RESEARCH ARTICLE

Effect of Topical Dipyridamole Gel in Comparison with Clobetasol on induced Psoriasis in Mice

Nihad H. Ahmed[1]*, Adeeb A. Kadhim Al-Zubaidy[2], Ban J. Qasim[3]

[1]*Department of Pharmacology and Toxicology, College of Pharmacy, Tikrit University, Tikrit, Iraq*
[2]*Department of Pharmacology and Therapeutics, College of Medicine, Al-Nahrain University, Baghdad, Iraq,*
[3]*Department of Pathology, College of Medicine, Al-Nahrain University, Baghdad, Iraq*

Received: 18th March, 2021; Revised: 10th April, 2021; Accepted: 29th May, 2021; Available Online: 25th June, 2021

ABSTRACT

Psoriasis is an immune disease that causes chronic inflammation. It is defined as a clinical entity, affects the skin, nails, mucous membranes, and joints. Psoriasis is primarily caused by the combined effect of several genetic sensitivities, an immune system disorder with prevalent environmental risk factors. Dipyridamole is an anti-platelet drug that acts as a phosphodiesterase-inhibitors that increases intracellular cyclic adenosine monophosphate and down-regulation of pro-inflammatory cytokines.

The present study aimed to evaluate the possible therapeutic effect of topical dipyridamole gel on imiquimod-induced psoriasiform skin inflammation in mice.

Forty male BALB/c albino experimental mice with an average age between 8 to 11 weeks and weight ranged 25–40 g were divided equally into four groups (ten mice/group) after their skin hair of the dorsal back and right ear being shaved for topical application:

Group (I) healthy mice without treatment. Group (II) in which mice received only a daily topical dose of 62.5 mg of imiquimod cream (5%) for seven days. In the following groups (III and IV), after being received imiquimod cream (5%) as mentioned in group II, mice were treated for a further two weeks with either clobetasol ointment (0.05%) topically once daily and dipyridamole gel (1%) topically once daily.

Skin samples were prepared for histopathological examination and biomarker assay, that is, the enzyme-linked immunosorbent assay for mouse tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17), interleukin-23 (IL-23), and transforming growth factor (TGF-), and vascular endothelial growth factor (VEGF).

Keywords: Cytokines, Dipyridamole, Imiquimod, Psoriasis, Topical gel.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.2.51

How to cite this article: Ahmed NH, Al-Zubaidy AAK, Qasim BJ. Effect of Topical Dipyridamole Gel in Comparison with Clobetasol on induced Psoriasis in Mice. International Journal of Drug Delivery Technology. 2021;11(2):524-529.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease-induced immune system.[1] It is defined as a clinical entity affecting the skin, nails, mucous membranes, and joints. Psoriasis is a disease of living signs and symptoms characterize by scaly, erythematous lesions with rigidly demarcated margins.[2]

A disturbed immune system, genetic allergies, and common environmental risk factors all have a common effect in causing psoriasis.[3] Environmental threat factors stimulate naive T cells' stimulation by the antigen-presenting cells (APC) in the epidermis, especially the Langerhans cells, through the body's immune response. Also, naive T cells are encouraged to differentiate in Th1 and Th17 cells, leading to secretion of cytokines such as interleukin IL-12 and IL-2w.[4] Then, the cytokines bind to the existing epidermal and skin cells and then cause changes, including keratinocyte proliferation and epidermal thickness.[5]

Cytokines are likely biomarkers of disease and play a vital role in the pathogenesis of psoriasis. Especially those produced by dendritic cells (IL-18, IL-20, TNF-α, and IL-23), and by Th1 cells (IFN-, IL-2, and TNF-α).[6]

The first line of treating dry skin gets psoriasis is emollients, which are the mainstay of psoriasis treatment.[7] Over the past years, anthralin has been the most active topical treatment for stable psoriasis that has not been cleared with other treatments.[8] Vitamin D types are prescribed as a treatment for psoriasis, as a single therapy, or in combination with topical corticosteroids.[9]

**Author for Correspondence: master.nihad@gmail.com*

Corticosteroids remain the primary treatment for psoriasis, and they are used as a single or combined therapy for systemic treatment.[10] Coal tar has been successful on chronic plaque psoriasis.[11]

Phototherapy comprises frequent contact of the skin to ultraviolet (UV) light to treat numerous inflammatory skin situations such as psoriasis, eczema, and vitiligo.[12] Methotrexate (MTX), an anti-metabolite, is similar in structure to folic acid and is commonly used to treat psoriasis patients.[13]

Retinoid has been used to treat severe psoriasis and is effective, especially for systemic pustular form.[14] Cyclosporine (CYC) is an immunosuppressive drug that was first used to prevent rejection in organ transplant patients and psoriasis.[15]

Psoriasis is mainly considered a T-cell mediated disease, and the T-cell targeting biologics, alefacept, and efalizumab substantiated the role of T-cells as primary modulators.[16]

Dipyridamole, a PDE_5 and PDE_6 inhibitor, was presented in the early 1960s as a coronary vasodilator, most often managed orally, and is currently approved for prophylactic thromboembolism prevention following surgery.[17] It acts by inhibiting the reuptake of adenosine, leading to increased adenosine concentration which causes coronary vasodilatation by acting on the A2-receptors.[18]

Dipyridamole (DIP) is an anti-platelet agent and acts as a phosphodiesterase (PDE) inhibitor that increases intracellular cAMP/cGMP.[19] The studies have shown that cyclic AMP act as a second messenger for a range of inflammatory mediators and cytokines and have been presented to control models of immune and non-immune inflammation *in vivo* and diversity of cellular processes *in vitro*.[20]

Aims of Study

To evaluate the anti-inflammatory and immunomodulatory effects of dipyridamole in mice models of induced psoriasis through its effects on serum IL-17, IL-23, VEGF, TGF-β, and TNF-α.

To compare the effect of dipyridamole drug with that of clobetasol ointment.

To compare the histopathological changes with those induced by topical clobetasol in mice models.

MATERIALS AND METHODS

The present study was done in the Department of Pharmacology in the College of Medicine, Al- Nahrain University, between April 2019 and June 2020.

Table 1: Effects of clobetasol ointment (0.05%) on skin tissues' biomarkers(TNF-α,IL17,IL23,VEGF, and TGF-β) in imiquimod-induced psoriasiform skin inflammation in mice

Biomarkers	*Control*	*Induction*	*Clobetasol ointment (0.05%)*
TNF-α(ng/g)	57.81 ± 17.13	94.45 ± 40.72[s]	53.74 ± 25.45[*]
IL-17 (pg/g)	29.95 ± 8.03	38.64 ± 12.44	28.36 ± 11.36*
IL-23 (pg/g)	12.85 ± 4.00	19.31 ± 4.85[s]	15.44 ± 5.28
VEGF(pg/g)	7.60 ± 2.61	12.41 ± 2.08[s]	9.62 ± 1.90*
TGF-β (pg/g)	77.78 ± 18.92	65.08 ± 17.75	86.52 ± 22.02

S: means $p \leq 0.05$ when being compared to group control. * : means $p \leq 0.05$ when being compared to induction group

Forty male BALB/c albino mice with an age range of 8–11 weeks and weight ranged 25–40 g; were divided equally into four groups (ten mice/group):

Group I (normal control) healthy mice without treatment. Group II (Induction group), in which mice received only a daily topical dose of 62.5 mg of imiquimod cream 5% for seven days.

The following groups (III and IV), after being received imiquimod cream (5%) as mentioned in the induction group, mice were treated for a further two weeks with either clobetasol ointment (0.05%) topically once daily (clobetasol group) and dipyridamole gel (1%) topically once daily (dipyridamole gel group).

RESULTS

Table 1 showed a significant elevation of tissue TNF-α and IL17 with a highly significant increase in IL23 and VEGF levels beside a reduction in the level of TGF-β in the induction group compared to the normal control Group I.

The Clobetasol group displayed a highly significant reduction in TNF-α, IL-17, and VEGF levels beside a reduction in IL-23 and no significant difference in the level of TGF-β when compared to the induction group.

This study demonstrated a significant reduction in both TNF-α and VEGF levels, a significant reduction in IL-17 and IL-23 levels, and a significant elevation of TGF-β level in the dipyridamole gel group compared with the induction group (Table 2).

DISCUSSION

Psoriasis, one of the most common immune-mediated inflammatory skin diseases, characterized by accelerated epidermal proliferation and massive infiltration of cells.[21] Imiquimod cream induces an immune response that creates numerous inflammatory mediators IL-1, IL-17, IL-23, and TNF-α;[22] causes erythema, large inflammatory cells leak out, and the skin swells due to the production of these cytokines through the maturation of Th17 cells.[23]

Certain studies have reported that effector cytokines such as IL-17 and IL-23 produced by Th17 cells are present in the peripheral blood of psoriasis patients and implicated in psoriasis pathogenesis.[24] Transforming growth factor-beta 1 (TGF-β1), a cornerstone mediator in many diseases, may induce the production of pro-inflammatory cytokines such as TNF-α.[25]

Clobetasol was used as a standard agent in this study and revealed a significant improvement in the symptoms of

Table 2: Effects of dipyridamole gel (1%) on skin tissues' biomarkers (TNF-α, IL17, IL23, VEGF, and TGF-β) in imiquimod-induced psoriasiform skin inflammation in mice

Biomarkers	*Induction*	*Clobetasol ointment (0.05%)*	*Dipyridamole gel (1%)*
TNF-α (ng/g)	94.45 ± 40.72	53.74 ± 25.45[*]	55.73 ± 28.55[*]
IL-17 (pg/g)	38.64 ± 12.44	28.36 ± 11.36[*]	30.30 ± 10.77
IL-23 (pg/g)	19.31 ± 4.85	15.44 ± 5.28	15.52 ± 4.15
VEGF(pg/g)	12.41 ± 2.08	9.62 ± 1.90[*]	7.94 ± 1.59[*]
TGF-β(pg/g)	65.08 ± 17.75	86.52 ± 22.02	97.68 ± 26.49[*]

* : means $p \leq 0.05$ when being compared to induction group

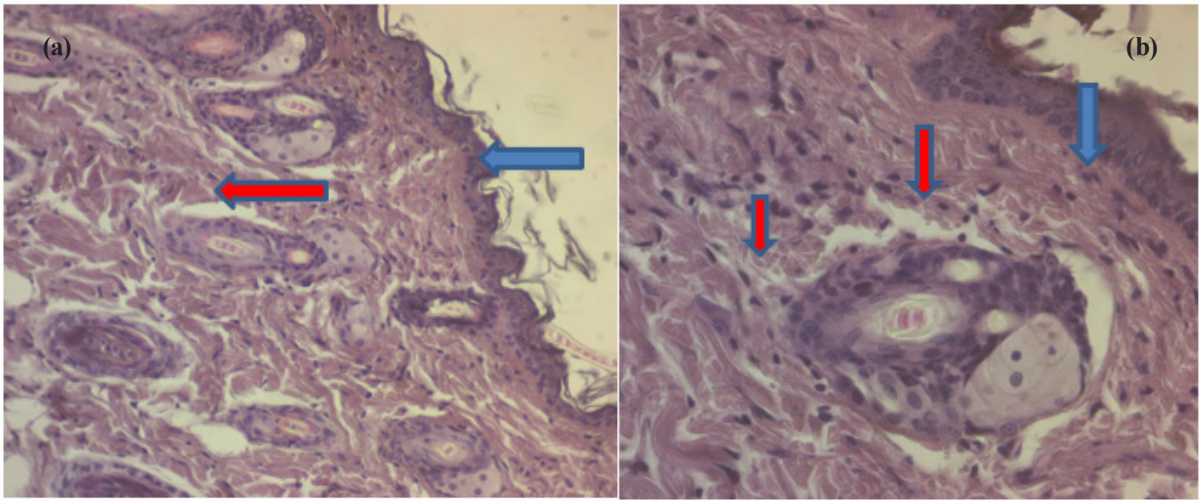

Figure (1 a, b): Histopathologic sections of skin sections from animals of a control group, sharing normal of epidermal (blue arrow) and dermis (red arrow). H&E 20X (a) and 40X(b)

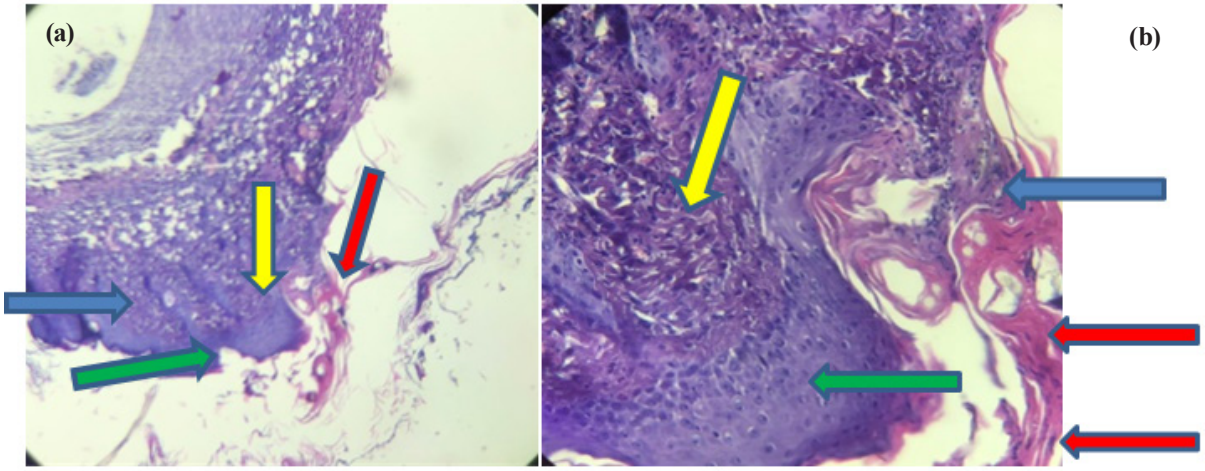

Figure (2 a, b): Histopathologic sections of skin from animals of the induction group shows acanthosis and elongation of rete ridges (green arrow), Munro's micro abscess (blue arrow), hyperkeratosis and parakeratosis (red arrow), and marked dermis lymphocytic infiltrate (yellow arrow). H&E 10X (a) and 40X (b)

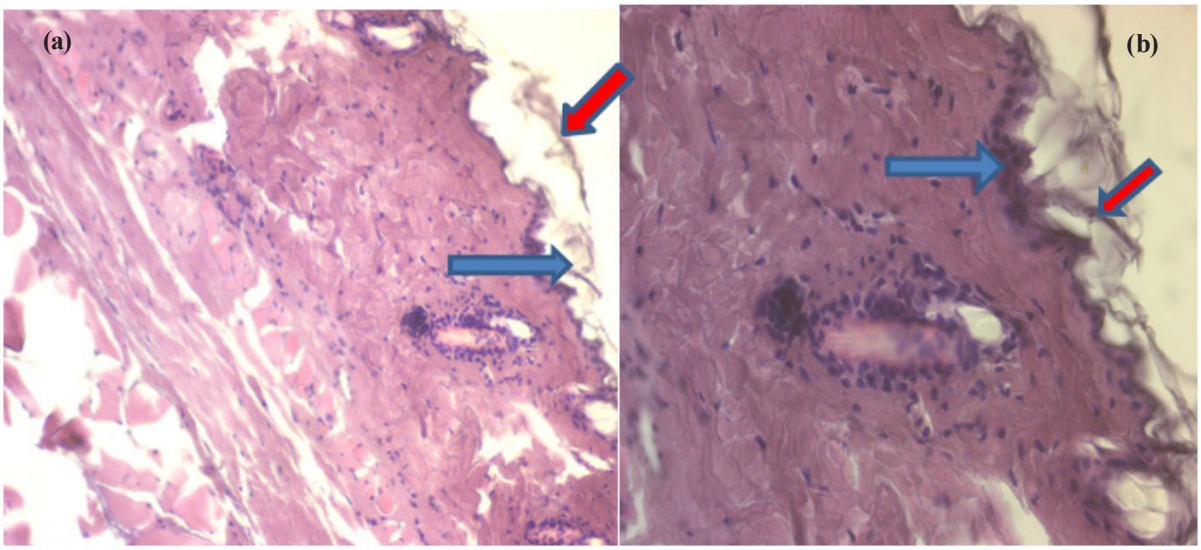

Figure (3 a, b): Histopathologic sections of skin sections from animals of the clobetasol group shows hyperkeratosis (red arrow) and epidermis thinning (blue arrow) above papillae. H&E 20 X(a) and 40X (b)

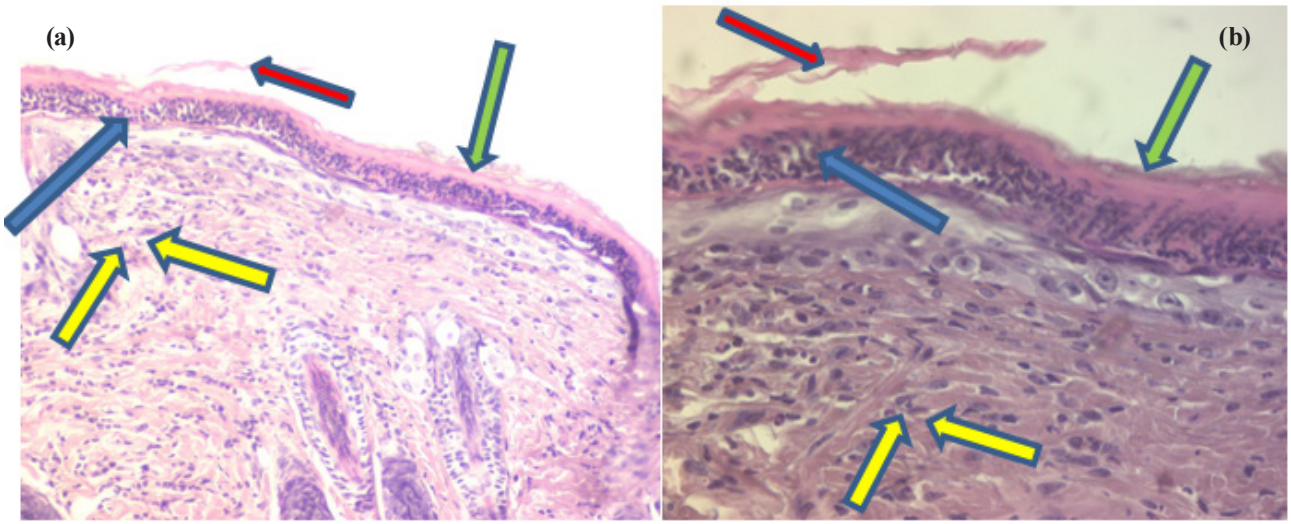

Figure (4 a, b): Histopathologic sections of skin sections from animals of dipyridamole gel group shows acanthosis (green arrow), hyperkeratosis (red arrow), Munro's micro abscess (blue arrow), and mild dermis lymphocytic infiltrate (yellow arrow). H&E 20 X(a) and 40X (b)

psoriasis.[26] This result agreed with other studies, which revealed that clobetasol causes a decrease in TNF-α level.[27]

The Clobetasol group displayed a highly significant reduction in IL-17 and VEGF levels besides a reduction in IL-23 and no significant difference in the level of TGF-β when compared to the induction group.

Also, the present study showed that treatment of clobetasol ointment significantly inhibits the histological changes in the ear and dorsal skin that IMQ induced. With clobetasol anti-inflammatory treatment, it was found that the number of epidermal T cells decreased afterward.[28] Clobetasol regulates gene transcription of numerous genes, particularly those that code for pro-inflammatory cytokines; thus, down-regulating the expression of interleukins and TNF-α.[8]

Dipyridamole is a phosphodiesterase inhibitor, which primarily functions to increase intracellular cAMP levels and block adenosine reuptake in cells, resulting in anti-platelet and vasodilator effects.[29]

Subsequently, the current study demonstrated that the TNF-α level significantly reduces the dipyridamole treated group compared to the induction group. Various cell types, including macrophages, neutrophils, lymphocytes, endothelial, and mast cells, produced pleiotropic cytokine such as TNF-α.[30] It was well known that TNF-α plays an important role in vascular inflammation and immune cell infiltration.[31]

Antioxidant and anti-inflammatory properties of dipyridamole positively affect blood flow and angiogenesis by decreasing the VEGF level.[32]

In the present study, IL-17 and IL-23 showed reduction when treated with dipyridamole gel compared to the induction group. In IL-17 cytokines, the use of dipyridamole was found to reduce the proliferation of T cells and activation.[33]

In this present study's topical administration of dipyridamole gel, a highly significant decrease in the VEGF level was compared to the IMQ group. Dipyridamole positively affects blood flow and angiogenesis and has pleiotropic pharmacological effects, such as antioxidant and anti-inflammatory properties.[34]

In the present study, the effect of topical dipyridamole showed a significant elevation of TGF-β levels in comparison with the induction group. The essential for the homeostasis of tissues and organs was a TGF-β which a pleiotropic cytokine and regulates numerous cell functions.[35]

The present study showed an increase in psoriatic skin parameters such as acanthosis, Munro microabscess (a neutrophil-rich stratum corneum), hyperkeratosis, Parakeratosis (retention of nuclei in the stratum corneum), thinning above papillae, lengthening and clubbing, and mild dermis lymphocytic in the induction group. These results are compatible with other studies.[36]

In the present study, topical application of dipyridamole gel led to improvement in histopathological changes induced by imiquimod, resulting in reduced acanthosis attributed to reduced angiogenesis and basal cell proliferation. The pharmacological agents that inhibit PDE activity elevate the intracellular cAMP content broad anti-inflammatory effects.[37]

CONCLUSION

The finding of the present study could be concluded that possible effect of anti-inflammatory activity of dipyridamole gel on the skin homogenate parameters in imiquimod-induced psoriasiform skin inflammation in mice model.

ACKNOWLEDGMENT

The authors would like to thank the staff of the pharmacology and therapeutics department, Medical Research Center staff, Dr. Mohammed Abdul-Jabbar at the college of Medicine/ Al-Nahrain University for his assistance in laboratory technique, and to all members of the College of Pharmacy/ University of Baghdad for their help and cooperation.

AUTHOR CONTRIBUTION

Mohammed Abdul-Jabbar: collection and analysis of data, interpretation, and discussion. Prof. Dr. Adeeb A. Al-Zubaidy and Prof. Dr. Ban J. Qasim: research reviewers.

LIST OF ABBREVIATION

IL = interlukin, TNF-α = tumor necrosis factor-alpha, PDE = phosphodiesterase, DIP = Dipyridamole, cAMP = cyclic adenosine monophosphate, VEGF = vascular endothelial growth factor, TGF-β = transforming growth factors-beta, ELISA = Enzyme-linked immunosorbent assay, and IMQ = imiquimod.

REFERENCE

1. Rachakonda TD, Schupp CW, Armstrong AW. Psoriasis prevalence among adults in the United States. J Am Acad Dermatol 2014;70:512–6.
2. Paller AS, Singh R, Cloutier M, Gauthier-Loiselle M, Emond B, Guerin A, Ganguli A. Prevalence of psoriasis in children and adolescents in the United States: a claims-based analysis. Journal of drugs in dermatology: JDD. 2018 Feb 1;17(2):187-194.
3. Raychaudhuri SK, Maverakis E, Raychaudhuri SP. Diagnosis and classification of psoriasis. Autoimmunity reviews. 2014 Apr 1;13(4-5):490-495.
4. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. Annual review of immunology. 2014 Mar 21;32:227-255.
5. Zeng J, Luo S, Huang Y, Lu Q. Critical role of environmental factors in the pathogenesis of psoriasis. The Journal of dermatology. 2017 Aug;44(8):863-872.
6. Molteni and E. Reali, 2012."Biomarkers in the pathogenesis, diagnosis, and treatment of psoriasis," Psoriasis: Targets and Therapy, vol. 2, pp. 55–66.
7. Croney S. Treating skin conditions in the community: know your emollients. British journal of community nursing. 2016 Sep 2;21(9):452-456.
8. Torsekar R, Gautam MM. Topical therapies in psoriasis. Indian dermatology online journal. 2017 Jul;8(4):235-245.
9. Hong SP, Oh Y, Jung M, Lee S, Jeon H, Cho MY, Lee SH, Choi EH. Topical calcitriol restores the impairment of epidermal permeability and antimicrobial barriers induced by corticosteroids. British Journal of Dermatology. 2010 Jun;162(6):1251-1260.
10. Svendsen MT, Andersen KE, Andersen F, Hansen J, Pottegård A, Johannessen H. Psoriasis patients' experiences concerning medical adherence to treatment with topical corticosteroids. Psoriasis (Auckland, NZ). 2016;6:113–119.
11. Koo J, Lebwohl M. Duration of remission of psoriasis therapies. Journal of the American Academy of Dermatology. 1999 Jul 1;41(1):51-59.
12. Nakamura M, Farahnik B, Bhutani T. Recent advances in phototherapy for psoriasis. F1000Research. 2016;5:1684.
13. Wald JM, Klufas DM, Strober BE. The use of methotrexate, alone or in combination with other therapies, for the treatment of palmoplantar psoriasis. Journal of drugs in dermatology: JDD. 2015 Aug;14(8):888-892.
14. Sevrain M, Richard MA, Barnetche T, Rouzaud M, Villani AP, Paul C, Beylot-Barry M, Jullien D, Aractingi S, Aubin F, Joly P. Treatment for palmoplantar pustular psoriasis: systematic literature review, evidence-based recommendations and expert opinion. Journal of the European Academy of Dermatology and Venereology. 2014 Aug;28:13-16.
15. Menter A, Griffiths CE, Tebbey PW, Horn EJ, Sterry W, International Psoriasis Council. Exploring the association between cardiovascular and other disease-related risk factors in the psoriasis population: the need for increased understanding across the medical community. Journal of the European Academy of Dermatology and Venereology. 2010 Dec;24(12):1371-1377.
16. Rønholt K, Iversen L. Old and new biological therapies for psoriasis. International journal of molecular sciences. 2017 Nov;18(11):2297.
17. Melani A, Cipriani S, Corti F, Pedata F. Effect of intravenous administration of dipyridamole in a rat model of chronic cerebral ischemia. Annals of the New York Academy of Sciences. 2010 Oct;1207(1):89-96.
18. Harel F, Finnerty V, Authier S, Pelletier-Galarneau M. Comparison of two dipyridamole infusion protocols for myocardial perfusion imaging in subjects with low likelihood of significant obstructive coronary artery disease. Journal of Nuclear Cardiology. 2020 Oct;27(5):1820-1828.
19. Gresele P, Momi S, Falcinelli E. Anti-platelet therapy: phosphodiesterase inhibitors. British journal of clinical pharmacology. 2011 Oct;72(4):634-646.
20. Moore AR, Willoughby DA. The role of cAMP regulation in controlling inflammation. Clinical and experimental immunology. 1995 Sep;101(3):387-389.
21. Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. Cellular & molecular immunology. 2012 Jul;9(4):302-309.
22. Weber A, Zimmermann C, Mausberg AK, Kieseier BC, Hartung HP, Hofstetter HH. Induction of pro-inflammatory cytokine production in thymocytes by the immune response modifiers Imiquimod and Gardiquimod™. International immunopharmacology. 2013 Oct 1;17(2):427-431.
23. El Malki K, Karbach SH, Huppert J, Zayoud M, Reißig S, Schüler R, Nikolaev A, Karram K, Münzel T, Kuhlmann CR, Luhmann HJ. An alternative pathway of imiquimod-induced psoriasis-like skin inflammation in the absence of interleukin-17 receptor a signaling. Journal of Investigative Dermatology. 2013 Feb 1;133(2):441-451.
24. Rizzo HL, Kagami S, Phillips KG, Kurtz SE, Jacques SL, Blauvelt A. IL-23–mediated psoriasis-like epidermal hyperplasia is dependent on IL-17A. The journal of immunology. 2011 Feb 1;186(3):1495-1502.
25. Di Fusco D, Laudisi F, Dinallo V, Monteleone I, Di Grazia A, Marafini I, Troncone E, Colantoni A, Ortenzi A, Stolfi C, Picardo M. Smad7 positively regulates keratinocyte proliferation in psoriasis. British Journal of Dermatology. 2017 Dec;177(6):1633-1643.
26. Boehncke WH, Brembilla NC. Unmet needs in the field of psoriasis: pathogenesis and treatment. Clinical reviews in allergy & immunology. 2018 Dec;55(3):295-311.
27. Reddy DV, Sreelatha P, Devi BR. A rapid novel RP-HPLC stability indicating assay method devel-opment and validation of dipyridamole in dipyridamole extended release capsules. Anal Chem. 2015;15(4):140-150.
28. Sun J, Zhao Y, Hu J. Curcumin inhibits imiquimod-induced psoriasis-like inflammation by inhibiting IL-1beta and IL-6 production in mice. PloS one. 2013 Jun 25;8(6):e67078.

29. Balakumar P, Varatharajan R, Nyo YH, Renushia R, Raaginey D, Oh AN, Akhtar SS, Rupeshkumar M, Sundram K, Dhanaraj SA. Fenofibrate and dipyridamole treatments in low-doses either alone or in combination blunted the development of nephropathy in diabetic rats. Pharmacological research. 2014 Dec 1;90:36-47.
30. Popa C, van den Hoogen FH, Radstake TR, Netea MG, Eijsbouts AE, Den Heijer M, van der Meer JW, van Riel PL, Stalenhoef AF, Barrera P. Modulation of lipoprotein plasma concentrations during long-term anti-TNF therapy in patients with active rheumatoid arthritis. Annals of the Rheumatic Diseases. 2007 Nov 1;66(11):1503-1507.
31. Park JH, Park YJ, Kim SK, Kwon JE, Kang HY, Lee ES, Choi JH, Kim YC. Histopathological differential diagnosis of psoriasis and seborrheic dermatitis of the scalp. Annals of dermatology. 2016 Aug 1;28(4):427-432.
32. Lana D, Ugolini F, Melani A, Nosi D, Pedata F, Giovannini MG. The neuron-astrocyte-microglia triad in CA3 after chronic cerebral hypoperfusion in the rat: protective effect of dipyridamole. Experimental gerontology. 2017 Oct 1;96:46-62.
33. Kyttaris VC, Zhang Z, Kampagianni O, Tsokos GC. Calcium signaling in systemic lupus erythematosus T cells: a treatment target. Arthritis & Rheumatism. 2011 Jul;63(7):2058-2066.
34. Palmer BF, Clegg DJ. Ascent to altitude as a weight loss method: the good and bad of hypoxia inducible factor activation. Obesity. 2014 Feb;22(2):311-317.
35. Fabregat I, Moreno-Càceres J, Sánchez A, Dooley S, Dewidar B, Giannelli G, Ten Dijke P, IT-LIVER Consortium. TGF-β signalling and liver disease. The FEBS journal. 2016 Jun;283(12):2219-2232.
36. Kawakita T, Landy HJ. Surgical site infections after cesarean delivery: epidemiology, prevention and treatment. Maternal health, neonatology and perinatology. 2017 Dec;3(1):1-9.
37. Page CP, Spina D. Selective PDE inhibitors as novel treatments for respiratory diseases. Current opinion in pharmacology. 2012 Jun 1;12(3):275-286.