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# Losartan ointment attenuates imiquimod-induced psoriasis-like inflammation

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ABSTRACT

*Background:* Psoriasis is a chronic skin condition associated with interleukin-23/interleukin-17 (IL-23/IL-17) pathway. Recent evidence declares that angiotensin II (Ang II) induces a potent IL-17-related inflammation. Meanwhile, Losartan, an angiotensin one receptor (AT1R) antagonist, attenuates the TH17-related responses. Therefore, we investigated the possible beneficial effects of topically applied Losartan1% ointment on imiquimod (IMQ)-induced psoriasis in mice.

*Method:* Psoriasis was induced in mice consecutively for five days by topical IMQ on the shaved back. The IMQ-induced psoriasis was treated via topical administration of Losartan1% twice a day. The severity of skin inflammation was evaluated employing Psoriasis Area and Severity Index (PASI) scores. Subsequently, the skin samples were assessed using Baker's scoring system, stereological studies, and biochemical assessment with real-time PCR and immunohistochemistry.

*Results:* IMQ administration induced plaque-type psoriasis and skin inflammation. We characterized psoriatic lesions by hyperkeratosis, Munro abscess, rete ridges, and marked T-cell infiltrates. IMQ significantly increased epidermal volume, mRNA expression of IL-17a, IL-23, Ang II, AT1R, and TNF-α levels compared with the Placebo group. Topical administration of Losartan1% on IMQ-induced psoriasis significantly reduced the PASI scores and alleviated the erythema and scaling. The treatment significantly decreased the psoriatic thickness and dermal T-cell infiltration. Regarding biochemical assessment, Losartan1% considerably reduced the IMQ-induced increase of IL-17a, Ang II, and AT1R expression in the skin.

*Conclusion:* Topical Losartan1% significantly alleviates psoriasis by reducing AT1R and IL-17a expression. Our results introduce AT1Rs as a promising therapeutic target in psoriasis and represent a link between angiotensin and TH17-related inflammation. However, the effects of AngII-AT1R systems on IL-17 signaling need to be confirmed by further investigations.

## 1. Introduction

Psoriasis is a common autoimmune skin disease characterized by red patches covered with thick silvery scales, frequently on the extensor surfaces, lower back, and scalp. The lesions are associated with itch, pain, burning sensation and occasionally cause cracking and bleeding.

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Psoriasis is an autoimmune T cell-mediated disease with unknown triggering factors. Stimulation of plasmacytoid and myeloid dendritic cells (DCs) results in overproduction of some cytokines such as type I interferons, tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-6. These cytokines are causing an increase in IL-12 and IL-23 secretion. Moreover, the pathogenic T helper cells type 17 produce high levels of IL-17 in response to IL-23 and acts on keratinocytes, leading to a feed-forward self-amplifying loop, arriving at epidermal hyperplasia, innate immune response activation, leukocytes recruitment to the skin, and production of proinflammatory cytokines (e.g., IL-1b, IL-6, and IL-8). Blocking IL-17 reverses the pathologic circuit and resolves the clinical disease. In addition to IL-17 (including IL-17A and IL-17F), TNF-α, IL-26, and IL-29 are also released by the skin T17 producing cells [1].

Considering the central role of the interleukin-23/T helper (TH) 17 axis, many IL-23/IL-17 antagonists are either approved or under investigation for the treatment of psoriasis; however, not all the patients have a satisfactory response to IL-17 or IL-23 blockade. Additionally, TNF-α acts synergistically with IL-17 to sustain the inflammation in a psoriatic lesion; hence, dual inhibition of TNF-α and IL-17 is a promising therapeutic approach [1,2]. In other words, targeting multiple cytokines and pathways might be a suitable strategy to have the best treatment outcome.

The renin-angiotensin-aldosterone system (RAAS) is known for its blood regulation functions [3]; whereas, recent investigations have reported its significant role in autoimmunity [4]. For example, experimental models of multiple sclerosis (MS) and autoimmune encephalomyelitis (EAE) showed that angiotensin II (Ang II) induces Th1 and Th17 related inflammation. On the other hand, ACE inhibitors or angiotensin one receptors (AT1R) antagonists suppress the autoreactive TH1 and TH17 cells [4]. Interestingly, Losartan, an AT1R blocker (ARB), exerts anti-inflammatory properties by reducing the production of TNF-α, IL-1β, and chemokine (C–X–C motif) ligand 1 in a mice model of arthritis [5].

The skin expresses all components of RAAS. AT1, Ang II, and angiotensin-converting enzyme (ACE) are found in primary keratinocytes, melanocytes, dermal microvascular endothelial cells, and fibroblasts. AT2 receptors are expressed by melanocytes [6]. Noteworthy, T cells have an endogenous RAAS and express AT1 receptors [7]. Given the anti-IL-17 and TNF-α properties of AT1R blocking, we evaluated the effect of topical Losartan1% on imiquimod-induced psoriasis in mice models.

## 2. Material and method

### 2.1. Drugs and reagents

We used Losartan potassium powder (Cipla Co. India) and imiquimod 5% cream (Meda Pharmaceuticals, UK). Five grams of Losartan potassium powder were dissolved in 1 ml of 2-propanol (Merck Co. Germany) and 6 ml of distilled water to prepare Losartan1% ointment. Then we dispersed the solution in Eucerin (Emad Darman Pars Co, Iran) to reach the total weight of 100 g. Further, we made the Placebo ointment using the same ingredients as mentioned above without Losartan powder. We kept the prepared ointments at 4 °C temperature.

### 2.2. Animals

We purchased male Naval Medical Research Institute (NMRI) mice (outbred, 5–6 weeks, 25–30 g) from Pasteur Institute, Tehran, Iran. Animals were habituated to the laboratory setting in a standard room temperature (23-25° C) and a regular 12-hr light/12-hr dark cycle with a humidity of 55 ± 2 percentage. Animals were housed solely in polycarbonate cages with free access to food and water.

We designed our experimental groups to consist of four mice, using each animal once in this study.

### 2.3. Experimental design and Psoriasis-like inflammation

We tested on four experimental groups; the IMQ group, the IMQ + Losartan1% group, the IMQ + Placebo group, and the Placebo group. To induce psoriasis, mice received topical IMQ cream 5% (Aldara; Meda Pharmaceuticals, UK) at the dose of 62.5 mg on the shaved back for five consecutive days [8]. In addition, IMQ-treated mice have been treated with topical Losartan1% or Placebo ointments on the shaved back twice a day with an interval of 6 h in the treatment groups. Mice, treated similarly with Placebo prepared in-house (without Losartan), were used as a control group.

We objectively scored the severity of skin inflammation based on the Psoriasis Area and Severity Index (PASI) daily during the treatment. The objective scoring system includes the erythema, scaling, and thickening with the score range of zero to four (zero, none; one, slight; two, moderate; three, marked; four, very marked). Then, we summed up all the scores as the cumulative score (scale 0–12) to indicate the severity of inflammation [8]. In addition, all animals were weighed regularly, and the thickness of the back skin was measured using a digital micrometer at the days indicated.

Subsequently, we sacrificed three mice in each group by lethal doses of ketamine followed by cervical dislocation and removed the back skin and spleen for further analysis.

### 2.4. Histopathological score

A blinded inspector evaluated Hematoxylin-eosin-stained skin sections (3–4 μm) for the histological parameters characteristic of psoriasis using Baker's scoring system. We graded the histopathological score on a scale from 0 to 10 [9].

In addition, we evaluated three different fields for epidermal and dermal vertical thickness and the number of neutrophils and lymphocytes under 100 × high power and calculated the mean scores.

### 2.5. Stereological study

#### 2.5.1. Number of lymphocyte and neutrophil

The numbers of inflammatory cells at the psoriasis-like inflammation site were estimated by the optical dissector method. Microcator was applied to measure the Z-axis movement of the microscope stage. To avoid biased counting, the only cells counted were nuclei placed within the frame completely without crossing the exclusion lines. The numerical density of cells was estimated by the following equation (1):

$$Nv = \left[\frac{\Sigma Q-}{h \times \frac{Xa}{f} \times \Sigma p}\right] \times \left(\frac{t}{BA}\right)$$

Where "ΣQ-" is the number of the cells' nuclei, "h" is the height of the disector, "a/f" is the frame area, "ΣP" is the total number of counting frame in all fields, "t" is the real section thickness measured using the microcator and BA is the section thickness.

#### 2.5.2. Volume of epidermis, dermis

The volume of the epidermis, dermis at the psoriasis-like inflammation site was estimated by Cavalieri's principle following equation (1):

$$V = \sum P \times \frac{a}{p} \times t$$

Where (t) is the distance between the sampled sections. The (ΣP) is estimated by the point-counting method. The a/p is the area associated with each point projected on the skin tissue [10].

### 2.6. Immunohistochemistry (IHC)

We immersed the samples in a 10% buffered formalin solution and placed them in 70%, 80%, and 90% alcohols, respectively, and then xylene solution to dehydrate the tissue. After putting the skin samples in paraffin, tissues were sectioned. Next, the five-micron thick sections of paraffin-embedded tissues were prepared by microtome and adhered to silane-coated slides. To deparaffinized and hydrate, the tissues were put down at 90 °C for 20 min, then xylene solution, different percentages of ethanol, and water, respectively. The slides were soaked in H2O2 0.03% diluted in methanol and then in an antigen retrieval solution. After adding the primary antibody (Kit number: ORB19079) diluted in PBS with a ratio of 1 to 100, the samples were incubated overnight at 2 to 8 °C. The samples were then washed with PBS and received 100 μl of Linker for twenty minutes. Next, polymer and DAB buffers were added to the skin samples for 30 min and 5 min, respectively. In the last step, the samples were counterstained and observed under a light microscope. The intensity of stained cells was measured using the freeware ImageJ software[11] (rsb.info.nih.gov/ij) in three representative high power fields (HPF) in each sample.

### 2.7. Real-time quantitative PCR

The skin samples were preserved as snap-frozen in −80 centigrade degrees before use. We measured mRNA expressions of IL-17A, IL-23, AT1R, Ang II, and TNF-α by real-time quantitative PCR analysis. RNA was extracted in different steps by adding TRIzol (Qiazol, KIAGENE. USA), chloroform, isopropanol, and ethanol. First, we eliminated Genomic DNA by RNase Free DNase I. Next, ten microliters of the RevertAid First Strand cDNA Synthesis kits' solution (Thermo scientific. the USA) were added to 10 μl of extracted RNA to synthesize cDNA. Then, tubes were placed in a thermocycler at 25°for 5 min and 60°for 60 min. In the next step, 5 μl Master Mix (RealQ Plus Master Mix Green high ROX™, Amplicon. Denmark), 2 μl cDNA, 0.5 μl forward and 0.5 μl reverse primers, 2 μl Nuclease-Free Water was mixed. The thermal cycling protocol was designed as follows: in the denaturation step, the microtubes were exposed to 95 °C for 15 min, and then in the annealing step, the specified cycle (15 s at 95 °C then 60 s at 60 °C) was repeated 40 times. Moreover, the products' melting curve was considered from 60 to 95° C. GAPDH was used to normalize Real-time PCR data. Further, the primers' TM temperatures were between 58 °C to 60 °C, and the maximum difference between the Tm temperature of the two primers was 1°. The competitive critical threshold (ΔΔCT) method was utilized to analyze data.

### 2.8. Statistical analysis

We analyzed data and drew the figures by GraphPad Prism 8. All data are presented as mean ± standard error of the mean (SEM) and were analyzed by one-way or two-way analysis of variance (ANOVA) alongside Tukey's multiple comparison test. $P < 0.05$ was considered significant. We used the biorender.com website platform to prepare the graphs.

### 2.9. Ethics approval statement

All experiments were in agreement with the Guidelines for the Care and Use of Laboratory Animal Ethics Committee of the national institute of medical research development (NIMAD) (Ethical code: IR.NIMAD.REC.1399.172) as well as the National Institutes of Health (NIH publication NO. 85–23; revised 1985). The committee for animal ethics and experiments at Tehran University of Medical Sciences, Tehran, Iran, approved the study protocol.

## 3. Results

### 3.1. Losartan treatment ameliorates the clinical appearance of IMQ-induced skin inflammation in mice

We first assessed whether topical IMQ application induces psoriasis-like skin inflammation in NMRI mice. Three to four days following the start of IMQ application on the shaved back skin, signs of erythema, scaling, and thickening appeared, which continually increased in severity until the end of the experiment (Fig. 1A-D).

Our data revealed that time was independently effective on the changes of erythema ($F(5, 72) = 20.40$; $p < 0.0001$), scaling ($F(5, 72) = 40.11$; $p < 0.0001$), skin thickness ($F(5, 72) = 35.18$; $p < 0.0001$) scores as well as the cumulative PASI score ($F(5, 72) = 86.11$; $p < 0.0001$). Comparison between the treatment groups over time showed a significant difference ($p < 0.0001$). Additionally, our results showed a significant difference in erythema ($F(3, 72) = 26.67$; $p < 0.0001$), scaling ($F(3, 72) = 41.00$; $p < 0.0001$), skin thickness ($F(3, 72) = 25.69$; $p < 0.0001$) and cumulative PASI score ($F(3, 72) = 70.24$; $p < 0.0001$) between different treatment groups (Fig. 1B-E). In addition, our findings indicated that the mean PASI score of the IMQ group was higher vs. the Placebo group on days 4 (mean difference = 3.5, S.E = 0.478, $p < 0.0001$), and this difference was worsened on days 5 (mean difference = 4.5, S.E = 0.478, $p < 0.0001$) and 6 (mean difference = 7.75, S.E = 0.478, $p < 0.0001$). Treatment with Losartan1% resulted in improvement of PASI score on days 5 ($p = 0.0026$) and 6 ($p < 0.0001$) when compared to the IMQ group, yet Losartan1% could not decrease the PASI score to the Placebo level ($p > 0.05$) (Fig. 1E).

Although the mean PASI score was also decreased in IMQ + Placebo group compared to the IMQ group on day 6 (mean difference = 2.75, S.E = 0.478; $p < 0.0001$), the mean PASI score was significantly less in IMQ + Losartan1% vs. IMQ + Placebo on days 5 (mean difference = 2.25, S.E = 0.478; $p = 0.0026$) and 6 (mean difference = 2.5, S.E = 0.478; $p = 0.0004$) indicating the effectiveness of Losartan1% on improving the PASI score (Fig. 1E).

Treatment with IMQ revealed a significant increase in the mean erythema (mean difference = 1.5, S.E = 0.288, $p = 0.0004$) and scaling scores (mean difference = 1, S.E = 0.216, $p = 0.0035$) from day 4 compared to the Placebo group, and this difference incremented by the end of the experiment (erythema: mean difference = 2.5, S.E = 0.288, $p < 0.0001$; scaling: mean difference = 2.75, S.E = 0.216, $p < 0.0001$) (Fig. 1B and C). Interestingly, the mean erythema score was significantly lower in the IMQ + Losartan1% group compared with the IMQ group (mean difference = 2, S.E = 0.288, $p < 0.0001$) on day 6; however, there was no significant difference between IMQ and IMQ + Placebo groups ($p = 0.11$) (Fig. 1B). The mean scaling score also showed an improvement in the IMQ + Losartan1% group compared with the IMQ-treated mice on days 5 (mean difference = 2.5, S.E = 0.216; $p < 0.0001$) and 6 (mean difference = 1.25, S.E = 0.216; $p < 0.0001$). Additionally, the difference between the IMQ + Losartan1% and Placebo groups on these days was not significant ($p > 0.05$) (Fig. 1C). The mean scaling score on day 6 was also lower in the IMQ + Placebo group than in the IMQ group (mean difference = 1.25, S.E = 0.216; $p < 0.0001$), indicating the therapeutic effect of Eucerin, which is the base ingredient of Placebo and Losartan1% ointments. Importantly, the mean scaling score was lower in the IMQ + Losartan1% group vs. the IMQ + Placebo group (mean difference = 1.25, S.E = 0.216; $p < 0.0001$; $p < 0.0001$) (Fig. 1C).

The results indicated an increase in the thickness with IMQ treatment after day 5 (mean difference = 1 S.E = 0.291, $p < 0.0001$) to the end of the experiment (mean difference = 2.5, S.E = 0.291, $p = 0.0005$); however, none of the treatments were effective to reduce the mean score of the thickness ($p > 0.05$) (Fig. 1D). We measured the results related to skin thickness with a micrometer and observed no significant difference between the experimental groups compared to the IMQ groups ($p > 0.05$). Additionally, time × treatment interaction was not significant ($F(15, 72) = 1.65$; $p = 0.08$) (Fig. 1F).

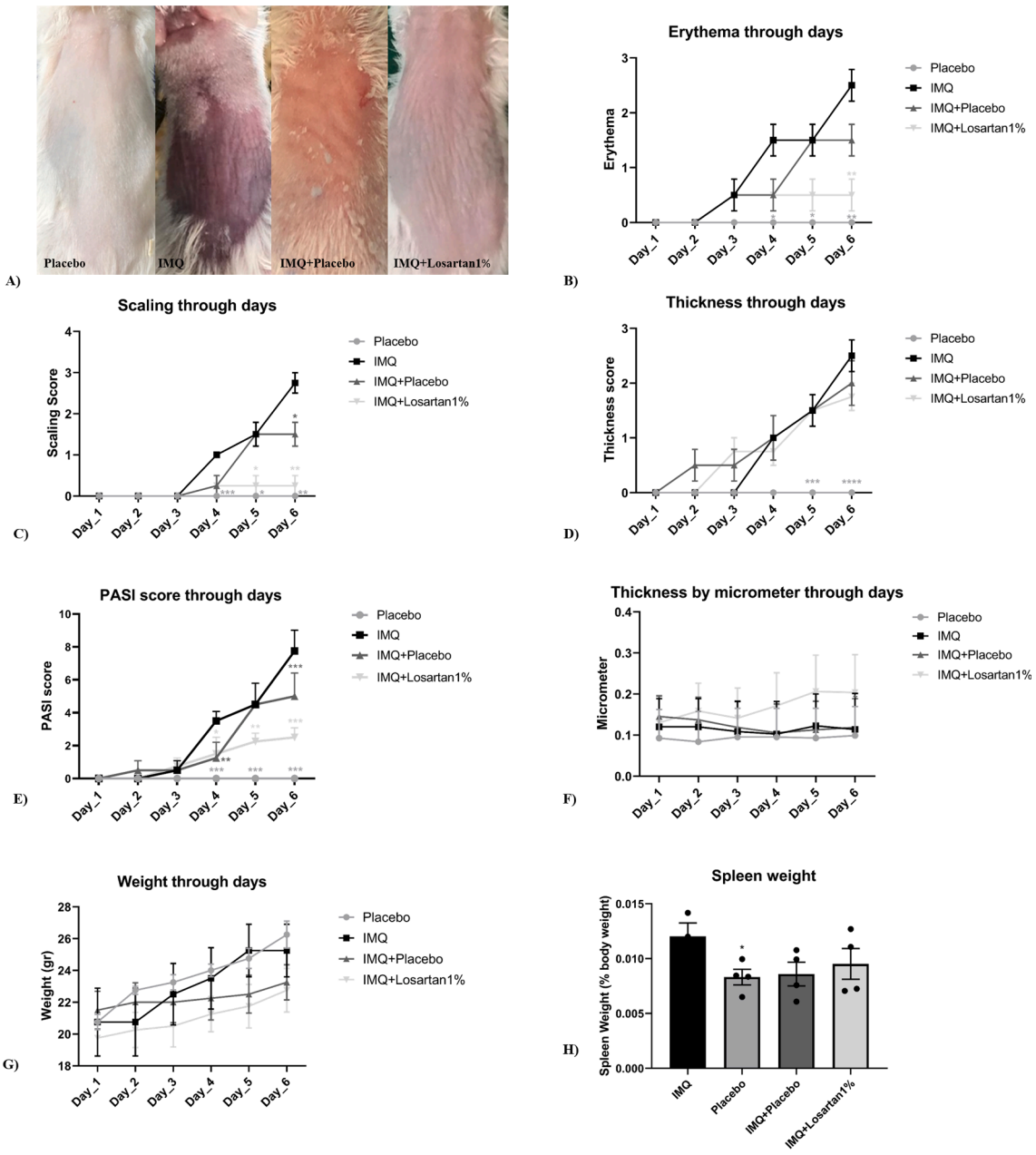


**Fig. 1.** Topical application of Losartan1% attenuates IMQ-induced skin inflammation in mice that resembles psoriasis. Mice have received daily IMQ cream or IMQ + Losartan1% or Placebo twice a day on the shaved back skin. **A.** Phenotypical presentation of mouse back skin after five days of different treatments. **B.** Erythema, **C.** Scaling, and **D.** Thickness of the shaved back skin were scored on a scale from 0 to 4 for five consecutive days. **E.** The cumulative score is the sum of erythema, thickness, and skin scaling scores on the sixth day (PASI score). **F.** Skin thickness and **G.** Mice's weight were measured daily. **H.** A non-significant increase in the mass of the spleen by applying topical IMQ. Symbols indicate the mean ± SEM of four mice per group. $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$ *compared to the IMQ-treated group.*

Our results displays an independent impact on changes of weight regarding time ($F(5, 72) = 4.214$; $p = 0.002$) and treatment ($F(3, 72) = 4.191$; $p = 0.008$). There was no significant difference between the groups over the time of the study ($F(15, 72) = 0.3312$; $p = 0.9$), indicating that the effect of treatment is not the same through days (Fig. 1G). Furthermore, we did not detect a significant spleen enlargement following IMQ treatment when compared the mean of spleen weight to body weight between the IMQ and Placebo groups ($p > 0.05$) (Fig. 1H).

### *3.2. Losartan treatment diminished pathologic characteristics of IMQ-treated skin*

Histopathologic findings showed that IMQ treatment caused a psoriatic-like reaction with hyperkeratosis, parakeratosis, acanthosis, presence of Munro micro-abscesses, moderate rete ridge formation, and a significant lymphocytic infiltrate (Fig. 2A). Further, it showed a statistically significant difference between groups ($F(3, 8) = 64.46$; $p < 0.0001$). Regarding pathological assessments, our results showed that the mean Baker's score of IMQ specimens (mean ± SEM = 4.3 ± 0.44) was significantly more than the Placebo group (0.16 ± 0.16; $p < 0.0001$). Furthermore, the histopathologic scores were markedly lower in IMQ + Losartan1% (0.5; $p < 0.0001$) and IMQ + Placebo (1.5; $p = 0.0001$) groups compared to the IMQ group. We did not observe any significant difference in the mean of Baker's score between IMQ + Losartan1% and Placebo groups ($p > 0.05$) (Fig. 2B).

While there were no significant changes in dermal volume among the

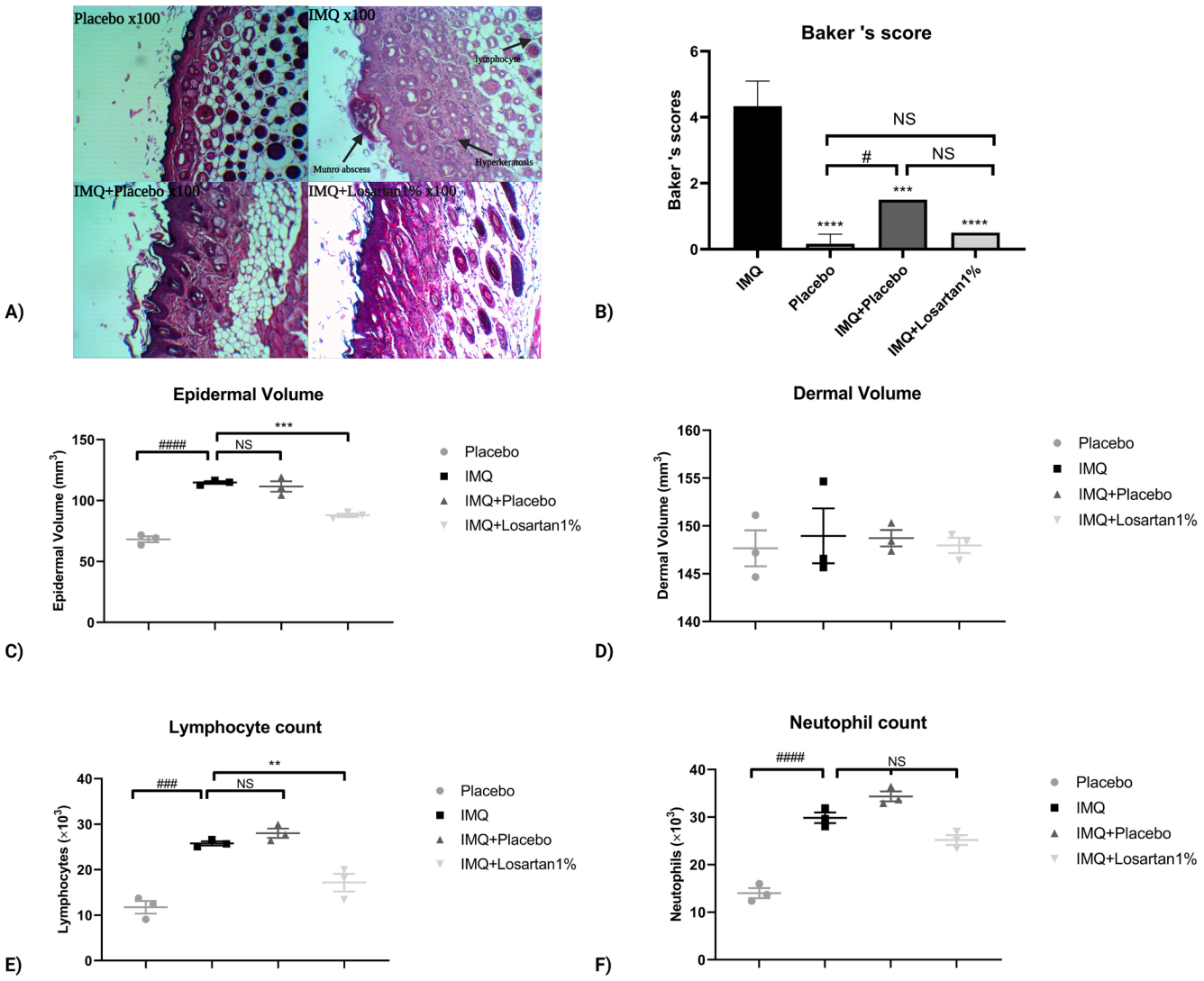


**Fig. 2.** Losartan1% reverses the IMQ-induces pathologic changes in the skin. Mice have received daily IMQ cream or IMQ + Losartan1% or Placebo twice a day on the shaved back skin. **A.** H&E staining of the back skin. In the IMQ group, we detected Munro's microabscesses in the keratin layer, lengthening and clubbing of rete ridges, and moderate-severe dermal lymphocytic infiltrate. IMQ + Losartan1% skin samples had only mild lymphocyte infiltrate in dermis **B.** Bars represent Baker's pathology score after five days of applying different treatments. **C.** Epidermal volume, **D.** Dermal volume, **E.** Lymphocyte count, and **F.** Number of neutrophils are defined with stereology technique. The data are presented as mean ± SEM of three mice per group. *$^{\#\#\#\#}$ $P < 0.0001$ and $^{\#\#\#}$ $P < 0.001$ and $^{\#}$ $P < 0.05$ compared to the Placebo group.* $^{****}$ *$P < 0.0001$,* $^{***}$ *$P < 0.001$ and* $^{**}$ *$P < 0.01$ compared to the IMQ treated group.*

groups, three-dimensional histological results using a stereology method demonstrated the significant difference in epidermal volume ($F(3, 8) = 69.63$; $p < 0.0001$), neutrophil count ($F(3, 8) = 67.25$; $p < 0.0001$) and lymphocyte infiltrates ($F(3, 8) = 32.85$; $p < 0.0001$) between the study groups ($F(3, 8) = 0.1163$; $p = 0.94$) (Fig. 2C-F). Compared with the Placebo group, IMQ administration resulted in a significant increase in mean of the epidermal volume (mean difference = 46.55, S.E = 3.7, $p < 0.0001$), neutrophil (mean difference = 15.84, S.E = 1.5, $p < 0.0001$) and lymphocyte count (mean difference = 14.04, S.E = 1.86, $p < 0.0001$) (Fig. 2 C, E, and F). The stereological study results showed that the epidermal volume (mean difference = 26.84, S.E = 3.7, $p = 0.0004$) and the mean number of infiltrated lymphocytes (mean difference = 8.6, S.E = 1.86, $p = 0.007$) were significantly lower in the IMQ + Losartan1% group vs. IMQ; however, there was no significant difference in these scales between IMQ + Placebo and IMQ groups ($p > 0.05$) (Fig. 2 C and E). Further, our findings revealed that Losartan1% diminished the number of infiltrated lymphocytes to the Placebo level, as there was no significant difference in lymphocyte count between the IMQ + Losartan1% and Placebo groups ($p > 0.05$) (Fig. 2E). Additionally, Losartan1% was ineffective in reducing the IMQ-induced high number of cutaneous neutrophils ($p = 0.056$) (Fig. 2F).

### 3.3. Losartan treatment reduced the high levels of AT1R in IMQ-treated skin

Our data revealed that IMQ treatment increased AT1R in the skin ($F(3, 8) = 74.38$; $p < 0.0001$) (Fig. 3A). Analysis of immunohistochemical staining of skin samples with ImageJ showed an increase in AT1R expression in the dermis with IMQ treatment compared with Placebo ($p < 0.0001$); however, Losartan1% significantly decreased the elevated levels of AT1R in comparison to the IMQ-treated skin ($p = 0.0005$). Although the difference between IMQ + Placebo and IMQ groups was also significant ($p = 0.035$), the AT1R levels were significantly less in the samples of the IMQ + Losartan1% group than IMQ + Placebo group ($p = 0.032$) (Fig. 3B).

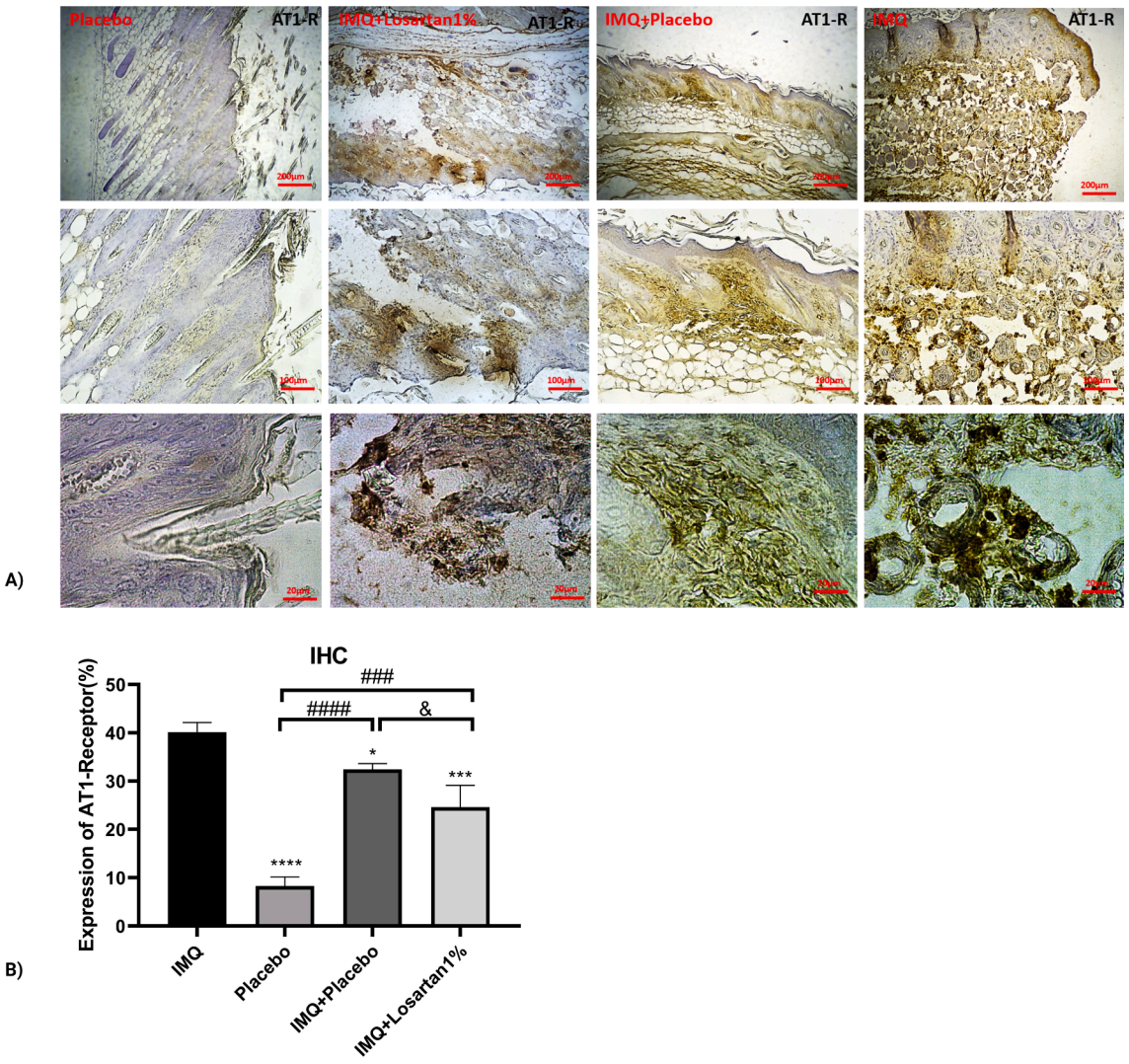


**Fig. 3.** Losartan1% reduces the increased expression of dermal AT1R following IMQ administration. **A.** Immunohistochemical analysis of AT1R in back skin samples. **B.** Bars graph shows the AT1R positive cells measured by ImageJ. Losartan1% significantly reduced the expression of AT1R in the dermis of IMQ-treated mice; however, there was still a significant difference between IMQ + Losartan1% and Placebo groups. The data are displayed as mean ± SEM of three mice per group. $^{****}$ *P < 0.0001,* $^{***}$ *P < 0.001, and* $^{*}$ *P < 0.05 compared to the IMQ-treated group.* $^{\#\#\#\#}$ *P < 0.0001 and* $^{\#\#\#}$ *P < 0.001 compared to the Placebo group.* $^{\&}$ *P < 0.05 shows the comparison between IMQ + Placebo* vs. *IMQ + Losartan1% groups.*

### *3.4. Losartan treatment inhibits the IMQ-induced increase of IL-17A and Ang II expression in the skin*

The IL-23/IL-17 axis and TNF-α involve in the pathogenesis of IMQ-induced psoriasis-like inflammation [8,12]. Therefore, we determined the expression of these key mediators by the RT-PCR technique. We also assessed the expression of Ang II and AT1R in the skin samples. The data showed a statistically significant difference between the study groups in terms of IL-23 ($F(3, 8) = 4.95$; $p = 0.03$), IL-17a ($F(3, 8) = 38.25$; $p < 0.0001$), TNF-α ($F(3, 8) = 6.28$; $p = 0.017$), Ang II ($F(3, 8) = 9.07$; $p = 0.006$), and AT1R ($F(3, 8) = 6.81$; $p = 0.013$) expression (Fig. 4A-D). Our results remarks a significantly higher expression of IL-23 ($p = 0.035$), IL-17a ($p < 0.0001$), TNF-α ($p = 0.033$), Ang II ($p = 0.008$), and AT1R ($p = 0.015$) with IMQ treatment compared to the Placebo (Fig. 4A-D). The expressions of IL-17a ($p = 0.001$) and Ang II ($p = 0.038$) were significantly lower in IMQ + Losartan1% compared with the IMQ group (Fig. 4B and D). Moreover, treatment with IMQ + Losartan1% significantly increased the IL-17a expression level in mice compared with Placebo ($p = 0.04$); yet the level of IL-17a was lower compared with the IMQ + Placebo group ($p = 0.009$) (Fig. 4B).

## 4. Discussion

Our results showed that the topical application of Losartan1%, an Ang II antagonist, significantly improved imiquimod-induced psoriasis-like skin lesions in mice by suppressing IL-17a expression. Interestingly, we found an increase in the cutaneous levels of Ang II and AT1R in the IMQ-treated mice, representing the involvement of the angiotensin system in psoriasis. Interestingly, Losartan1% significantly decreased the expression of Ang II.

IMQ-induced inflammation in mice simulates the features of psoriatic inflammation in humans. This model induces an IL-17/IL-23 dependent skin inflammation with erythematous plaques with overlying scale, hyperkeratosis, increasing skin thickness, and significant lymphocytic and neutrophilic infiltrates.

Aligned with previous studies, the PASI score started to rise significantly from day four after the IMQ application. While the topical application of Losartan1% significantly decreased the PASI score, Losartan1% significantly suppressed erythema and wholly resolved the scaling symptoms caused by IMQ. Although Placebo decreased the scaling score, the difference between IMQ + Losartan1% and IMQ + Placebo was statistically meaningful. Topical application of IMQ results

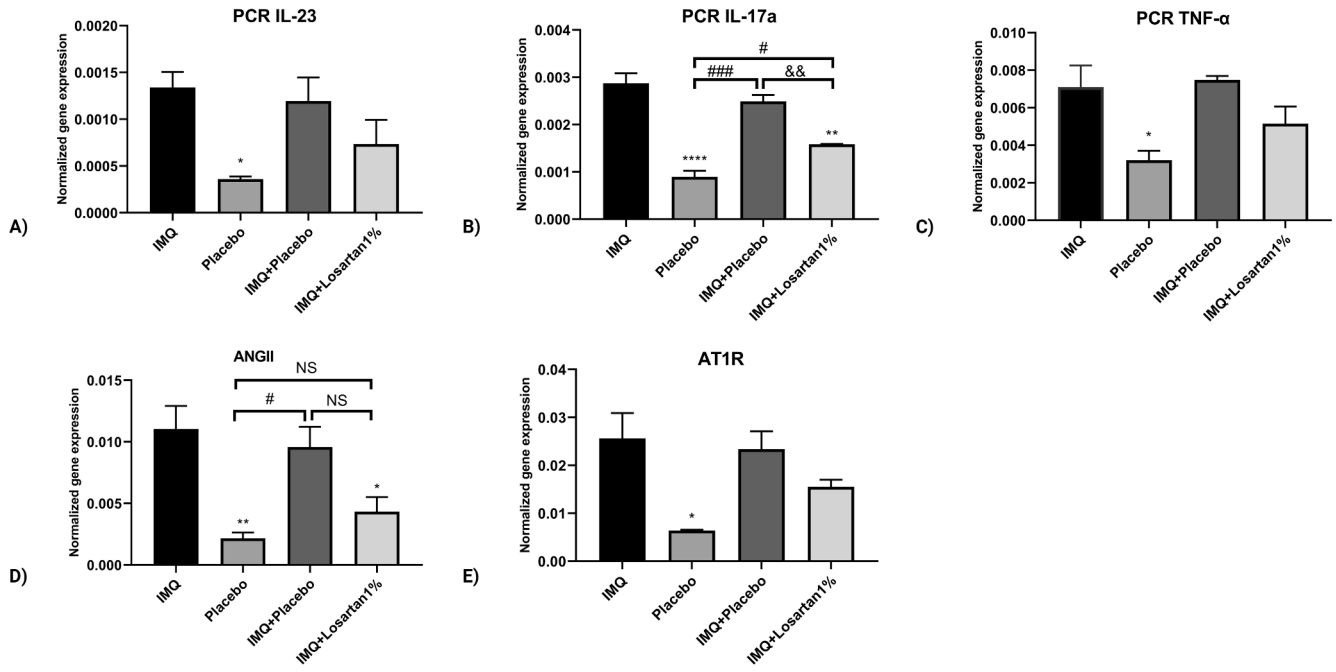


**Fig. 4.** Losartan1% reduces the IMQ-induced increase in the cutaneous expression of IL-17A and Ang II. RNA was extracted from back skin, and the expression of **A.** IL-23, **B.** IL-17a, **C.** TNF-α, **D.** ANGII, and **E.** AT1R was determined by quantitative RT-PCR. The data are presented as mean ± SEM of three mice per group. $^{****}P < 0.0001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ compared to the IMQ-treated group. $^{###}P < 0.001$ and $^{#}P < 0.05$ compared to the Placebo group. $^{\&\&}P < 0.01$ demonstrates the comparison between IMQ + Placebo and IMQ + Losartan1% groups.

in an increased spleen weight due to the activation of T cells and systemic inflammation [8]. On the other hand, our results did not show a significant spleen enlargement following IMQ treatment; nonetheless, further immunologic analysis on spleen samples is needed to determine the systemic inflammation. In addition, it would be worth addressing the systemic absorption of Losartan1% ointment.

Consistent with the changes in the clinical features, Baker's pathology score of the skin samples taken from the IMQ + Losartan1% group was significantly lower than the IMQ group. Pathologic results of IMQ-treated skin represented significant psoriatic-related changes, including hyperkeratosis, development of Munro micro-abscesses, acanthosis, and marked T cell dermal infiltrations. On the contrary, we detected only a mild lymphocytic infiltration in Losartan1%-treated skin samples. We also evaluated the skin changes with 3D stereological evaluation based on dermal T cell infiltration and epidermal and dermal thickness. Our results did not show any significant changes in dermal volume, neither by IMQ only treatment nor by Losartan1% cream. On the other hand, Losartan1% effectively reduced the epidermal thickness to the Placebo level, while IMQ + Placebo was ineffective. Increased epidermal thickening happens due to the hyperproliferation of keratinocytes [8]. Therefore, Losartan1% was effective in suppressing inflammation and keratinocyte hyperproliferation. The skin thickness measurement by micrometer showed no significant difference between IMQ and Placebo groups, which indicates the insensitivity of micrometer for measuring the epidermal thickness. Measuring epidermal thickness is challenging macroscopically, and although the increased thickness was obvious with IMQ treatment, we could not detect any changes in the skin thickness with IMQ + Losartan1% treatment.

IMQ-induced dermatitis is dependent on the presence of T cells [8]. While Losartan1% significantly reduced infiltrated T cells, three-dimensional histological analysis confirmed the increased T cells in the IMQ treated skin. In line with these changes, although our data showed that Losartan1% reduced the skin expression of IL-17a compared with IMQ and IMQ + Placebo groups, it was not to the level of Placebo. IL-23/IL-17 axis has a pivotal role in psoriasis and IMQ-induced dermatitis. Skin-resident dendritic cells (DCs) and macrophages rather than migratory myeloid cells are the primary sources of IL-23 [13]. In addition, IL-23 stimulates skin-resident γδ T cells, IL-17-producing CD4 + T helper cells (TH17), and IL-17 producing CD8 + T cells (Tc17), to secrete IL-17 and IL-22, cytokines that contribute to inflammatory leukocyte recruitment and epidermal hyperplasia, respectively [14,15].

TNF-α is another key mediator underlying the pathology of psoriasis that regulates the function of antigen-presenting cells [16]. Blockade of TNF-α has been effective in alleviating psoriatic severity in the clinic [15]. The qPCR results showed the increased cutaneous TNF-α in IMQ-induced dermatitis; however, Losartan1% did not significantly affect the expression level of TNF-α. The main source of TNF-α is a subset of myeloid inflammatory DCs, which acts as an activator of IL-23 synthesis in these cells [15]. Given that Losartan1% was not effective on IL-23 and TNF-α, we do not speculate that it targets DCs; nevertheless, due to its effect on suppressing skin IL-17a expression and epidermal hyperplasia, lymphocytes, specifically the IL-17-producing αβ and γδ T cells, IL-17a might be the primary target for Losartan1%, which would be valuable to be identified by further experiments.

Using other IL-17 dependent pathological models in mice, such as 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, Losartan had effectively ameliorated the mucosal TH17 responses [17]. Further, Ang II, directly and indirectly, promotes TH17 polarization via JAK2/STAT1/3 [18]. Madhur *et al.* found that Ang II infusion increases IL-17 production in the aortic media. Vascular dysfunction in response to Ang II was also abolished in IL-17$^{-/-}$ mice [19]. Moreover, a high salt diet in rats has been shown to trigger renal Th17 activation, which was significantly reduced by Losartan [20]. Our data with qPCR analysis showed that while Losartan1% significantly decreased the potentiated *Ang II* gene expression to the Placebo level, IMQ increased the skin expression of *AT1R* and *Ang II*. Our findings strongly confirm the role of *Ang II* in psoriasis pathogenesis. Interestingly, *meta*-analysis results stated that angiotensin-converting enzyme gene insertion/deletion polymorphism is linked to psoriasis susceptibility [21–24].

## 5. Conclusion

We found that topical administration of Losartan1% significantly improved IMQ-induced psoriasis-like dermatitis in mice. Treatment with IMQ in mice showed an increased expression of AT1R and Ang II. Losartan1% significantly suppressed Ang II expression to the Placebo levels. Interestingly, Losartan1% decreased the IL-17a levels, the critical immune regulator of psoriatic inflammation, in the skin of IMQ-treated mice, demonstrating the probable association of the angiotensin system with IL-17 type inflammation and psoriasis. Further studies are needed to elucidate the role of AT1R and IL-17a by administering Losartan in IL-17a knockout mice or pretreatment with anti-IL-17 antibody to eliminate all the possible variations. Since Ang II stimulates TH17 polarization via JAK2/STAT1/3 signaling pathway, it is recommended to evaluate the involvement of this pathway. It is also suggested that the future experiment investigate the effects of Losartan in psoriasis in the *in vitro* and clinical studies.

## CRediT authorship contribution statement

**Maryam Shokrian Zeini:** Data curation, Methodology, Formal analysis, Investigation, Writing – original draft. **Nazgol-Sadat Haddadi:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Maryam Shayan:** Data curation, Methodology, Investigation, Formal analysis, Writing – review & editing. **Mohadese Shokrian Zeini:** Data curation, Methodology. **Kiarash Kazemi:** Data curation. **Shahabaddin Solaimanian:** Data curation. **Mohammad-Amin Abdollahifar:** Formal analysis. **Keshvad Hedayatyanfard:** Resources. **Ahmad-Reza Dehpour:** Conceptualization, Project administration, Supervision.

## Declaration of Competing Interest

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

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## References

[1] J.E. Hawkes, T.C. Chan, J.G. Krueger, Psoriasis pathogenesis and the development of novel targeted immune therapies, J. Allergy Clin. Immunol. 140 (3) (2017) 645–653.
[2] T. Torres, M. Romanelli, A. Chiricozzi, A revolutionary therapeutic approach for psoriasis: bispecific biological agents, Taylor & Francis (2016).
[3] L. Ghazi, P. Drawz, Advances in understanding the renin-angiotensin-aldosterone system (RAAS) in blood pressure control and recent pivotal trials of RAAS blockade in heart failure and diabetic nephropathy, F1000Research 6 (2017).
[4] M. Platten, S. Youssef, E.M. Hur, P.P. Ho, M.H. Han, T.V. Lanz, L.K. Phillips, M. J. Goldstein, R. Bhat, C.S. Raine, R.A. Sobel, L. Steinman, Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1-and TH17-mediated autoimmunity, Proc. Natl. Acad. Sci. 106 (35) (2009) 14948–14953.
[5] K.D. Silveira, F.M. Coelho, A.T. Vieira, L.C. Barroso, C.M. Queiroz-Junior, V. V. Costa, L.F.C. Sousa, M.L. Oliveira, M. Bader, T.A. Silva, R.A.S. Santos, A.C.S. e. Silva, M.M. Teixeira, Mechanisms of the anti-inflammatory actions of the angiotensin type 1 receptor antagonist losartan in experimental models of arthritis, Peptides 46 (2013) 53–63.
[6] U.M. Steckelings, T. Wollschläger, J. Peters, B.M. Henz, B. Hermes, M. Artuc, Human skin: source of and target organ for angiotensin II, Exp. Dermatol. 13 (3) (2004) 148–154.
[7] N.E. Hoch, T.J. Guzik, W. Chen, T. Deans, S.A. Maalouf, P. Gratze, C. Weyand, D. G. Harrison, Regulation of T-cell function by endogenously produced angiotensin II, American Journal of Physiology-Regulatory, Integrative and Comparative, Physiology (2009).
[8] L. van der Fits, S. Mourits, J.S.A. Voerman, M. Kant, L. Boon, J.D. Laman, F. Cornelissen, A.-M. Mus, E. Florencia, E.P. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis, J. Immunol. 182 (9) (2009) 5836–5845.
[9] B.S. BAKER, L. BRENT, H. VALDIMARSSON, A.V. POWLES, L. AL-IMARA, M. WALKER, L. FRY, Is epidermal cell proliferation in psoriatic skin grafts on nude mice driven by T-cell derived cytokines? Br. J. Dermatol. 126 (2) (1992) 105–110.
[10] L. Zahedi, P.G. Beigi, M. Shafiee, F. Zare, H. Mahdikia, M. Abdouss, M.-A. Abdollahifar, B. Shokri, Development of plasma functionalized polypropylene wound dressing for betaine hydrochloride controlled drug delivery on diabetic wounds, Sci. Rep. 11 (1) (2021) 1–18.
[11] E.C. Jensen, Quantitative analysis of histological staining and fluorescence using Image, Anatomical Record 296 (3) (2013) 378–381.
[12] Z. Liu, H. Liu, P. Xu, Q.i. Yin, Y. Wang, Y.K. Opoku, J. Yang, L. Song, X.u. Sun, T. Zhang, D. Yu, X. Wang, G. Ren, D. Li, Ameliorative effects of a fusion protein dual targeting interleukin 17A and tumor necrosis factor α on imiquimod-induced psoriasis in mice, Biomed. Pharmacother. 108 (2018) 1425–1434.
[13] L. Riol-Blanco, J. Ordovas-Montanes, M. Perro, E. Naval, A. Thiriot, D. Alvarez, S. Paust, J.N. Wood, U.H. von Andrian, Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation, Nature 510 (7503) (2014) 157–161.
[14] Y. Zheng, D.M. Danilenko, P. Valdez, I. Kasman, J. Eastham-Anderson, J. Wu, W. Ouyang, Interleukin-22, a TH 17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis, Nature 445 (7128) (2007) 648–651.
[15] M.A. Lowes, C.B. Russell, D.A. Martin, J.E. Towne, J.G. Krueger, The IL-23/T17 pathogenic axis in psoriasis is amplified by keratinocyte responses, Trends Immunol. 34 (4) (2013) 174–181.
[16] L.S. deLuca, J.L. Gommerman, Fine-tuning of dendritic cell biology by the TNF superfamily, Nat. Rev. Immunol. 12 (5) (2012) 339–351.
[17] Y. Shi, T. Liu, L. He, U. Dougherty, L.i. Chen, S. Adhikari, L. Alpert, G. Zhou, W. Liu, J. Wang, D.K. Deb, J. Hart, S.Q. Liu, J. Kwon, J. Pekow, D.T. Rubin, Q. Zhao, M. Bissonnette, Y.C. Li, Activation of the renin-angiotensin system promotes colitis development, Sci. Rep. 6 (1) (2016), https://doi.org/10.1038/srep27552.
[18] L. He, J. Du, Y. Chen, C. Liu, M. Zhou, S. Adhikari, D.T. Rubin, J. Pekow, Y.C. Li, Renin-angiotensin system promotes colonic inflammation by inducing TH17 activation via JAK2/STAT pathway, American Journal of Physiology-Gastrointestinal and Liver, Physiology 316 (6) (2019) G774–G784.
[19] M.S. Madhur, H.E. Lob, L.A. McCann, Y. Iwakura, Y. Blinder, T.J. Guzik, D. G. Harrison, Interleukin 17 promotes angiotensin II–induced hypertension and vascular dysfunction, Hypertension 55 (2) (2010) 500–507.
[20] P. Mehrotra, J.B. Patel, C.M. Ivancic, J.A. Collett, D.P. Basile, Th-17 cell activation in response to high salt following acute kidney injury is associated with progressive fibrosis and attenuated by AT-1R antagonism, Kidney Int. 88 (4) (2015) 776–784.
[21] G.G. Song, S.-C. Bae, J.-H. Kim, Y.H. Lee, The angiotensin-converting enzyme insertion/deletion polymorphism and susceptibility to rheumatoid arthritis, vitiligo and psoriasis: A meta-analysis, J. Renin-Angiotensin-Aldosterone system 16 (1) (2015) 195–202.
[22] T. Liu, Y. Han, L. Lu, Angiotensin-converting enzyme gene polymorphisms and the risk of psoriasis: a meta-analysis, Clin. Exp. Dermatol. 38 (4) (2013) 352–359.
[23] T. Xia, J. Diao, H.e. Huang, J. Li, L. Sun, H. Li, S. Lv, Evaluation of the association between CD143 gene polymorphism and psoriasis, Cell Biochem. Biophys. 70 (3) (2014) 1617–1623.
[24] M. Ramezani, E. Zavattaro, M. Sadeghi, Angiotensin-converting enzyme gene insertion/deletion polymorphism and susceptibility to psoriasis: a systematic review and meta-analysis, BMC Med. Genet. 21 (1) (2020) 1–10.