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Research article

Effects of Lycopene as Monotherapy or Combined with Clobetasol on Spleen Index and Inflammatory Markers in Mouse Model of Imiquimod-Induced Psoriasis

Samer Shukur Mohammed[1]*, Wael Waleed Mustafa[2]

[1] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Rafidain University College, Baghdad 10052, Iraq;*
[2] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Turath University College, Baghdad, Iraq*

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Abstract

Background: Lycopene has demonstrated favorable impacts on diverse health conditions, particularly those of an inflammatory nature. ***Objective***: To evaluate the impact of topical application of lycopene on the spleens of mice with psoriasis induced by imiquimod (IMQ). ***Methods***: The study employed a sample size of 48 mice, divided into six groups of eight mice each. The experimental protocol involved the application of IMQ to each of the five groups twice. The first application was administered for six days to induce psoriasis, followed by a second application for another six days while the tested medications were concurrently administered, with the exception of the first group, which served as the control group. Following the six-day induction phase, group 2 was treated exclusively with petrolatum jelly. On the other hand, groups 3-6 were subjected to different treatments, including clobetasol propionate, lycopene at concentrations of 0.125 mg/ml and 0.25 mg/ml, and a combination of 0.05% clobetasol and 0.25 mg/ml of lycopene. Each of the examined pharmaceutical substances was administered topically once daily for a duration of six days at the IMQ application site. ***Results***: Lycopene at a concentration of 0.25 mg/ml resulted in a significant reduction in the spleen index and a restoration of the tissue levels of inflammatory mediators (TNF-α, IL-23, NF-κB, and IL-17) that were close to normal. ***Conclusion***: The anti-inflammatory properties of lycopene can significantly aid in the normalization of spleens in patients who have developed psoriasis due to imiquimod.

Keywords: Lycopene, Psoriasis, Imiquimod-induced psoriasis, Mice, Spleen

تأثير اللايكوبين كعلاج وحيد أو جنبا إلى جنب مع كلوبيتاسول على مؤشر الطحال وعلامات الالتهاب في نموذج الصدفية المستحثة في الفئران بواسطة الأكويمود

الخلاصة

الخلفية: أظهر اللايكوبين تأثيرات إيجابية على الظروف الصحية المتنوعة، لا سيما تلك ذات الطبيعة الالتهابية. **الهدف**: تقييم تأثير التطبيق الموضعي لليكوبين على طحال الفئران المصابة بالصدفية التي يسببها إيميكومود. **الطريقة**: استخدمت الدراسة عينة بحجم 48 فأرا مقسمة إلى ست مجموعات من ثمانية فئران لكل منها. تضمن البروتوكول التجريبي استخدام IQM على كل مجموعة من المجموعات الخمس مرتين. تم إعطاء الأعطاء الأول لمدة ستة أيام للحث على الصدفية، تلاه اعطاء ثان لمدة ستة أيام أخرى بينما تم إعطاء الأدوية المختبرة في وقت واحد، باستثناء المجموعة الأولى، التي كانت بمثابة المجموعة الضابطة. بعد مرحلة الحث التي استمرت ستة أيام، عولجت المجموعة 2 حصريا بهلام الفازلين. خضعت المجموعات 3-6 لعلاجات مختلفة، بما في ذلك بروبيونات كلوبيتاسول، والليكوبين بتراكيز 0.125 مجم/مل و 0.25 مجم/مل، ومزيج من 0.05٪ كلوبيتاسول و 0.25 مجم/مل من الليكوبين. تم إعطاء كل مادة من المواد الصيدلانية التي تم فحصها موضعيا مرة واحدة يوميا لمدة ستة أيام في موقع أعطاء ألأيميكويمود. **النتائج**: أدى الليكوبين بتركيز 0.25 مجم / مل إلى انخفاض كبير في مؤشر الطحال واستعادة مستويات الأنسجة للمؤشرات الألتهابية TNF-αو IL-23 و NF-κB و IL-17 التي كانت قريبة من المعدل الطبيعي. **الأستنتاج**: الخصائص المضادة للالتهابات لدى اللايكوبين يمكن أن تساعد بشكل كبير في حماية انسجة الطحال في الفئران المصابة بالصدفية بعد التعرض للأكويمود.

* Corresponding author: Samer S. Mohammed. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Rafidain University College, Baghdad 10052, Iraq; Email: samer.shukur@ruc.edu.iq

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INTRODUCTION

Psoriasis, which is genetically inherited, is a chronic condition characterized by widespread and persistent symptoms. It is transmitted dominantly and can present with varying degrees of inflammation and invasion, as well as debilitating and proliferative skin manifestations [1,2]. The etiology of psoriasis is multifactorial. Psoriatic arthritis has a prevalence ranging from 1.3% to 34.7% among individuals with psoriasis, leading to joint deformities and functional limitations [3]. Psoriasis can be classified into different clinical manifestations such as inverse, erythrodermic, guttate, pustular, and plaque. Plaque-like lesions have the potential to impact diverse anatomical regions and are observable in 85-90% of individuals diagnosed with psoriasis. Psoriasis can hinder the proliferation and differentiation of skin layer keratinocytes by attenuating the immune system in multiple skin layers [4]. The pathogenesis of psoriasis involves the significant contributions of neutrophils, T-lymphocytes, natural killer cells, and dermal dendritic cells. It is possible to perceive them as a constituent of an intrinsic mechanism that identifies external antigens. The immune system's mediators in the skin comprise of mature CD4+ and CD8+ T-cells present in high concentrations. Histocompatibility complexes facilitate the interaction between lymphocytes (T-cells) and dendritic cells of myeloid origin in the lymph nodes. The heightened synthesis of pro-inflammatory cytokines by myeloid dendritic cells will stimulate the maturation and proliferation of CD4+-type lymphocytes, resulting in their differentiation into T-helper 17 and T-helper 1 subsets. Psoriasis pathogenesis is believed to be modulated by additional cytokines, including TNF-α and IL-12. The persistence of psoriatic inflammation during the maintenance phase necessitates activation of the adaptive arm of the immune system. The activation of the JAK/STAT pathway is facilitated by cytokines such as IL-21, IL-22, and IL-17, which are released by Th-17 cells. This pathway is known to stimulate the proliferation of keratinocytes in the epidermal layer of the skin. The activation of pro-inflammatory genes leads to the phosphorylation and regulation of their respective transcription factors [5]. Psoriasis is characterized by a systemic inflammatory response that manifests in various organs, including the spleen and lymph nodes. The spleen holds significant value as a secondary lymphoid organ in the study of the systemic inflammatory response and immunological response. Numerous investigations have explored the potential of secondary lymphoid organs as indicators of inflammation. Nonetheless, only a limited number of these inquiries have successfully established a correlation between these modifications and the extent to which psoriasis is affected by inflammation. In order to assess the potential of this association as an indicator of psoriasis severity, the examination took into account the spleen, lymph nodes, and germinal center staining [7]. Lycopene, an endogenous compound, is present in several red-hued foods such as tomatoes, guavas, and watermelon. Additional sources include pink guava and pink grapefruit. Lycopene has exhibited promising results in inhibiting the growth of various cancer cell lines, particularly those located in the oral cavity and pancreas [8]. Furthermore, prior research has suggested potential benefits for individuals with cardiovascular disease [9]. Furthermore, a research study has indicated that an elevated concentration of lycopene in the bloodstream may mitigate the consequences of oxidative stress and enhance the efficacy of endothelial cells [10]. Additionally, it has been demonstrated that lycopene is efficacious in diminishing the activity of nuclear factor-κB and the production of adhesion molecules in endothelial cells [11]. A research findings suggest that lycopene has the potential to protect the human keratin layer from the harmful effects of full-spectrum UVR. This is in accordance with previous studies [12]. The administration of IMQ has been observed to impede the functioning of both the innate and adaptive immune systems, with a specific emphasis on the IL23/IL17 axis. Additionally, it has been noted that IMQ can promptly induce dermatitis that bears a striking resemblance to psoriasis in humans. The utilization of this method has been commonly employed in the development of a rapid psoriasis model. Its application facilitates the evaluation of novel therapeutic interventions and pharmaceuticals, as well as the induction of dermatitis that closely mimics psoriasis [6]. The present study examined the impact of topical lycopene on the spleens of mice exhibiting psoriasis induced by IMQ.

METHODS

The IMQ 5% cream was procured from Meda®, Germany, while the lycopene powder was obtained from Hyperchem®. Additionally, the clobetasol ointment (0.05% w/v) was acquired from GlaxoSmithKline®, UK. An ointment containing lycopene at concentrations of 0.125 mg/g and 0.25 mg/g was formulated through the process of grinding glycerin (at a weight percentage of 10% w/w) as a moistening agent with lycopene. Subsequently, a commensurate amount of petrolatum jelly was incorporated into the amalgam of lycopene and glycerin. The process of dilution was executed through the utilization of the geometric dilution methodology, culminating in a total mass of 100 grams [13]. The study was conducted at the Department of Pharmacology, Al-Nahrain University in Baghdad. The protocol of the study underwent evaluation and received approval from the college's local review board. A total of 48 Albino mice, aged between 8 to 11 weeks and weighing approximately 25 to 40 grams, were procured from the animal house of Al-Razi Center, Ministry of Industry, Iraq. Prior to the commencement of the experiment, the mice were subjected to a period of acclimatization within polypropylene cages, during which they were provided with ad libitum access to food and water. Subsequently, the mice were segregated into six distinct cohorts, with each group comprising of eight mice.

Study design

Prior to the induction of plaque-type psoriasis, the dorsal region of the mice was depilated and subsequently treated with IMQ. The aforementioned lesions exhibit discernible differentiation and increased proliferation of the epidermis. The mice were randomly allocated into six distinct groups. The first group (normal) consisted of healthy mice that were not subjected to any form of treatment. The second group (induction) involved the topical application of IMQ 5% cream on the shaved dorsal skin for twelve consecutive days. Furthermore, the experimental protocol involved the topical application of petrolatum jelly from day seven for a duration of six days in addition to the administration of IMQ 5% cream as a positive control in group 2, followed by the topical application of clobetasol 0.05% once daily from the seventh day for six consecutive days [14]. In group 4, IMQ was applied for twelve days, while lycopene (0.125 mg/g) ointment was topically applied for six days starting from day seven [15]. Similarly, in group 5, IMQ cream was applied for twelve days as in group 2, and lycopene (0.25 mg/g) ointment was applied dorsally once daily for six days starting from the seventh day. Finally, in group 6, IMQ was applied for 12 days as previously mentioned, while a combination of clobetasol and lycopene 0.25 mg/g was topically applied as an ointment from the seventh day.

Sampling and measurements

At the end of treatment, animals were euthanized with diethyl ether (using a piece of cotton ether soaked in an anesthetic jar). Spleens were obtained and kept in 10% phosphate buffered saline. Then the mixture was harvested, prepared for spleen homogenate using an electric homogenizer, and then centrifuged at 5,000 rpm for ten minutes to get the supernatant, which was kept frozen at -80 degrees Celsius in a freezer for biomarker analyses [17]. At the time of sacrifice, the spleen of each mouse was extracted, weighed, and measured. The spleen index was calculated using the following formula: average spleen weight/total body weight.

Laboratory analysis

The Sandwich-ELISA practice module is applied to this ELISA kit to test the levels of TNF-α, IL-17, IL-23, and NF-κB in spleen tissue homogenate following the manufacturer's instructions. At 450 nm wavelength, the optical density (OD) was measured spectrophotometrically [18–21].

Statistical analysis

Microsoft Excel was used to analyze the data using Windows 11. All data were presented as a mean with a standard error of the mean (SEM). To compare variables, a paired t-test was employed, with a p-value less than 0.05 considered significant [22].

RESULTS

The present study revealed a statistically significant ($p < 0.05$) increase in the spleen index of the induction group (group 2) when compared to the control group (group 1), as presented in Table 1. In comparison to the induction group (group 2), a statistically significant reduction ($p < 0.05$) in the spleen index was observed in the cohorts that were treated with 0.25 mg/g lycopene (group 5), clobetasol (group 3), and combination therapy (group 6). On the contrary, the recorded spleen index did not exhibit a significant decrease in subjects administered with lycopene 0.125 mg/g (group 4) as compared to the induction group.

Table 1: Spleen index comparison among different treatment groups

Marker	G1 (normal)	G2 (induction)	Group G3 (clobetasol)	G4 (lycopene 0.125 mg/g)	G5 (lycopene 0.25 mg/g)	G6 (clobetasol + lycopene 0.25 mg/g)
Spleen index	6.26±1.24	18.39±3.98#	8.22±1.67*	12.53±2.19	9.29±1.54*	7.59±1.06*

Values were expressed as mean±SEM, # $p<0.05$ when group 2 compared to group1, * $p<0.05$ when treatment groups compared to induction group using unpaired *t*-test.

Table 2 presents the concentrations of IL-17, IL-23, TNF-α, and NF-κB biomarkers in the homogenized tissue of the spleen. The concentrations of all inflammatory biomarkers analyzed were significantly elevated in the IMQ group as compared to the normal group. The examined inflammatory biomarkers exhibited a significant reduction in levels among the participants who were administered clobetasol (group 2). The inflammatory biomarkers in groups 4 and 5, which were administered with lycopene in two distinct dosages (0.125 mg/g and 0.25 mg/g, respectively), exhibited a significant reduction to varying extents. The combination group (group 6) that incorporated lycopene at a concentration of 0.25 mg/g into clobetasol exhibited the most effective normalization of the evaluated biomarkers, in comparison to the remaining groups.

Table 2: Effects of topical lycopene alone or in combination with clobetasol ointment on the levels of inflammatory markers in spleen tissue homogenate of IMQ-induced psoriasis in mice

Group	Spleen tissue homogenate levels			
	IL-17 (pg/ml)	IL-23 (pg/ml)	TNF-α (pg/ml)	NFκB (pg/ml)
G1	15.04±3.2	5.45±1.43	13.21±2.87	58.41±7.3
G2	49.12±9.03#	36.56±3.59#	224.27±14.28#	289.85±32.95 #
G3 clob	25.49±2.15*	13.87±2.44**	85.34±7.98**	149.32±18.5 **
G4 lyc 0.125	31.15±3.22*	23.45±5.4*	145.42±8.32**	219.43±18.7*
G5 lyc 0.25	27.00±2.75*	15.41±4.5**	118.19±6.99**	167.28±15.45**
G6 Clob+0.25 lyc	19.18±3.29**	10.89±2.39**	54.21±2.69**	124.52±19.29**

Data were expressed as mean±SEM. GI= normal; G2= induction; G3= clobetasol 0.05%; G4= lycopene 0.15 mg/g; G5= lycopene 0.25 mg/g; G6= lycopene 0.25 mg/g and clobetasol; # $p< 0.05$ when compared to control group, * $p< 0.05$ when compared to induction group, ** $p< 0.01$ when compared to the induction group.

DISCUSSION

According to the available literature, psoriasis is an immune-mediated condition that can result in erythema, scaling, and thickening of the affected area. The occurrence may arise due to the interaction between the activated dendritic cells of the skin and the adjacent keratinocytes situated on the surface [23]. The utilization of mouse tail models has been widely employed due to the propensity of mice with the plaque-type genotype to develop skin that bears resemblance to psoriasis. Mouse models have exhibited human-like traits, including cutaneous inflammation, hyperkeratosis, and dendritic CD4 cell appearance [14]. The administration of IMQ to mice is a frequently employed procedure. The aforementioned compound is a Toll-like receptor 7/8 agonist with a high degree of selectivity. Its mechanism of action involves the activation of dendritic cells, macrophages, and monocytes, which in turn elicit an innate response. This process is facilitated by the activation of several proinflammatory cascades, leading to the release of numerous inflammatory cytokines. Elevated levels of T-helper 1 cells elicit an immune response that exhibits antiviral and antitumor properties [24]. Despite inducing psoriasis-like symptoms, the IMQ model exhibits self-resolving effects following a six-day therapeutic regimen. The results of the study indicate that mice possess no genetic anomalies and possess the ability to reverse the inflammatory response that is triggered by IMQ stimulation, as evidenced by previous research [14,25]. The mouse dorsal region was subjected to IMQ administration for a duration of twelve days, as per the aforementioned rationale. The present investigation demonstrates that IMQ induced a significant alteration in the spleen mass index as compared to the control group. This finding is consistent with the results reported by several other researchers [23,27,28]. The findings indicate that IMQ has the potential to markedly increase various pro-inflammatory markers and inflammatory signals, as evidenced by the outcomes of an enzyme-linked immunosorbent assay (ELISA) for spleen inflammatory markers. Additionally, the administration of IMQ to mice can induce plaque psoriasis that bears resemblance to the clinical presentation of the disease in humans. Several publications in the past have investigated the effect of IMQ on tissue inflammatory biomarkers [27-29]. Topical corticosteroids with high potency have become the prevailing treatment method for psoriasis due to their efficacy in preventing inflammation and immunosuppression [30]. There are several theories pertaining to the mechanism of action of clobetasol, which can be categorized into genome and non-genome mechanisms. Typically, the glucocorticoid receptor (GR) exists in the cytoplasm in an inactive state, where it is complexed with two subunits of the heat shock protein 90 (hsp90). Upon binding to the inactive receptor, the steroid prompts the dissociation of hsp90, thereby enabling the nuclear localization of the active steroid/receptor complex. This complex subsequently binds to and obstructs transcription factors that facilitate the expression of inflammatory genes [31]. The rapid onset of corticosteroids' action, however, appears to be attributed to their non-genomic pathway. Research has demonstrated that the administration of glucocorticoids results in an elevation of Annexin A1, a distinctive protein that has the ability to form a complex with phospholipids and impede the production of pro-inflammatory prostanoids. Furthermore, it has been suggested that there may be an augmentation in the expression of nitric oxide, a reduction in the expression and activation of cytokines that promote inflammation, and an impact on the activity of mast cells [4]. Glucocorticoids have the ability to augment the transcription of multiple genes, thereby mitigating inflammation. Additionally, they inhibit the concurrent transcription of genes that promote inflammation. The present study demonstrated a significant reduction in the inflammatory response induced by IMQ with the administration of clobetasol. The group treated with clobetasol exhibited a significant decrease in inflammatory cytokines, namely IL-17, IL-23, TNF-α, and NF-κB, in comparison to the induction group [32]. Despite being the primary treatment for psoriasis, clobetasol is associated with various local adverse effects such as skin atrophy, striae on the applied area, and telangiectasia [33]. Due to the aforementioned concerns, the prolonged use of clobetasol is not advised, thus highlighting the necessity for alternative therapies that exhibit reduced adverse

effects. Lycopene [34] has been suggested as a potential supplement to complement existing treatments for the prevention or treatment of specific conditions, such as psoriasis. The activation of DNA repair has been found to yield several benefits for the skin, such as a robust antioxidant effect and a safeguarding mechanism against the deleterious impacts of UV radiation [35]. Several studies have demonstrated the advantageous impact of lycopene on plaque-type psoriasis in mice, as determined by clinical and histological evaluations [36]. The results of an ELISA-based histochemical investigation suggest that the IL-17/IL-23 pathway could exert a noteworthy influence on the mechanism by which IMQ triggers psoriasis in mice. Upon activation, dendritic cells located in the dermal layer secrete IL-23, thereby facilitating the activation and differentiation of type 17 T-helper lymphocytes. The aforementioned process results in the production of pro-inflammatory cytokines, which trigger the immune system, attract additional immune cells, and facilitate the growth and differentiation of the keratin layer [37]. The present study revealed that lycopene administration resulted in a significant elevation of the levels of IL-17, IL-23, and NF-κB in the spleen, particularly at higher doses (Group 5). TNF-α, a cytokine believed to be implicated in psoriasis, has been recognized as the primary inflammatory signal that initiates the innate immune system due to its multiple sources and targets. TNF-α is commonly released by T-helper cells, macrophages, monocytes, natural killer (NK) cells, and antigen-presenting cells. The aforementioned cytokine possesses the ability to augment the angiogenic factor recognized as vascular endothelial growth factor (VEGF), instigate the proliferation of the keratin layer in the epidermis, and stimulate the generation of additional pro-inflammatory cytokines. In comparison to the induction group (group 2), lycopene exhibited a potential reduction in TNF-α levels over the course of the study. The results of this study are consistent with previous research and indicate that lycopene has the ability to suppress the levels of IL-17 and TNF-α in mice [38]. Furthermore, it was found that adhesion molecules, which were previously activated by IL-23, were suppressed [15]. A separate investigation has indicated that the compound lycopene may impede the activation of NF-κB in endothelial cells, thereby eliciting its observed physiological responses [11]. The practice of combining steroidal and non-steroidal therapies for the treatment of psoriasis has been in use for a considerable period of time, with the aim of reducing the adverse effects of high dosages of steroids and enhancing outcomes through synergism [39,40]. The results of this study indicate that the group receiving the combination treatment (group 6) exhibited the most significant enhancement in the spleen tissue index. Furthermore, the co-administration of lycopene at a concentration of 0.25 mg/g and clobetasol at a concentration of 0.05% demonstrated superior inhibition of inflammatory biomarkers in the spleen compared to the application of clobetasol alone in a topical treatment of IMQ-induced psoriasis. The positive outcomes observed could be attributed to the synergistic effects of clobetasol and lycopene, which are known to possess anti-inflammatory properties. This intervention exhibits efficacy in halting the advancement of psoriasis and mitigating its associated outcomes.

Conclusion

The findings of the research indicate that lycopene exhibits anti-psoriatic properties and has the ability to heal the spleen, which is affected by psoriasis through an anti-inflammatory molecular pathway, especially when administered in high doses. Lycopene exhibits potential as a potent adjunct or alternative to conventional therapies in mitigating regional inflammation associated with psoriasis.

Conflict of interests

The author declares no conflict of interests.

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Data sharing statement

Data can be provided based on a reasonable request to the corresponding author.

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