Effect of Topical Tadalafil Gel in Imiquimod-induced Psoriasiform Skin Inflammation in Mice

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Abstract

To evaluate the possible beneficial effect of tadalafil gel(0.05%) in imiquimod-induced psoriasiform skin inflammation in mice model. forty male BALB/c albino mice with age range of 8-11 weeks and weight ranged 25-40 g; were divided equally into four groups (ten mice / group) after their skin of dorsal back and right ear being shaved for topical application: Group I (normal control) healthy mice without treatment. Group II (Induction group) in which mice received only a daily topical dose of 62.5mg of imiquimod cream (5%) for seven days. The following groups (III and IV), after being received imiquimod cream (5%) as mentioned in induction group, mice were treated for further two weeks with either clobetasol ointment (0.05%) topically once daily (clobetasol group), tadalafil gel (0.05%) topically once daily(tadalafil gel group)

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 induction group as compared to normal control group . Clobetasol group treated 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 being compared to induction group (table 2).

This study demonstrated a significant reduction in both TNF-α and IL-17, with a highly significant decrease in IL23 level. Beside a no significant decrease in both VEGF and of TGF-β levels in tadalafil gel group in comparison with induction group. The possible effect of anti-inflammatory activity of tadalafil gel on the skin homogenate parameters in imiquimod-induced psoriasiform skin inflammation in mice model.

Keywords: *Psoriasis, Tadalafil, Topical gel, Imiquimod, Cytokines*

Introduction

Psoriasis is an immune-mediated chronic inflammatory skin disease[1,2]. It is defined as a clinical entity, affecting the skin, nails, mucous membranes, and joints. Psoriasis is a chronic common disease, affecting 2–4 % of the population in countries, with incidence rates influenced by age, geographic position, and hereditary background[1].

Psoriasis is a disease of living signs and symptoms characterized by scaly, erythematous lesions with rigidly demarcated margins[3]. There has been a huge concentration on the association of psoriasis with predictable cardiovascular risk factors including metabolic syndrome, obesity, low physical activity, smoking, alcohol, lipid abnormalities, and hypertension[4].

The pathogenesis of psoriasis primarily shares the combined effect of several gene susceptibilities, the disordered immune system together with pervasive environmental risk factors[5].

Environmental threat factors generate the immune response of the body, where naive T cells are stimulated by antigen-presenting cells (APC) in the epidermis, especially Langerhans cells. APC can also discharge

cytokines such as interleukin (IL)-12 and IL-23, which encourage naive T cells to differentiate into Th1 and Th17 cells[6].

Then, the activated T cells transfer from lymph nodes to the skin, where they are stimulated to produce sufficient cytokines. Therefore, these cytokines interrelate with the occupant epidermal and dermal cells and then cause changes, including keratinocyte proliferation and epidermal thickness[7].

Cytokines have a very important role in the pathogenesis of psoriasis, especially those produced by Th1 cells (IFN-γ, IL-2, and TNF-α) and those produced by dendritic cells (IL-18, IL-20, TNF-α, and IL-23). All those cytokines are likely biomarkers for psoriasis[8].

In addition, many drugs, such as imiquimod, antivirals, lithium, beta-blockers, TNF-alpha, and anti-cytokine treatments (anti-TNF antibodies), have all been clinically related to the beginning and exacerbation of psoriasis[9].

Tadalafil is an actual selective strong competitive inhibitor of phosphodiesterase type 5 (PDE-5)[10], which particularly inhibits nitric oxide (NO)/GMP pathway that blocking cGMP degradation in smooth muscles inducing vascular dilation and inhibiting platelets aggregation[11].

Tadalafil has a prolonged half-life of 17.5hrs and is operative for 36hrs after dosing [12]. The drug relaxes smooth muscle by reducing PDE-5 levels and prevent breakdown the cyclic guanosine monophosphate [13]. The PDE-5 inhibitors are usually well accepted and active in the treatment of erectile dysfunction (ED) [14]. Tadalafil is used in the monitoring of ED, of benign prostatic hyperplasia, and of pulmonary arterial hypertension [15]. The chief adverse effect is a headache. Whereas, the rare adverse effects include dyspepsia, back pain, myalgia, nasal congestion, and flushing [16].

Aims of the Study

1- To evaluate the anti-inflammatory effects of tadalafil in mice models of psoriasis through its effects on serum IL-17, IL-23, VEGF, TGF-β, TNF-α, histopathology score, and observational severity score.

2- To compare the effect of tadalafil drug with that of clobetasol ointment on serum IL-17, IL-23, VEGF, TGF-β, TNF-α in mice model.

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

Forty male BALB/c albino mice with age range of 8-11 weeks and weight ranged 25-40 g; were divided equally into four groups (ten mice / group) after their skin of dorsal back and right ear being shaved for topical application:

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

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

At the end of the experiment, all of the mice were anesthetized by chloroform and then, they were sacrificed. The skin samples were arranged for histopathological examination and assay of biomarkers, i.e., enzyme-linked immunosorbent assay for mouse tumor necrosis-α, interleukin 17, interleukin 23, transforming growth factor-β, and vascular endothelial growth factor. The one-way analysis of variance (ANOVA) independent sample t-test was used to determine the statistical significance of differences of laboratory parameters between healthy and treated groups.

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 induction group as compared to normal control group I. 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 being compared to induction group (table 2).

This study demonstrated a significant reduction in both TNF-α and IL-17, with a highly significant decrease in IL23 level. Beside a no significant decrease in both VEGF and of TGF-β levels in tadalafil gel group in comparison with induction group.

Table (1): Effect of Imiquimod on skin tissues' biomarkers(TNF-α ,IL17,IL23,VEGF and TGF-β) in imiquimod-induced psoriasiform skin inflammation in mice

Biomarkers	Control	Induction
TNF-α (ng/g)	57.81±17.13	94.45±20.72s
IL-17 (pg/g)	29.95±8.03	38.64±12.44
IL-23 (pg/g)	12.85±4.00	19.31±4.85s
VEGF (pg/g)	7.60±2.61	12.41±2.08s
TGF-β (pg/g)	77.78±18.92	65.08±17.75

S: means p ≤ 0.05 when being compared control group

Table(2): Effect 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±20.72s	53.74±15.45*
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±4.85s	15.44±5.28
VEGF(pg/g)	7.60±2.61	12.41±2.08s	9.62±1.90*
TGF-β (pg/g)	77.78±18.92	65.08±17.75	86.52±22.02

S: means p ≤ 0.05 when being compared to group control . * : means p ≤ 0.05 when being compared to induction group

Table (3): Effect of Tadalafil gel (0.05%) 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%)	Tadalafil gel (0.05%)
TNF-α(ng/g)	94.45±20.72	53.74±15.45*	59.48±15.86*
IL-17 (pg/g)	38.64±12.44	28.36±11.36*	27.08±8.77*
IL-23 (pg/g)	19.31±4.85	15.44±5.28	10.72±3.13*
VEGF(pg/g)	12.41±2.08	9.62±1.90*	11.10±4.73
TGF-β(pg/g)	65.08±17.75	86.52±22.02	77.92±20.52

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

Discussion

Psoriasis, which is one of the most common immune-mediated inflammatory skin diseases, characterized by accelerated epidermal proliferation and massive infiltration of cells[17]. Imiquimod induce an immune response that makes the creation of numerous cytokines, such as IL-1, TNF-α, IL-23, and IL-17 [18].

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[19]. Transforming growth factor-beta 1 (TGF-β1), a cornerstone mediator in many diseases, may induce the production of proinflammatory cytokines such as TNF-α [20].

Clobetasol was used as a standard agent in this study and revealed a significant improvement in the symptoms of psoriasis[21]. Clobetasol group treated showed a reduction in TNF-α level when compared to the imiquimod group. Clobetasol group displayed a highly significant reduction in IL-17, and VEGF levels beside a reduction in IL-23 and no significant difference in the level of TGF-β when being compared to induction group.

This present study was agree with other studies observed that clobetasol was able to decreasing the IL-23/IL-17A axis of psoriasis-like inflammation in mice[22]. Clobetasol belongs to the corticosteroids group, which have a vasoconstriction, anti-proliferative, anti-inflammatory, and immunosuppressive effect by binding to intracellular corticosteroid receptors[23].

In this study, with tadalafil gel treated group, the level of TNF-alpha reduced significantly when being compared to imiquimod group. These results is compatible with other studies which showed a reduction of local and systemic of TNF-α production [24] .

Our results were in agreement with further studies showing the anti-inflammatory and anti-oxidative potential of tadalafil[25] . These present study agrees with, who demonstrated that tadalafil possesses anti-inflammatory action in addition to its vasodilator property and that PDE5 inhibitors suppressed other TNF-α induced genes related to inflammation[26] .

In the current study, there was a significant reduction in IL-17 level in the tadalafil gel group was compared with the induction group. In addition, comparing tadalafil gel with the IMQ group, there was a high significance decrease in level IL-23.

In other studies, an oxidative stress leads to the production of inflammatory cytokines and tadalafil showed the antioxidant property[27] . The PDE inhibitors

have been to be effective in the management of autoimmune diseases, such as rheumatic arthritis[28].

In the tadalafil gel group, there was a non-significant reduction in VEGF level as compared to induction group. Tadalafil has been to demonstrate an anti-inflammatory action by the increased tissue serum levels of nitric oxide (NO) and by the increased serum activity of antioxidant enzymes, and showed significant antioxidant, anti-inflammatory, and anti-apoptotic effects[29] . Tadalafil seems a good candidate for targeting ulcerative colitis diseases in which inflammation plays a central role [30] .

The effect of topical tadalafil gel on growth factors showed no significant difference in the TGF-beta level. The biological functions of TGF-β were studies in inflammation, tissue repair, and embryonic development. In addition, it has been found that TGF-β plays an important role in regulating cell growth, differentiation, and immune function[31] .

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List of abbreviation

IL=interlukin, TNF-α=tumor necrosis factor-alpha, PDE= phosphodiesterase, TD=Tadalafil, cGMP=cyclic guanosine monophosphate, VEGF=vascular endothelial growth factor,

TGF-β=transforming growth factors-beta, ELISA=Enzyme-linked immunosorbent assay, IMQ=imiquimod

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