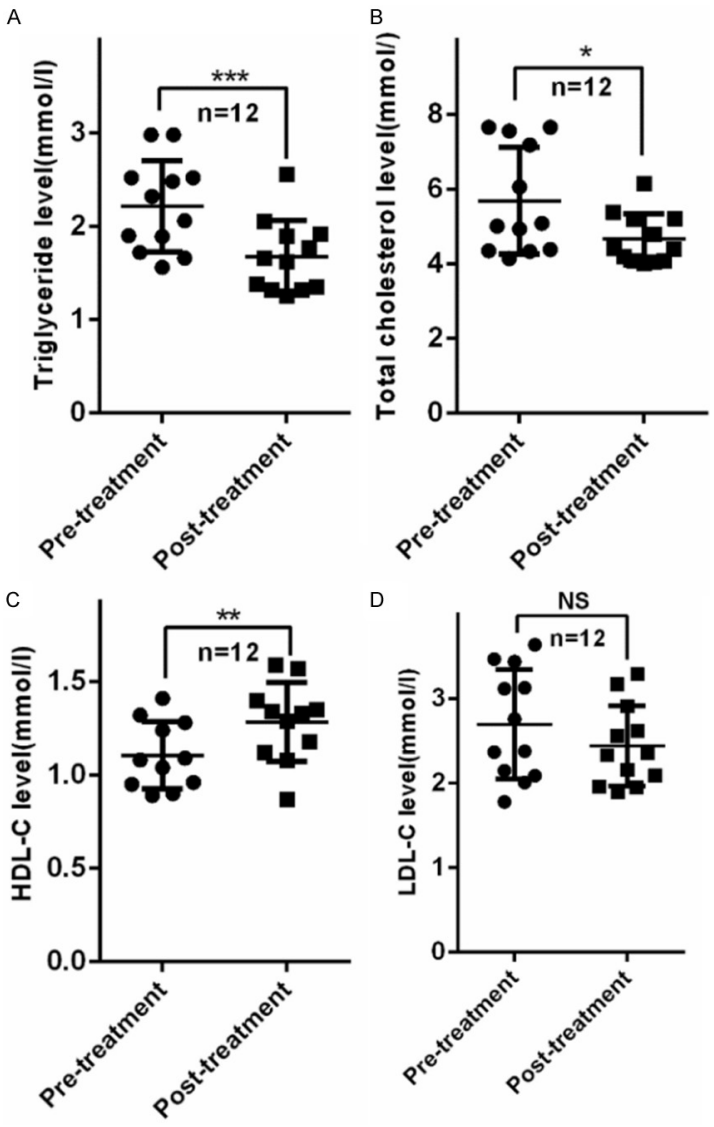

Figure 2. Changes in the serum level of TG, TC, HDL-C, and LDL-C before and after treatment. The changes of TG (A), TC (B), HDL-C (C), and LDL-C (D) in serum before and after treatment were examined by Hitachi 7060 automatic biochemical analyzer and analyzed with the 2-tailed paired Student's *t* test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, no statistical significance.

15]. In turn, these proinflammatory cytokines act on keratinocytes, leading to keratinocytes abnormal proliferation and differentiation. These abnormal keratinocytes can produce more chemokines and adhesion molecules to recruit neutrophils and form neutrophil-associated inflammatory enhancement loop in psoriatic plaques [16]. Thereby, T cell mediated immunity plays a key role in the occurrence and persistence of psoriasis lesions.

Emerging studies indicated that significant abnormal lipid metabolism is a common phenomenon in patients with psoriasis, which seriously affected the disease outcome and the life quality of patients [17]. It is generally believed that hyperlipidemia sustains chronic low inflammatory response in psoriasis patients, inducing or aggravating the progression of the disease. The inflammation response in local skin lesions of psoriasis is accompanied by a systematic cascade reaction. Immune cells released into systemic circulation lead to increased risk factors for metabolic diseases. Systemic inflammation induced by obesity, metabolic syndrome, and other diseases can aggravate local skin lesions, forming a vicious cycle [18, 19]. A study conducted by Naldi *et al*. [20] found that obesity was a modifiable, independent, psoriasis-associated risk factor, accounting for 16% of all psoriatic episodes. Further study by Wolk and Sabat [21] stated that the risk of psoriasis onset increased by 9% for every unit increase in body mass index. In obesity-related metabolic diseases, the number of macrophages, $CD8^+$ T cells, and helper T (Th) 1 cells increases and a large amount of IL-6, TNF cytokines, interferon-gamma (IFN-γ), and other pro-inflammatory cytokines were produced [22]. Whereas in psoriasis, the activation of Th1, Th17, and Th22 cells prompts the lymphocytes and keratinocytes in local skin lesional areas to produce a variety of inflammatory mediators, which is mainly initiated by pro-inflammatory cytokines and adipokines produced by adipose tissue; all above mentioned changes may lead to insulin resistance and endotheliocytes injury, further motivating glucose and lipid metabolism disorders, vascular dysfunction, and immune cell infiltration [23, 24]. Thus, hyperlipidemia and associated inflammatory reaction are believed to be the major risk factors contributing to the onset of psoriasis.

PPAR-γ is a nuclear receptor regulating transcription factor activated by adipose tissue specific ligand. PPAR-γ expression can be trig-

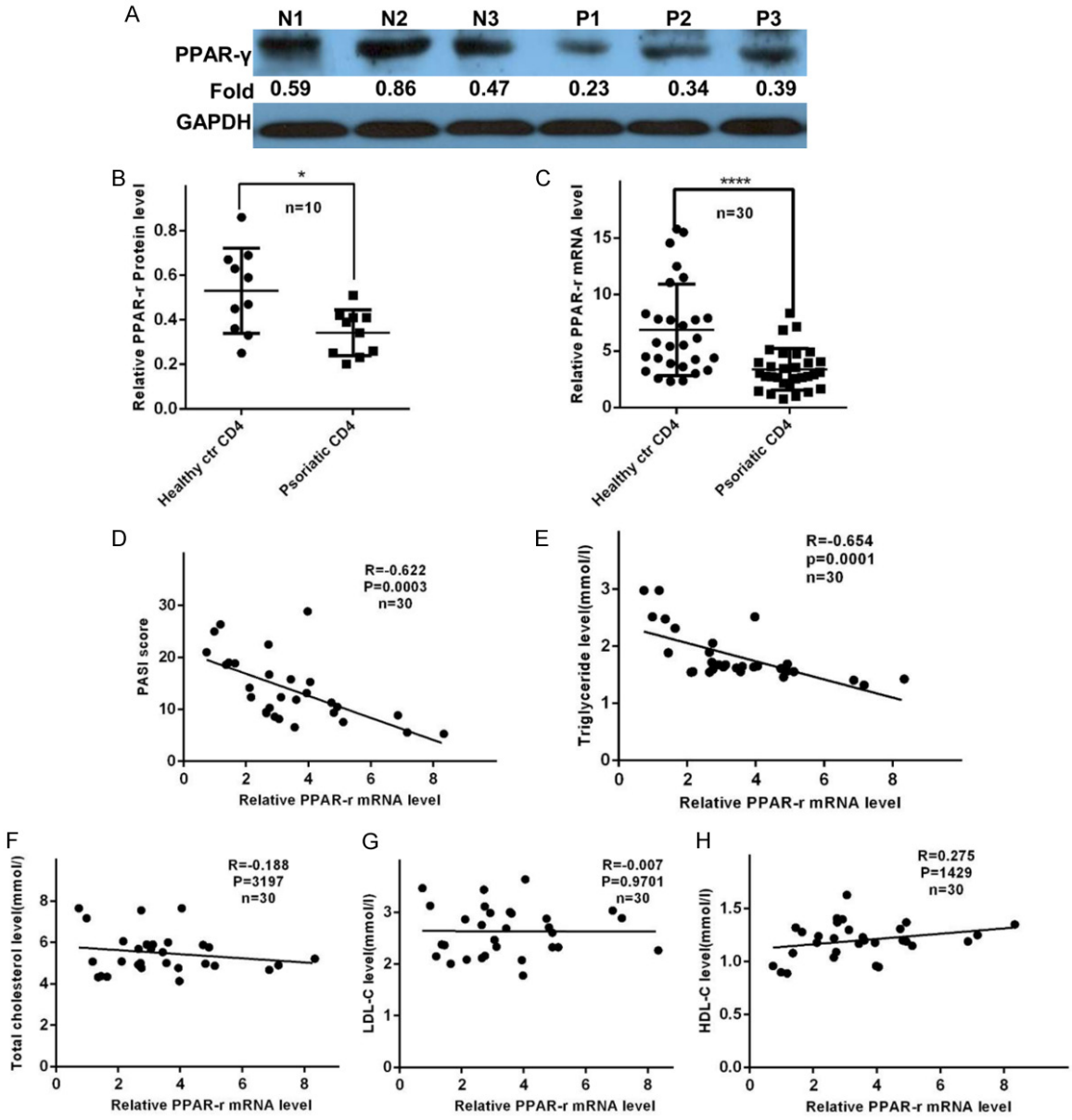

Figure 3. The changes of PPAR-γ expression level in $CD4^+$ T cells from the peripheral blood of patients with psoriasis and its correlation with PASI score and blood lipid level. The changes of PPAR-γ expression level in $CD4^+$ T cells from the peripheral blood of patients with psoriasis was examined by Western blot (A and B) and qPCR (C). The correlation of PPAR-γ expression level with PASI score (D), triglyceride (E), total cholesterol (F), LDL-C (G), and HDL-C (H) was also assessed with absolute value of R greater than 0.05 and $P < 0.05$ considering to be correlative.

gered by fatty acids and exogenous peroxisome proliferator, by which to regulate the expression of certain enzymes involved in lipid metabolism and gene transcription, so as to regulate blood lipid levels and adipocyte differentiation [25]. In addition, it also mediates the expression of multiple nuclear target genes, such as nuclear factor (NF)-κB [26], signal transducer and activator of transcription (STAT) [27] and activator protein (AP)-1 [28], thus blocking the transcription of pre-inflammatory factors and exhibiting multiple biological effects. Studies have confirmed that PPAR-γ plays a critical role in regulating Th17/Treg cell balance. PPAR-γ agonists inhibit the differentiation of Th17 cells while promoting Treg polarization, and suppress inflammatory responses by inducing the production of IL-10 and inhibiting the generation of

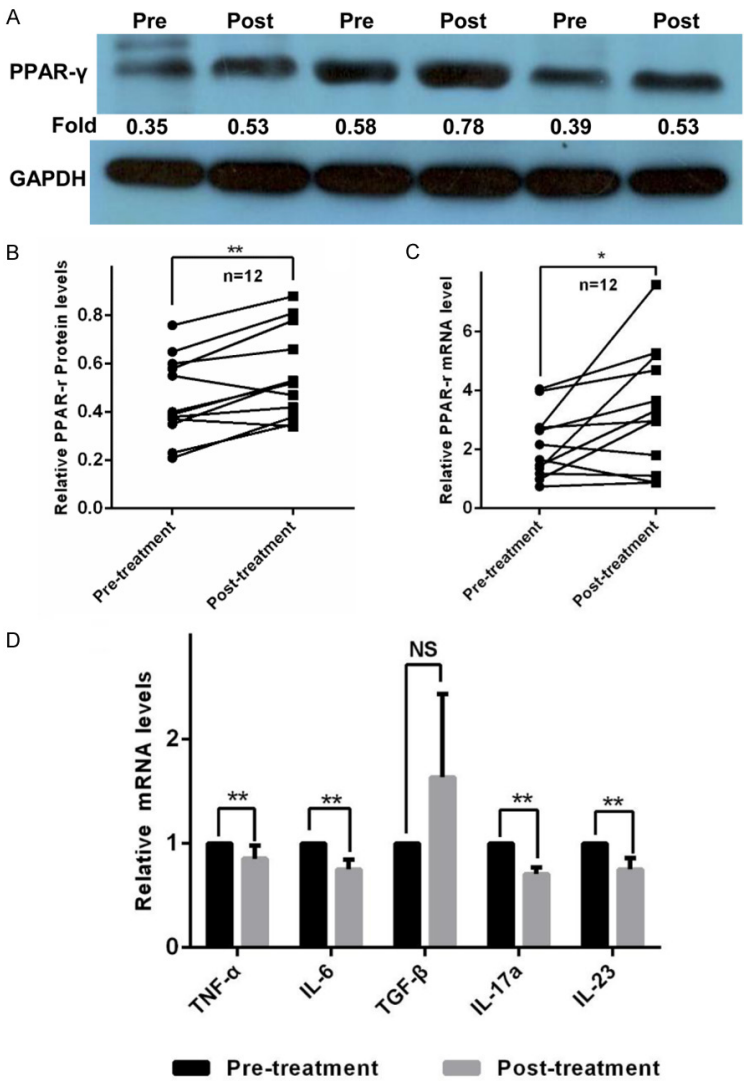

Figure 4. The impact of OAHT on PPAR-γ expression. The impact of OAHT on PPAR-γ expression in $CD4^+$ T cells from peripheral blood of patients with psoriasis was examined by Western blot (A and B) and qPCR (C); and the changes of the relative cytokines in $CD4^+$ T cells from peripheral blood of patients with psoriasis was determined by q-PCR (D).

IL-17a, IL-17f, IL-22, and IL-23 [29]. Activating PPAR-γ action on Treg cells can alleviate inflammatory response and increased insulin sensitivity in mouse [30]. Activation of PPAR-γ can induce moderate increases in HDL-C levels in human in two large clinical trials [31, 32]. The mechanism underlying the HDL-C and PPAR-γ is not well known. One possible explanation is that PPAR-γ upregulates expression of ABCA1 in macrophage, which leads to flux of cholesterol from cells to form nascent HDL, thus increasing HDL-C levels [33]. Our data verified that the expression level of PPAR-γ in $CD4^+$ T cells in psoriasis vulgaris as well as the increase in serum HDL-C levels, indicating their potential interacting mechanisms upon OAHT. Our study substantiated that ozone-mediated PPAR-γ level plays a key role in modulating the state of the inflammatory response to treat psoriasis.

Ozone therapy is playing an increasing role in many inflammatory diseases due to its bacteriostasis, oxidative stress, immune regulation, and epigenetic regulation. Our previous studies have found that topical ozone therapy is safe and effective for the treatment of stable psoriasis vulgaris, with an efficacy equivalent to that of intermediate-acting glucocorticoids, and we found that ozonated oil is suitable for the long-term care and management of psoriasis [34]. Compared with topical ozone application, systematic ozone therapy has clearly advantages in improving metabolism, blood hypercoagulable, angiosclerosis, insomnia and rejuvenation of the body. A study showed that the application of OAHT had achieved an inspiring response in the field of anti-oxidation and anti-aging [35]. Since medical OAHT was initiated in Germany in the 1950s, some scholars have applied it for the treatment of chronic fatigue and anti-aging, due to its anti-oxidation action. In 2001, Tylicki innovatively confirmed that atherosclerotic ischemic diseases benefit from OAHT treatments. In his trial, he explained the mechanisms by referring to lowering fibrinogen concentration and blood viscosity and decreasing plasma cholesterol level [36]. However, there is no enough data to support its safety, we deprecate systemic ozone treatment for pregnant women and children. In consideration of its invasiveness, patients with local vessel intolerant treatment are not recommended to use this therapeutic strategy. Collectively, multiple studies have shown that ozone can improve the level of lipid peroxidation and increase the

activity of lipid metabolic enzymes, thus participating in lipid metabolism and decelerating blood lipids in the body. In this study, we found after ozone treatment, PPAR-γ level was significantly increased in psoriasis patients, and inflammatory factor expression level was also decreased obviously. Moreover, there was a marked improvement in skin lesions, which might be associated with ozone-induced PPAR-γ expression to inhibit the transcription and expression of inflammatory factors. The specific mechanism that ozone decreases blood lipid level is related to the up-regulating PPAR-γ expression in patients with psoriasis, which provides more theoretical basis for ozone therapy in psoriasis. Our study substantiated that ozone-mediated PPAR-γ level plays a key role in controlling the state of the inflammatory response to treat psoriasis.

The current study demonstrated that short term OAHT treatment attenuated the severity of disease in psoriatic patients and lower the level of blood lipids. Simultaneously, we found that PPAR-γ level in $CD4^+$ T cells of patients was increased by OAHT. Based on the fact that the PPAR-γ expression markedly reduced in $CD4^+$ T cells in psoriasis vulgaris in comparison with healthy controls, which was negatively correlated to the PASI score and the level of blood lipids in patients. Thus, we speculated OAHT treatment improves psoriasis by inducing PPAR-γ expression, which deserves further popularizing and applying in clinic settings.

Limitations

There are a few limitations of this study. 1) The changes of serum lipid profiles may be associated with dietary habits among individuals which data is not available; 2) Whether there is a causal correlation between PPAR-γ in $CD4^+$ T cells and lipid level is yet to be determined, which will be one of our future goals; 3) Whether and how OAHT affects lipid synthesis and metabolism associated tissues or cell types such as liver, intestine epithelial cells, adipocytes and macrophages, etc., are unknown. We also don't know whether OAHT affects other lipid synthesis related genes in addition to PPAR-γ. These potential causal correlations between OAHT and lipid profiles in patients will be investigated in future; 4) We cannot exclude the possibility that OAHT will cause oxidation of cholesterols and lead to cholesterol degradation. However, our data also shows HDL-C increases in psoriasis patients, indicating OAHT indeed regulates lipoproteins synthesis; the detailed mechanisms will be our focus for future studies.

Conclusion

Collectively, we found that OAHT treatment decreases blood lipid level and attenuates inflammatory responses in psoriasis by upregulating the expression of PPAR-γ, suggesting that OAHT is an effective treatment for psoriasis and is worthy of further clinical evaluation and application.

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Disclosure of conflict of interest

None.

Abbreviations

PPAR-γ, Peroxisome proliferator activated receptor-gamma; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; NF-κB, nuclear factor-κB; OAHT, ozone autohemotherapy; PASI, psoriasis area and severity index; RCM, reflectance confocal microscopy; SOD, superoxide dismutase; DCs, dendritic cells; Lp-PLA2, lipoprotein-related phospholipase A2.

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Table S1. Primer sequences for quantitative PCR

H-IL17A-F: 5'-ATTACTACAACCGATCCACCTC-3'	H-IL17A-R: 5'-TGGTAGTCCACGTTCCCAT-3'
H-IL6-F: 5'-AATTCGGTACATCCTCGACGGC-3'	H-IL6-R: 5'-GCCAGTGCCTCTTTGCTGCTTT-3'
H-TNFα-F: 5'-GGACACCATGAGCACTGAAAGC-3'	H-TNFα-R: 5'-TGCCACGATCAGGAAGGAGAAG-3'
H-TGFβ-F: 5'-GCAACAATTCCTGGCGATAC-3'	H-TGFβ-R: 5'-AAGGCGAAAGCCCTCAAT-3'
H-GAPDH-F: 5'-ATGGGGAAGGTGAAGGTCG-3'	H-GAPDH-R: 5'-GGGGTCATTGATGGCAACAATA-3'
H-IL23-F: 5'-GAGCCTTCTCTGCTCCCTGATA-3'	H-IL23-R: 5'-GACTGAGGCTTGGAATCTGCTG-3'
H-PPAR-γ-F: 5'-CCGCAGATTTGAAAGAAG-3'	H-PPAR-γ-R: 5'-AAGGAGTGGGAGTGGTCT-3'

Note: H = human; F = forward primer; and R = reverse primer.