International Immunopharmacology 73 (2019) 442–450

Contents lists available at ScienceDirect

International Immunopharmacology

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

Efficacy and safety of etanercept combined plus methotrexate and comparison of expression of pro-inflammatory factors expression for the treatment of moderate-to-severe plaque psoriasis

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ARTICLE INFO

Keywords:
Etanercept
Methotrexate
Psoriasis
Inflammatory factors

ABSTRACT

Etanercept has greatly improved management considerably. However, the efficacy and safety of combination therapy with methotrexate and the mechanism of action are not known. We aimed to describe the use of combined therapy of etanercept and methotrexate against moderate-to-severe plaque psoriasis in a Chinese population. We also wished to study the changes in expression of pro-inflammatory factors in peripheral blood mononuclear cells (PBMCs) and serum after the treatment to ascertain the mechanism of action. Thirty patients with moderate-to-severe plaque psoriasis were assigned into a monotherapy group and combination group randomly and equally. All patients received etanercept (50 mg, s.c., weekly), whereas patients in the combination group also received oral methotrexate (7.5–15 mg, p.o., weekly). Serum levels of interleukin (IL)-17A, IL-23, tumor necrosis factor (TNF)-α and their mRNA expressions in PBMCs were measured by ELISA and qRT-PCR. In the monotherapy group, Psoriasis Area and Severity Index (PASI) 50/75/90 responses were achieved by 86.7/66.7/40% after 24 weeks of treatment whereas, in the combination group, they were achieved by 93.3/80/60%, respectively. Although the overall prevalence of adverse effects (AEs) was higher in the combination group (60%) than in the monotherapy group (33.3%), the AEs were mild to moderate. The serum levels of IL-17A, IL-23, TNF-α, IL-33 and their mRNA expression in the PBMCs of the two groups were significantly higher than those of healthy controls ($P < 0.05$). Compared with the monotherapy group, serum levels of IL-17A, IL-23, TNF-α and their mRNA expression in PBMCs were decreased significantly in the combination group ($P < 0.05$). These data suggest that the efficacy of etanercept could be improved upon combination with methotrexate in the treatment of moderate-to-severe plaque psoriasis without increasing the risk of serious AEs. The mechanism of action might be associated with down-regulation of IL-17A, IL-23, and TNF-α.

1. Introduction

Psoriasis is a T cell-mediated chronic inflammatory disorder of the skin characterized by erythematous, scaly, sharply demarcated plaques, Also, it involves excessive proliferation of keratinocytes and infiltration of immune cells into the skin [1]. According to the epidemiological studies, the prevalence of psoriasis in China was 0.47%. The global prevalence of psoriasis is 2%–3% [2].

It has been hypthesized that T-helper (h)1 and Th17 cells participate in psoriasis pathogenesis [3,4]. These T-cell subsets, and the cytokines that they secrete, form an inflammatory environment that promotes the occurrence and development of psoriasis [5].

Increasing numbers of biologic agents are being approved by US Food and Drug Administration for psoriasis treatment. Compared with conventional treatment, biologic agents can relieve psoriasis and slow its progress.

Etanercept is a blocker of tumor necrosis factor (TNF)-α. It has been shown to improve disease symptoms and health-related quality of life (QoL) while maintaining acceptable safety in patients with moderate-to-severe plaque psoriasis [6–11].

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https://doi.org/10.1016/j.intimp.2019.05.042
Received 5 January 2019; Received in revised form 10 May 2019; Accepted 22 May 2019
Available online 30 May 2019
1567-5769/

Methotrexate is an immunosuppressant demonstrated to be efficacious against moderate-to-severe psoriasis [12]. In recent years, a combination of etanercept and methotrexate has achieved good efficacy against autoimmune diseases such as rheumatoid arthritis [13,14] and juvenile idiopathic arthritis [15], as well as psoriasis [16–18]. Such combination therapy can not only shorten onset time and improve clinical efficacy, but also reduce the side-effects of immunosuppressive agents.

The pathogenesis of psoriasis remains unclear, but accumulating evidence suggests that immune-mediated overproduction of inflammatory cytokines and amplified inflammation by keratinocytes are the major pathophysiologic characteristics [19,20].

Interleukin (IL)-17A plays a vital part in psoriasis development [19]. IL-17A is produced by immune cells in psoriatic lesions and directly affects expression of the keratinocyte genes involved in innate immune defenses [21]. By releasing chemokines and cytokines, keratinocytes actively maintain inflammatory microenvironments and sustain plaque development. Furthermore, TNF-α, which is highly expressed in psoriatic skin and serum [22], can amplify the effects of IL-17A on keratinocytes. Moreover, the serum and lesions of psoriasis patients have increased expression of IL-23, IL-27, IL-33 and IL-12 [23–25]. Therefore, the interplay among these cytokines expressed in psoriatic skin appears to be important for development and/or maintenance of psoriasis clinical features [26].

We wished to investigate the efficacy and safety of combination therapy of etanercept with methotrexate in moderate-to-severe psoriasis among a Chinese population. We measured expression of several related pro-inflammatory cytokines to explore the potential mechanism of action of this drug combination.

2. Materials and methods

2.1. Ethical approval of the study protocol

The study protocol was approved by the Ethics Committee of Shanghai Tenth People's Hospital (Shanghai, China). All patients and healthy controls (HCs) provided written informed consent before study enrollment.

2.2. Inclusion criteria

The inclusion criteria were patients: with moderate-to-severe plaque psoriasis by clinical features and/or histopathology with a Psoriasis Area and Severity Index (PASI) score ≥ 10; who had not undergone systemic immunotherapies within the preceding 2 months; who had not taken topical glucocorticoids within the preceding 2 weeks.

2.3. Exclusion criteria

Patients were excluded if they: had been treated with TNF-α inhibitors; had other autoimmune diseases, or significant renal/hepatic disease; had contraindications for phototherapy; were pregnant or breastfeeding.

2.4. Patients

Thirty patients (20 males and 10 females; mean age 51.93 ± 14.72 years) were enrolled in the present study. Fifteen sex- and age-matched HCs formed the control group for measurement of expression of related cytokines.

Randomization was undertaken with the use of computer-generated random numbers. The analysis points were weeks 2, 6, 12, 18, and 24 after treatment.

2.5. Tests

Besides measurement of body temperature, chest radiographs and electrocardiograms were examined. The following laboratory tests were also undertaken in all eligible patients 4 weeks before study commencement: routine blood examination; urinalysis; serum levels of creatinine, blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, total protein, alkaline phosphatase and bilirubin. The other tests carried out were: purified protein derivatives; interferon gamma release assay; antibody against the human immunodeficiency virus; hepatitis-B antigen and antibody; hepatitis-C antibody; human chorionic gonadotrophin. These tests were done at every analysis point as well.

2.6. Treatment regimens

Patients suffering from psoriasis were injected with etanercept (50 mg, s.c.) every week. The initial dose of methotrexate was 7.5 mg/week, and was increased to 10 mg/week after 2 weeks of treatment. After 4 weeks of therapy, the methotrexate dose was increased to 12.5 mg/week, and the dose was maintained at 15 mg/week from week-6 to the endpoint (Fig. 1). During the entire treatment period, no other therapy was allowed.

2.7. Outcomes

We wished to evaluate the efficacy (change in PASI score, static Physician's Global Assessment (sPGA), Patient's Global Assessment (PtGA), Dermatology Life Quality Index (DLQI) and recurrence prevalence) as well as safety (clinical and laboratory abnormalities) of etanercept treatment and etanercept + methotrexate. We also made a comparison between etanercept monotherapy and etanercept + methotrexate.

The PASI score was determined by a dermatologist at 2, 6, 12, 18 and 24 weeks of treatment. Patients with a reduction in the PASI score > 50% were considered to be “moderate responders” (PASI 50). A “significant response” was defined as a reduction in the PASI score of > 75% (PASI 75). “Almost complete clearing” was defined as a reduction in the PASI score of > 90% (PASI 90). “Recurrence” was defined as an increase of 50% in the PASI score from baseline after drug withdrawal for 12 weeks. The DLQI is a validated instrument for dermatologic conditions. Scores range from 0 points to 30 points, with higher scores indicating a greater effect upon QoL [27].

2.8. Preparation of peripheral blood mononuclear cells (PBMCs)

PBMCs were collected from 5 mL of heparinized blood samples and isolated by density gradient centrifugation using Ficoll-Hypaque™ (PAA Laboratories, Vienna, Austria). PBMCs were suspended in 90% fatal bovine serum (PAA Laboratories) with 10% dimethyl sulfoxide (Sigma–Aldrich, Saint Louis, MO, USA).

2.9. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from thawed PBMCs using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) and converted to cDNA using a PrimeScript™ RT Reagent kit (TaKaRa Biotechnology, Shiga, Japan). cDNA was used as a template for qRT-PCR employing SYBR® Premix Ex Taq II (TaKaRa Biotechnology) on a PCR 7500 system (Applied Biosystems, Foster City, CA, USA). PCRs were cycled 40 times after an initial denaturation (95 °C for 30 s), followed by 95 °C for 5 s, and 60 °C for 30 s. The relative fold change of mRNA expression was calculated by the $2^{-Ct\triangle\triangle}$ method.

The gene-specific primer pairs (forward and reverse, respectively) were: 5′- TTGATGCTCTCGCTCTTC-3′ and 5′-CTTCCTCCTCTTCCT

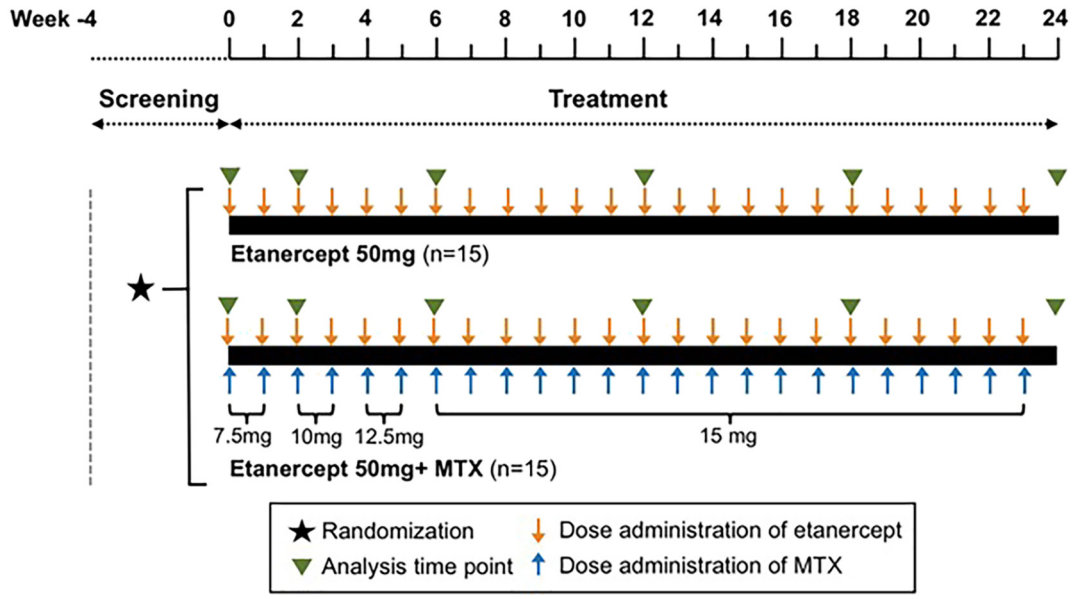

Fig. 1. Study design.

CCA-3′ for IL-17; 5′- TCCTTCAGACACCCTCAACC-3′ and 5′-AGGCCCC AGTTTGAATCCTT-3′ for TNF-α; 5′- TGTTGTGAAAATGCTACAGG-3′ and 5′-GCTTGTGTTCTGGGATGA-3′ for IL-23; 5′-ACCGCTTTGCGGAA TCTCA-3′ and 5′-AGGTCAGGGAAACATCAGGGA-3′ for IL-27; 5′-TAG GAGAGAAACCACCAAAA-3′ and 5′-ATACCAAAGGCAAAGCAC-3′ for IL-33; 5′- CCTTGCACTTCTGAAGAGATTGA-3′ and 5′-ACAGGGCCATC ATAAAAGAGGT-3′ for IL-12; 5′-AGAGCTACGAGCTGCCTGAC-3′ and 5′-AGCACTGTGTTGGCGTACAG-3′ for actin.

2.10. Quantification of serum levels of cytokines

Serum was centrifuged from 5 mL of blood samples and stored at −80 °C until use. Serum levels of IL-17, IL-23, TNF-α, IL-27, IL-33 and IL-12 were measured using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer (eBioscience, San Diego, CA, USA) instructions.

2.11. Statistical analyses

Statistical analyses were undertaken using SPSS 20 (IBM, Armonk, NY, USA). Measurements are expressed as the mean ± standard deviation. The Student's *t*-test was used to evaluate the difference between the two groups for quantitative variables with a normal distribution. One-way ANOVA was employed to analyze continuous variables. The Spearman correlation regression test was used to assess the correlation between quantitative variables. $P < 0.05$ was considered significant.

3. Results

3.1. Clinical assessment

The 30 patients were divided randomly and equally into a monotherapy group and combination group (Table 1). Demographic and baseline disease characteristics were similar across both groups. All 30 patients completed 24 weeks of treatment according to the study protocol.

3.2. Efficacy

The PASI score (mean ± SD) at baseline was 30.31 ± 9.32 in the monotherapy group and 28.97 ± 7.09 in the combination group. After 24 weeks of treatment, the PASI scores decreased to 7.10 ± 2.94 and 5.31 ± 2.35, respectively (Fig. 2a, Table S1). The change in the PASI score from baseline to 24 weeks was 86.4 ± 3.8% in the monotherapy group and 92.8 ± 2.6% in the combination group. There were significant differences in the change of the PASI score from baseline to 2, 6, 12 and 18 weeks ($P < 0.05$ for all). However, the difference in the response between the two groups was not significant at 24 weeks ($P = 0.194$) (Fig. 2b, Table S1). Fig. 3 shows the changes in skin lesions of patients before and after treatment in both groups.

Table 1
Clinical characteristics of psoriasis patients and healthy controls.

	Healthy controls (n = 15)	Psoriasis patients (n = 30)	P value
Sex(male/female)	9/6	20/10	> 0.05
Age(years)	37.47 ± 12.72	51.93 ± 14.72	> 0.05
Duration of psoriasis (years)	N/A	19.40 ± 10.27	N/A
PASI score	N/A	26.64 ± 8.95	N/A

Plus–minus values are means ± SD.
PASI, Psoriasis Area and Severity Index.

PASI 50 was achieved in 86.7% of the monotherapy group and by 93.3% in the combination group at week-24 (Fig. 4a–c). PASI 75 was achieved in 66.7% of the monotherapy group and by 80.0% in the combination group at week-24. Almost complete clearing (PASI 90) was achieved in 40.0% of the monotherapy group and by 60.0% in the combination group at week-24. Detailed information is provided in Table S2.

A significantly decreased sPGA score was found in the combination group compared with the monotherapy group at week-12 (1.87 ± 0.68 vs. 2.86 ± 0.64; $P < 0.05$), week-18 (1.25 ± 0.43 vs. 1.86 ± 0.83; $P < 0.05$) and week-24 (0.25 ± 0.23 vs. 0.71 ± 0.45; $P < 0.05$) (Fig. 4d).

A significantly higher PtGA score was observed in the combination group than that in the monotherapy group at week-12 (2.36 ± 1.16 vs. 2.36 ± 1.16; $P < 0.05$), week-18 (1.93 ± 0.94 vs. 3.10 ± 0.85; $P < 0.05$) and week-24 (0.49 ± 0.19 vs. 2.30 ± 0.63; $P < 0.05$) (Fig. 4e).

The DLQI score declined in the combination group compared with that in the monotherapy group at week-12 (9.28 ± 2.16 vs. 2.36 ± 1.16; $P < 0.05$), week-18 (1.93 ± 0.94 vs. 3.10 ± 0.85; $P < 0.05$) and week-24 (0.49 ± 0.19 vs. 2.30 ± 0.63; $P < 0.05$)

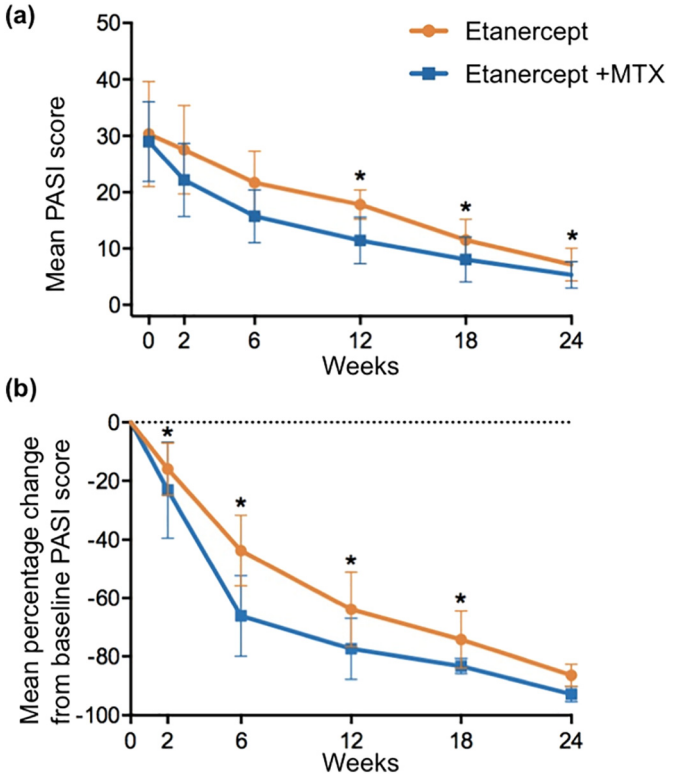

Fig. 2. Mean PASI score (a) and percentage change from baseline PASI score (b) over time for patients randomized to monotherapy and combination therapy groups. PASI, Psoriasis Area and Severity Index. * $P < 0.05$ for monotherapy group vs. combination group.

(Fig. 4f). Table S3 presents the detailed data.

Only one patient (6.7%) had disease recurrence in the combination group. Three patients (20.0%) in the monotherapy group suffered disease recurrence (Table S4).

3.3. Safety

The side-effects were mild and moderate in both groups during the entire treatment period (Table 2). At week-24, adverse effects (AEs) were reported in 60% of patients in the combination group and in 33% in the monotherapy group. No serious AEs were reported. None of the patients discontinued treatment because of AEs.

3.4. mRNA expression of cytokines in PBMCs

qRT-PCR demonstrated that the relative mRNA expression of IL-17A, TNF-α, IL-23 and IL-33 in PBMCs of all patients was significantly higher than those in HCs before treatment ($P < 0.05$ for all) except that of IL-27, which was significantly lower ($P < 0.05$) (Fig. 5). The relative mRNA expression of IL-12 was not significantly different before and after treatment. Expression of these cytokines (except IL-27 and IL-12) decreased significantly after treatment ($P < 0.05$ for all). The relative mRNA expression of IL-17A, IL-23 and TNF-α in the PBMCs of psoriasis patients after combination treatment declined significantly compared with that of the monotherapy group ($P < 0.05$ for all). Nevertheless, the relative mRNA expression of IL-33, IL-27 and IL-12 in PBMCs showed no significant difference between the monotherapy group and combination group after 24 weeks of therapy ($P < 0.05$ for all).

3.5. Serum levels of cytokines

ELISA indicated that the serum levels of IL-17A, TNF-α, IL-23 and IL-33 were significantly higher in all patients than those in HCs before study commencement ($P < 0.05$ for all), and that they all decreased significantly after treatment ($P < 0.05$ for all). The serum level of IL-23 showed opposite expression to that of the other cytokines, whereas the serum level of IL-12 did not change significantly before and after therapy ($P < 0.05$ for all) (Fig. 6). Similar to the results of qRT-PCR in PBMCs, the serum levels of IL-17A, TNF-α and IL-23 of patients with psoriasis decreased significantly after combination treatment when compared with those in the monotherapy group ($P < 0.05$ for all). The serum levels of IL-33, IL-27 and IL-12 of psoriasis patients after treatment showed no significant difference between the two groups.

3.6. Expression of TNF-α, IL-17A, IL-23, IL-27, IL-33 and IL-12 mRNA in PBMCs

Quantitative real-time PCR demonstrated that the relative mRNA expressions of IL-17A, TNF-α, IL-23 as well as IL-33 in PBMCs of all patients were significantly higher than those in healthy controls before the treatment ($P < 0.05$), however, the down-regulated mRNA expression of IL-27 was measured in pre-treatment group ($P < 0.05$) (Fig. 5). When it comes to IL-12, the relative mRNA expression of it was not significantly different before and after the administration. As we predicted, the expression of these cytokines (except IL-27 and IL-12) decreased after the treatment ($P < 0.05$). The relative mRNA expressions of IL-17A, IL-23 and TNF-α in PBMCs of psoriasis patients after combination treatment significantly declined compared to monotherapy group ($P < 0.05$). Nevertheless, the relative mRNA expressions of IL-33, IL-27 and IL-12 in PBMCs seemed had no significant difference between monotherapy group and combination group after 24 weeks therapy.

3.7. Serum levels of TNF-α, IL-17A, IL-23, IL-27, IL-33 and IL-12

ELISA results indicated that the serum levels of IL-17A, TNF-α, IL-23 and IL-33 were significantly higher in all patients than those in healthy controls before the trial began, and they all decreased significantly after the treatment ($P < 0.05$). The serum level of IL-23 still showed opposite expression to other cytokines, while the serum level of IL-12 did not change significantly before and after the therapy. (Fig. 6). Similar to the results of qRT-PCR in PBMCs, the serum levels of IL-17A, TNF-α and IL-23 of patients with psoriasis were significantly decreased after the combination treatment when comparing to monotherapy group ($P < 0.05$), while the serum levels of IL-33, IL-27 and IL-12 of psoriasis patients after treatment showed no significant difference between two post-treatment groups.

4. Discussion

Biologic agents have become vital tools in psoriasis management. Etanercept is an anti-TNF biologic in which many efficacy and safety studies have been completed. It is manufactured by linking the extracellular portion of human TNF receptor P75 to the Fc segment of immunoglobulin-G1. It can bind to soluble TNF-α and TNF-β to inactivate their activities. It was the first TNF antagonist listed in China. In 2005, it was approved by the China Food and Drug Administration (CFDA) for treatment of rheumatoid arthritis, with specifications of 12.5 mg/bottle and 25 mg/bottle. In 2007, etanercept received CFDA approval for the treatment of moderate-to-severe plaque psoriasis in adults, as well as ankylosing spondylitis. According to the updated psoriasis guideline set by Alexander Nast et al. in 2015, consensus suggests that there is additional benefit of adding methotrexate to etanercept compared with etanercept monotherapy [28].

Etanercept monotherapy has been reported to have good effects for

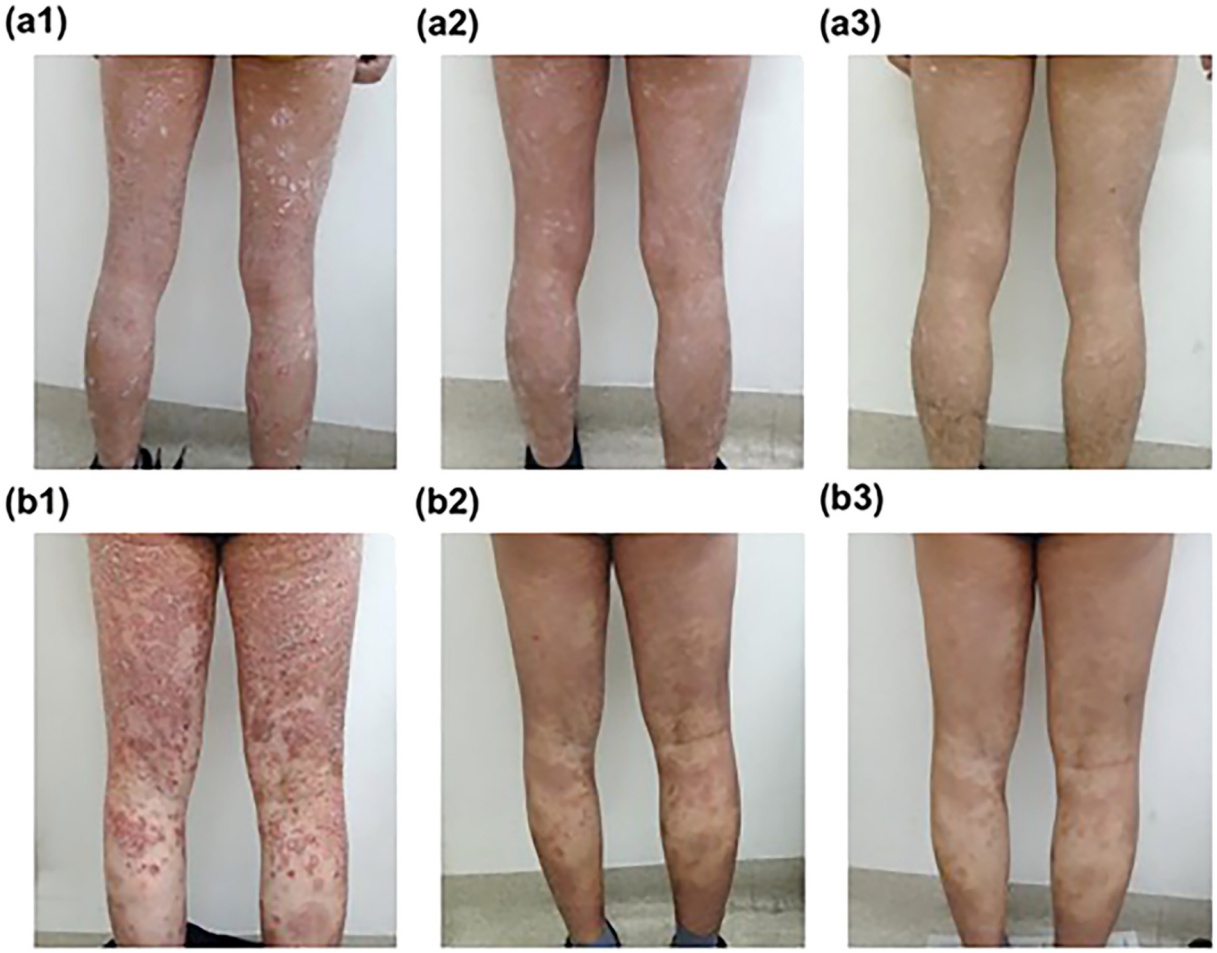

Fig. 3. Changes in skin lesions before and after treatments of patients in both groups. (a1): In the monotherapy group, there were large erythema and superficial hypertrophic scales existed at the lower extremities before treatment; (a2): Skin lesion reduced after 12-week etanercept monotherapy; (a3): skin lesions were almost cleared after 24 weeks etanercept mono-treatment. (b1): In the combination group, large erythema was covered by hypertrophic scales at the lower extremities before treatment; (b2): After 12 weeks of combined treatment, the skin lesions were almost cleared; (b3): After 24 weeks of combination treatment, there was only pigmentation left.

psoriasis treatment. In a randomized, double-blind study involving patients with moderate-to-severe psoriasis [29], patients received 12 weeks of treatment using etanercept (50 mg, s.c., twice a week) and the control group received placebo. The PASI-75 response was achieved by 58.8% of patients in the treatment group at week-12, which was significantly higher than that in the placebo group (5.6%). After 12 weeks of treatment, patients tolerated etanercept well, and AEs were mild and had a low prevalence.

Combination therapy of etanercept and methotrexate could improve the efficacy of psoriasis management. In a randomized, double-blind, placebo-controlled study undertaken by Gottlieb et al. [18], the mean PASI score of the combination group (etanercept + methotrexate) at the endpoint was 5.31, which was lower than that (7.10) in the monotherapy group (etanercept + placebo).

In our study, etanercept combined with methotrexate could increase the response of PASI 50/75/90. It could also improve the DLQI significantly in a shorter time with a lower prevalence of disease recurrence. These data suggest that combination therapy was more efficacious than etanercept monotherapy.

Interestingly, it has been reported that combination treatment may be more efficacious in patients who do not respond to etanercept monotherapy. In a retrospective analysis by Graziella et al. [30], methotrexate was used in 22 patients who had a poor response to etanercept treatment. The mean PASI score decreased from 12.5 to 2.5 after 22 weeks of treatment, which emphasized the importance of combination therapy.

Although biologics combined with immunosuppressive therapy have shown good clinical efficacy, physicians remain worried about the side-effects after combination therapy. According to Zachariae et al. [17], the prevalence of AEs was similar in the etanercept monotherapy group ($n = 28$) and the etanercept + methotrexate group ($n = 31$). Another clinical study showed that the combination group had a higher prevalence of AEs (75%) than the monotherapy group (60%) [18], but most of the AEs were mild-to-moderate. In addition, it has been reported that concomitant administration of methotrexate could reduce the formation of anti-drug antibodies (ADAs) [31]. Although the production of ADAs to etanercept is not high, they could still be produced, and possibly their number reduced, by methotrexate to some extent. In the present study, the prevalence of AEs was 33.3% and 60% in the monotherapy group and combination group, respectively. The proportion of patients with increased levels of transaminases in the combination group was 33.3%, whereas no patient in the monotherapy group had increased levels of transaminases. However, the increased levels of transaminases in the combination group were low, and did not result in withdrawal of the study agent; continuously increased levels of transaminases were not observed during follow-up.

The TNF-α/IL-23/IL-17 axis plays a significant part in the pathology of psoriasis [32–34]. TNF-α is as a pro-inflammatory cytokine secreted by Th17 cells and other types of inflammatory cells (keratinocytes, dendritic cells, dermal macrophages) [35]. TNF-α has complicated

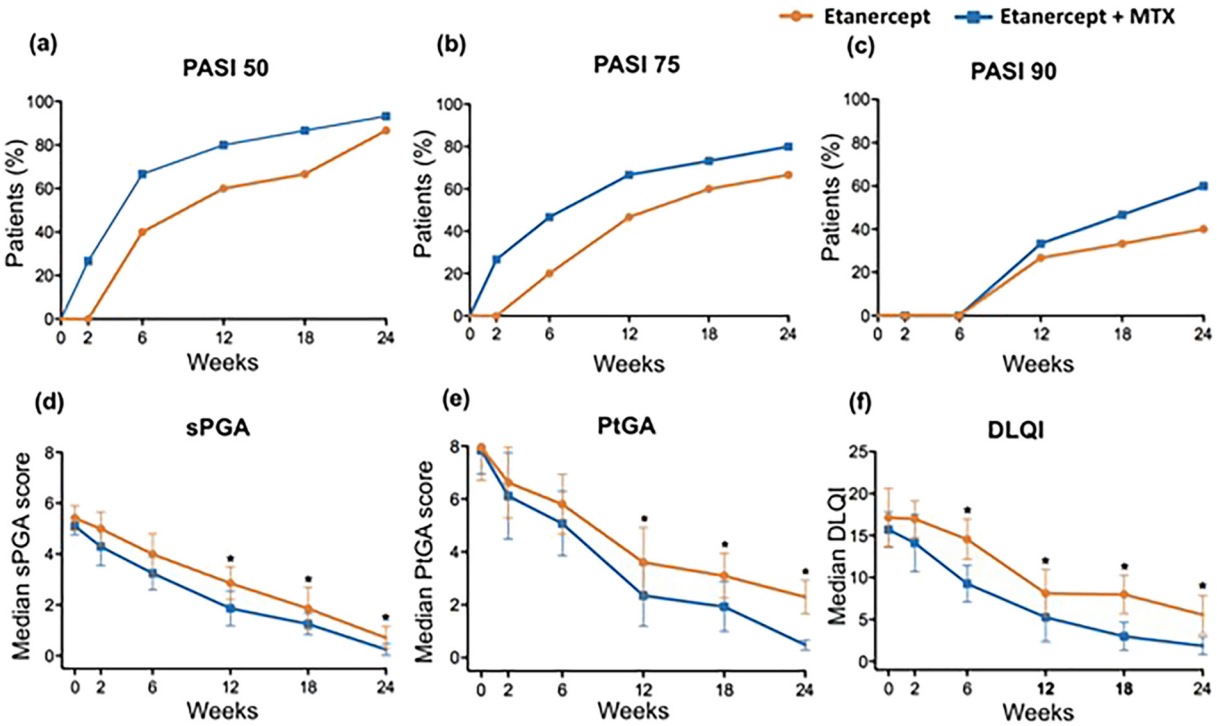

Fig. 4. Efficacy over time for patients randomized to etanercept or combination treatment by multiple imputation. Clinical response rates for (a) PASI 50, (b) PASI 75, (c) PASI 90, (d) sPGA, (e) PtGA, (f) DLQI from baseline to week 24. PASI 75/90/100, 75%/90%/100% improvement from baseline in Psoriasis Area and Severity Index; sPGA, static Physician's Global Assessment; PtGA, patient's global assessment; DLQI, dermatology life quality index. * $P < 0.05$ for monotherapy group vs. combination group.

Table 2
Adverse events occurring in patients in both treatment groups through 24 weeks.

	Etanercept	Etanercept + MTX
Elevated aminotransferase, *n* (%)	0 (0.0%)	5 (33.3%)
pyrexia, *n* (%)	1 (6.7%)	1 (6.7%)
Proteinuria, *n* (%)	0 (0.0%)	1 (6.7%)
Aberrant glycosuria, *n* (%)	1 (6.7%)	1 (6.7%)
Headache, *n* (%)	1 (6.7%)	0 (0.0%)
Insomnia, *n* (%)	1 (6.7%)	1 (6.7%)
Drowsiness, *n* (%)	1 (6.7%)	0 (0.0%)
Any AEs, *n* (%)	5 (33.3%)	9 (60.0%)

AE: Adverse effect.

effects on cell differentiation and expression of pro-inflammatory-associated genes. IL-17A can stimulate keratinocytes to secrete pro-inflammatory mediators that recruit additional inflammatory cells (neutrophils, Th17 cells, dendritic cells, innate lymphoid cells). Moreover, IL-17-regulated genes show high expression in skin with psoriatic lesions, and *IL-17* expression is down-regulated in psoriasis patients who respond to etanercept treatment [36,37]. IL-23 is produced by antigen-presenting dendritic cells. It is needed for the activation and survival of Th17 and Th22 cells, and its expression is increased significantly in psoriatic lesions [34]. IL-23 can stimulate the activation of Th17 cells and thereby secrete IL-17A.

The cytokines mentioned above are important for the progression of psoriasis. Hence, antibody-based treatments targeting these cytokines have shown remarkable success against moderate-to-severe psoriasis [38–40] . Therefore, we measured expression of TNF-α, IL-17A and IL-23 in the PBMCs and serum of patients to ascertain the mechanism of action. We demonstrated that expression of TNF-α, IL-17A and IL-23 was reduced significantly in the combination group compared with that in the monotherapy group. These data suggest that TNF-α, IL-17A and IL-23 might be the targets of combination treatment of etanercept and methotrexate in moderate-to-severe plaque psoriasis.

IL-33 is a recently discovered member of the IL-1 family. IL-33 is considered to be a dual-role cytokine that can promote and reduce inflammation depending on the tissue and cytokine environment [41]. We found that serum levels of IL-33 in patients with psoriasis were markedly higher than those in HCs, that this phenomenon was correlated with serum TNF-α levels in patients with psoriasis, and decreased after anti-TNF-α therapy. Initially, IL-27 was regarded to be a pro-inflammatory cytokine which acts by promoting differentiation of Th1 cells. However, accumulating evidence suggests that IL-27 has an anti-inflammatory role because it suppresses differentiation of Th17 cells and their IL-17 production [42]. IL-27 can also suppress the reinforcement of IL-17 expression on TNF-α-induced secretion of psoriasis-related chemokines by keratinocytes. IL-12 is an important pro-inflammatory cytokine that provides the principal signal for development of Th1 cells through the induction of interferon-γ synthesis. It has been reported that high levels of IL-12 are related to the clinical activity and severity of psoriasis [43,44]. However, we found that expression of IL-33, IL-27 and IL-12 had little or no effect in the combination group or monotherapy group. Hence, IL-33, IL-27 or IL-12 might not contribute to the improvement in treatment efficacy by etanercept + methotrexate for moderate-to-severe plaque psoriasis.

5. Conclusions

Our study suggested that combination therapy of etanercept and methotrexate could improve the PASI 50/75/90 response as well as the QoL of patients with moderate-to-severe psoriasis compared with those of etanercept monotherapy, and could also decrease the prevalence of disease recurrence. The prevalence of AEs in the combination group was higher than that in the monotherapy group, but all were mild AEs and did not result in treatment cessation. qRT-PCR and ELISAs revealed that combination treatment could down-regulate expression of IL-17A, TNF-α and IL-23 in PBMCs and serum more efficaciously than

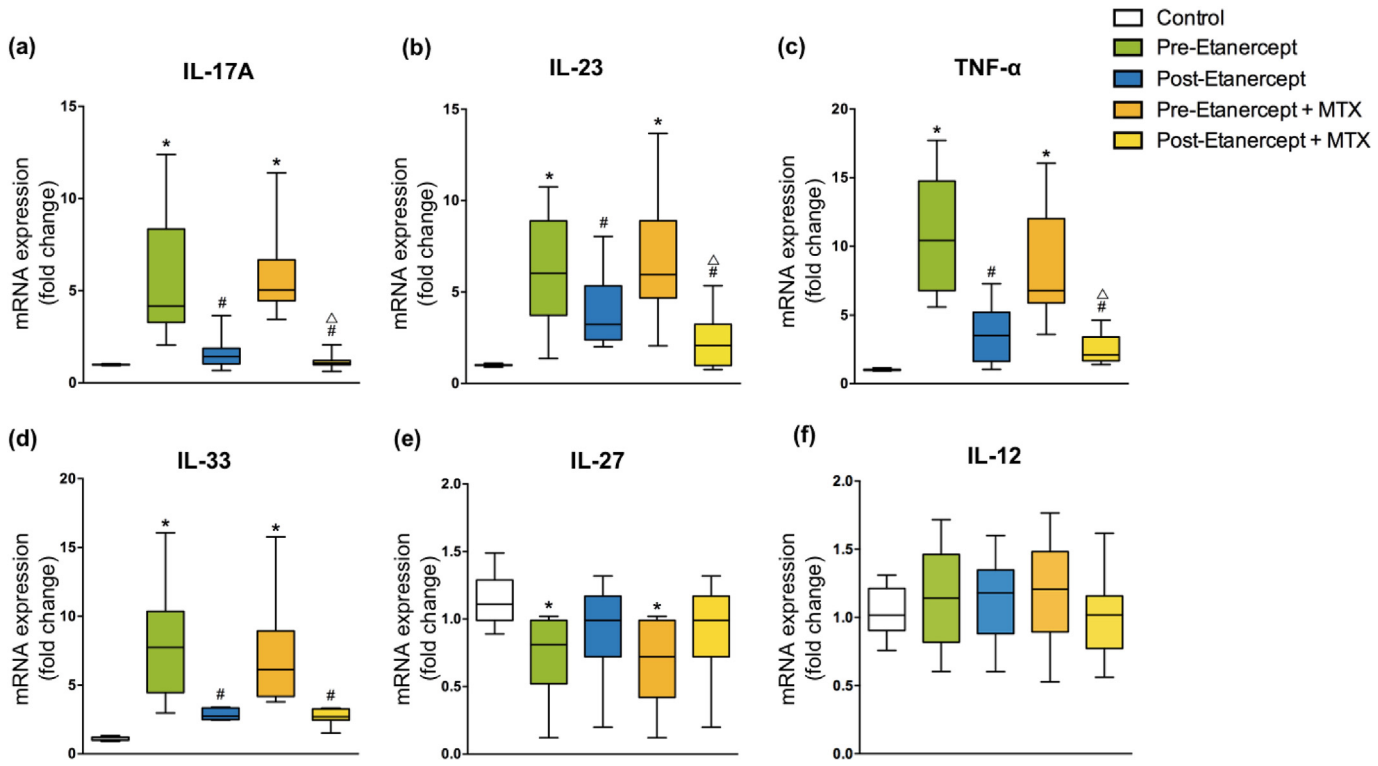

Fig. 5. Different mRNA expressions of IL-17A, IL-23, TNF-α, IL-33 and IL-27 in PBMCs. Relative mRNA expressions of IL-17A (a), IL-23 (b), TNF-α (c), IL-33 (d), IL-27 (e) and IL-12 (f) were measured by qRT-PCR in PBMCs of healthy controls and psoriasis patients before and after the monotherapy or the combination treatment. Data are presented as mean ± SD ($n = 15$), * $P < 0.05$ for pre-treatment or post-treatment group vs. control group; # $P < 0.05$ for post-treatment group vs. pre-treatment group; △ $P < 0.05$ for post-treatment of combination group vs. monotherapy group.

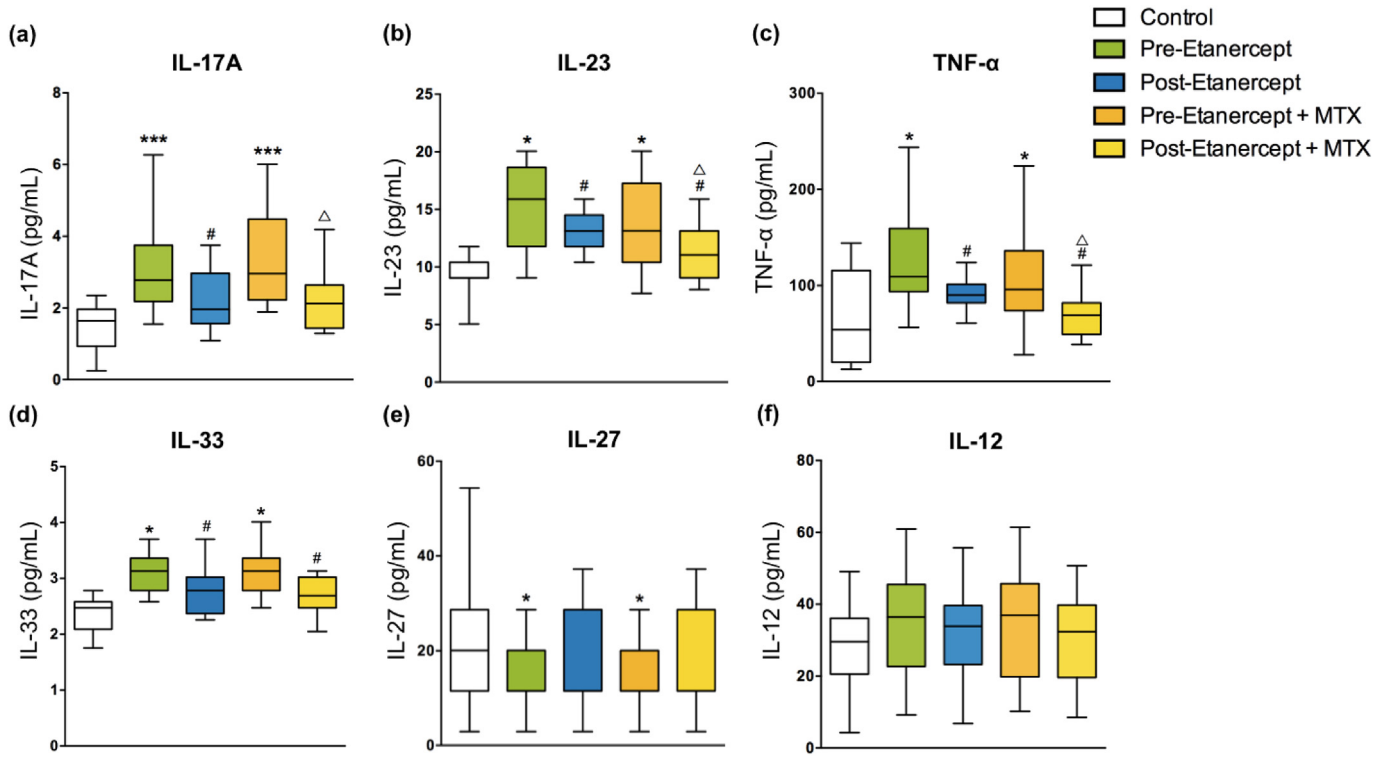

Fig. 6. Different serum levels of IL-17A, IL-23, TNF-α, IL-33 and IL-27 in healthy controls and patients. Serum levels of IL-17A (a), IL-23 (b), TNF-α (c), IL-33 (d), IL-27 (e) and IL-12 (f) were measured by ELISA in healthy controls and psoriasis patients before and after the monotherapy or the combination treatment. Data are presented as mean ± SD ($n = 15$), * $P < 0.05$, *** $P < 0.001$ for pre-treatment or post-treatment group vs. control group; # $P < 0.05$ for post-treatment group vs. pre-treatment group; △ $P < 0.05$ for post-treatment of combination group vs. monotherapy group.

monotherapy. Neutralizing the effects of IL-17A, TNF-α and IL-23 using etanercept plus methotrexate could be a successful strategy against psoriasis.

Declaration of Competing Interest

There is no conflicting interest.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (No. 81673050, 81872522), the Program of Science and Technology Commission of Shanghai Municipality (No. 18140901800), Innovation Program of Shanghai Municipal Education Commission (No.2019-01-07-00-07-E00046), Excellent Subject Leader Program of Shanghai Municipal Commission of Health and Family Planning (No. 2018BR30), Clinical Research Program of Shanghai Hospital Development Center (No. SHDC12018X06).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.05.042.

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