The Ameliorating Effect of Oral Paquinimod Administration against Imiquimod Induced Psoriasis-like Inflammation in Mice
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Abstract
Psoriasis is a chronic, inflammatory condition that primarily affects the skin, hair, and joints and is associated with significant humanistic and economic consequences. Psoriasis was induced in mice in this work using an imiquimod 5% cream, an immune response modifier that can cause psoriasis-like skin inflammation when given orally. Paquinimod is prepared as a suspension and has been orally given to mice before imiquimod application. In this study, albino mice were allocated into five groups and treated as follows: the control group received only daily application of cream based on shaved back (62.5mg/2cm) with a daily oral dose of vehicle for 14 consecutive days with the oral vehicle. Imiquimod group received a daily oral dose of vehicle one hour before imiquimod 5% application on shaved back (62.5mg/2cm) for 14 consecutive days. The paquinimod-treated group received different daily oral doses of paquinimod one hour before imiquimod 5% application on shaved back (62.5mg/2cm) for 14 consecutive days. Dexamethasone -treated group received a daily oral dose of dexamethasone oral solution (2mg/5ml) one hour before imiquimod 5% application on shaved back (62.5mg/2cm) for 14 consecutive days. Paquinimod only group received a daily oral dose of paquinimod for 14 consecutive days. Paquinimod is prepared as a suspension and has been orally given to mice before imiquimod using an imiquimod 5% cream, an immune response modifier that can cause psoriasis associated with significant humanistic and economic consequences.

Keywords: Psoriasis, Paquinimod, Imiquimod 5%, TNF

The current study found that the administration of paquinimod suspension resulted in a significant decline in TNF-α, IL-17, IL-23, IL-17 level expression of TNF-α, IL-1B, IL-17 in the (Paquinimod suspension+imiquimod) group substantially more than that in the (vehicle suspension+imiquimod) groups. In conclusion, paquinimod has a strong ameliorating effect as compared with dexamethasone against imiquimod-induced psoriasis-like inflammation in mice.

Accepted: 15/2/2022
Received: 19/4/2022

Iraqi Journal of Pharmaceutical Science
**Introduction**

Psoriasis is a chronic, inflammatory condition that primarily affects the skin, hair, and joints and is associated with significant humanistic and economic consequences. It occurs in about 1% to 3% worldwide, with a higher prevalence in western countries\(^{(3)}\). The precise cause of psoriasis is still unclear. Psoriasis is closely related to genetics, but environmental exposure plays an essential role in disease development\(^{(3)}\). This immune-mediated disease entails a dysregulated interplay between immune cells and keratinocytes. While the pathogenesis is not fully understood, intense research has been ongoing to explore the underlying mechanisms involved. The topical application of imiquimod (IMQ), a ligand of Toll-like receptors (TLR) 7 and 8, has been reported to induce psoriasis-like dermatitis with many hallmarks of human psoriasis, such as the formation of micro abscesses, skin thickening, hyperkeratosis, acanthosis, scaling, and erythema\(^{(3)}\). Paquinimod is an immunomodulatory agent. In vivo, paquinimod reduces splenic counts of Ly6ChICD115 inflammatory monocytes and CD11b+CD11c+ dendritic cells in a mouse model of experimental autoimmune encephalomyelitis\(^{(5)}\). Frane'n Pettersson N, et al. (2018) has been reported that the immunomodulatory quinoline-3-carboxamide paquinimod reverses established fibrosis in a novel mouse model for liver fibrosis\(^{(5)}\). In 2015 Martin Stenström also reported that Paquinimod reduces skin fibrosis in tight skin 1 mice, an experimental model of systemic sclerosis\(^{(5)}\). Jong-Uk Lee (2021) approved that the Inhibitory effect of Paquinimod on a Murine Model of Neutrophilic Asthma Induced by Ovalbumin with Complete Freund’s Adjuvant\(^{(6)}\). Tahvili S, et al (2018) has been reported that Paquinimod prevents the development of diabetes in the non-obese diabetic (NOD) mouse\(^{(7)}\). In this work, paquinimod was prepared as a suspension and given orally to mice before psoriasis induction via imiquimod. In view of the above considerations, this study was conducted to examine the ameliorating effect of oral paquinimod administration against imiquimod induced psoriasiform-like inflammation in mice and to compare this effect with the dexamethasone administered orally pointing to its ability to cause significant reduction in PASI score and gene expression of TNF-α, NF-KB, IL-1B, IL-17.

**Methodology**

**Chemicals and kits**

The chemicals that were used in this work include formalin (merk chemicals, Germany), diethyl ether (BDH chemicals, India), xanthan as a suspending agent (Samara drug industry Iraq), Imiquimod cream (MEDA - Germany), Paquinimod (Sigma - Aldrich USA), Dexamethasone syrup (Samara drug industry Iraq), and Dulbecco’s phosphate-buffered saline (Euro Cyclone S.p.A, Italy). The kits used in this study include (Interleukin-23, Interleukin-17, Tumor necrosis factor Alfa) all of them from (Bioassay technology laboratory, China) also RNA purification kit (GENEzolTM TriRNA Pure Kit, General, India), gDNA remover and cDNA synthesis super Mix (Transgen, China) and Green qPCR supermix (Transgen, China).

**Methods**

**Animal treatment**

Forty (40) BALB/c albino mice (male, 8wk) (22-40 gm wt.) They were obtained from and maintained in the Animal House at the College of Pharmacy/University of Baghdad under conditions of controlled temperature, humidity and light periodicity (12-hour light/dark cycle). They were fed commercial pellets and tap water ad libitum throughout the experimental period with ethical and animal care approval.

**Preparation of paquinimod suspension:**

Paquinimod 7.5mg was triturated in the mortar with 5ml of glycerin until a smooth paste is formed. The smooth paste was then diluted gradually by adding distilled water with continuous mixing until a homogenous mixture is formed. The mixture is transferred to the graduated cylinder. The preservative (methylparaben 0.09gm, propyl paraben 0.015gm) is dissolved in 10ml distilled water and added to the mixture in the cylinder. The volume is completed to 100ml. Final concentration of paquinimod is 7.5 mg/100ml

**Dose of dexamethasone**

Human dose (mg / kg) = Animal does (mg / kg) × (Animal Km / Human Km)

As the Km factor for each species is constant, the Km ratio is used to simplify calculations\(^{(8)}\).

Km ratio = 0.162

Human dose (mg / kg) = Animal does (mg / kg) × 0.081

Human dose of dexamethasone = 18 mg /day

18 mg / 60 kg (reference body weight for human) = 0.3 mg /kg

Animal does (mg / kg) = 0.3 (mg/kg) / 0.081

Animal does (mg / kg) = 3.7 (mg/kg)

**Experimental design**

This study was started in February; 2021 to September; 2021. Approximately forty (40) mice have shaved their back area using a traditional manual eraser (trimming not zero shaving), and all of the mice were weighed at zero time and ear thickness of the right ear was measured using vernier every two days for 14 days consecutively. The Oral experiment includes 5 groups of mice (each group includes 8 mice), as the following:
The Control group were received only daily application of cream based on shaved back (62.5mg/2cm) and right ear (5mg) with a daily oral dose of vehicle for 14 consecutive days with the oral vehicle.

Imiquimod group in which mice have received a daily oral dose of vehicle one hour before imiquimod 5% application on shaved back (62.5mg/2cm) and right ear (5mg) for 14 consecutive days.

Paquinimod -treated groups in which mice were received different daily oral doses of paquinimod one hour before imiquimod 5% application on shaved back (62.5mg/2cm) and right ear (5mg) for 14 consecutive days.

Dexamethasone -treated group in which mice have received a daily oral dose of dexamethasone oral solution (2mg/5ml) one hour before imiquimod 5% application on shaved back (62.5mg/2cm) and right ear (5mg) for 14 consecutive days.

Paquinimod only group in which mice have received a daily oral dose of paquinimod for 14 consecutive days.

To determine the severity of inflammation of the back skin, an objective the scoring system was developed based on the clinical PASI score. Erythema, scaling, and thickening were independently scored on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. A higher PASI score was associated with poorer quality of life and poorer satisfaction with a skin condition. Furthermore, an improvement in PASI was associated with an improvement in conventional PASI score for general bodily pain for mice (9).

Measurements of spleen index
All mice have been weighed and when mice have been sacrificed, the spleen index is calculated by dividing the spleen weight of the mice in mg by body weight in gm (10).

Measurements of skin thickness
At the end of the experiment, the skin thickness of the back area has been measured in mm via digital Vernier caliper and comparing measured thickness between groups (11).

Assessment of inflammation
Approximately 1 to 1.5 ml of blood was collected from a retro-orbital vein placed in an Eppendorf tube and centrifuged at 3500 rpm for 15 minutes to obtain serum, which was separated and stored at -20 °C until the day of analysis. Serum was utilized for the estimation of serum IL-23 and IL-17 levels by ELISA kits according to the manufacturer’s protocols (12).

Tissue homogenate
Skin tissue from the back area was rinsed in PBS (PH7.4) to thoroughly remove excess blood and weighed before homogenization. The tissue was homogenized in PBS (pH 7.4) by homogenizer on ice, and thawed at 2-8 °C or frozen at -20 °C. Finally the homogenized tissue was centrifuged at 2000-3000RPM for approximately 20 minutes (13).

Polymerase chain reaction procedure
Total RNAs were extracted using Trizol from (GENEzolTM TriRNA Pure Kit, Genelad, India). Reverse transcriptase PCR was performed following standard procedures. The primer pairs of the expected products were shown in table 1. All primers were purchased from Applied Bio system (13), and are designed according to online tool of integrated DNA technology (14).

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Primer sequence (forward/reverse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gapdh</td>
<td>Forward: 5 - CTTTGTCAGTCATTTTCCTGG-3 Reverse: 5 - TCTTTGCTCAGTGTCCTTG-3</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>Forward: 5 - TAGCCCACGTCGTAGCAAAC-3 Reverse: 5 - ACAAGGTCACACCATCGGC-3</td>
</tr>
<tr>
<td>NF-kB</td>
<td>Forward: 5 - AAGACACGAGGAGCAATGG-3 Reverse: 5 - AGCAACATCATTCACATCCC-3</td>
</tr>
<tr>
<td>IL-1B</td>
<td>Forward: 5 - AGGGGCTCCAAAGATGAAG-3 Reverse: 5 - TTCTCCACAGCCAAATGAG-3</td>
</tr>
<tr>
<td>IL-17</td>
<td>Forward: 5 - TGGGACGAGGTCAACC-3 Reverse: 5 - TATCAGGTTCCATGGCGG-3</td>
</tr>
</tbody>
</table>
**Histopathological examination**

The skin of the back area and the right ear was removed from mice. Then it was washed with normal saline solution for the preparation tissue to histopathological examination. After washing tissues were fixed with formaldehyde (10% of formaldehyde in water). The thin section slide for tissues was stained with hematoxylin and eosin stain and mounted with the protective coverslip. 

**Statistical analysis**

Data were expressed as mean values, the mean± standard error of the mean (SEM). Where One-way ANOVA analysis was used for testing the significant difference between the means of groups. Differences were considered statistically significant when the P-value was less than 0.05.

**Results**

**Oral effect of paquinimod on the PASI score**

The PASI score was significantly increased in the model animal treated by imiquimod in comparison with the control group. The anti-inflammatory effect of paquinimod was successfully decreased the PASI score compared to model animals. Dexamethasone suspension administered orally significantly decrease the PASI score compared to IMQ treated group (Figure 1).

**Effect of oral administration of paquinimod on skin thickness in imiquimod-induced psoriasis in mice.**

Each value represents mean ± standard deviation (SD). * Significantly different (P<0.05) concerning the control group. # Significant compared to the IMQ treated group.

**Effect of oral administration of paquinimod on spleen index in imiquimod-induced psoriasis in mice.**

Each value represents mean ± standard deviation (SD).* significantly different (P<0.05) concerning the negative control group. # significant compared to the vehicle susp + IMQ group.

**Effect of oral administration of paquinimod on serum IL-23, IL-17, and TNF-α levels in imiquimod-induced psoriasis in mice.**

Figure 4 A & B showed that there was significant elevation (P<0.05%) in serum levels of IL-23 and IL-17 in the IMQ-treated group compared to the normal control group, as well as the treatment with dexamethasone showed significance in reduction serum IL-23 (P<0.05) compared to the model group. Also, the oral administration of paquinimod was significantly decreased the IL-23 and IL-17 serum levels in comparison with (Vehicle susp + IMQ) group (P<0.05). Serum IL-23 and IL-17 levels significantly decreased by the effect of oral paquinimod suspension in comparison with the normal control group (P<0.05). Figure 4 C revealed a significant elevation in tissue TNF-α level (P<0.05) in the group (Vehicle susp + IMQ) group as compared to the normal control group, there was approximately normal compared to the spleen index in the control group, while in the Dexamethasone-treated group the measured spleen index decreases significantly compared to the model group (Vehicle susp+IMQ) group.
also a significant reduction in tissue TNF-α level (P<0.05) in the group (Paquinimod susp + IMQ) group. (Vehicle susp+ IMQ) group, besides there was a significant reduction (P<0.05) in tissue TNF-α level in the group (Dexamethasone sol+ IMQ) group as compared to the group (Vehicle susp + IMQ) group. In the group (Paquinimod susp only) group there was a significant reduction (P<0.05) in tissue TNF-α level compared to the group (Vehicle susp + IMQ) group.

Effect of oral administration of paquinimod on gene expression of IL-1B, TNF-α, NF-κB, and IL-17 in imiquimod-induced psoriasis-like inflammation in mice.

Figure 5A displayed that gene expression for IL-1B in mice treated with IMQ was highly significantly expressed. The anti-inflammatory effect for paquinimod revealed a significant reduction for IL-1B’s expression is compared to the model group. On the other hand, the relative level of IL-1B mRNA in skin tissue for mice treated with dexamethasone or paquinimod alone showed significant inhibition compared to control mice.

In figure 5B, the model mice treated with IMQ have expressed the level for TNF-α in the skin in a highly significant increase than control mice. The gene expression of TNF-α for (Paq susp + IMQ) group was significantly reduced compared to the mRNA level for TNF-α in (Vehicle susp+IMQ) group. On other hand, gene expression of TNFα for mice of (Dexamethasone sol+IMQ) and (Paq point only) groups were showing significant differences compared to gene expression of TNF-α for mice of (Control) group.

Figure 5C manifested that the oral administration of paquinimod produces a significant reduction in mRNA level for NF-κB in the skin and inhibits the inflammatory effect for imiquimod. Dexamethasone has a clear anti-inflammatory effect on NF-κB level in comparison to the model group.

The gene expression for IL-17 in the back skin for the model group was elevated higher than that in a normal animal. Paquinimod successfully reduced the gene expression for IL-17 in comparison to model mice. Dexamethasone produces a paquinimod like an effect on the IL-17 level. (Figure 5D).

Figure 4. Effect of oral administration of paquinimod on (A: Serum IL-23 level, B: IL-17 level, and C: TNF alfa level) in imiquimod-induced psoriasis in mice.

Each value represents mean ± standard deviation (SD). * Significantly different (P<0.05) concerning the negative control group. # significant compared to the vehicle susp + IMQ group.
Oral paquinimod administration against imiquimod-induced psoriasis

Effect of oral administration of paquinimod on histological examination in imiquimod-induced psoriasis in mice.

In the sections of the skin of the back area of the normal control group, the histological sections showed a normal appearance as in Figure 6 A. While in the skin of the section of the back area (vehicle oint + IMQ), the histological section showed marked hyperkeratosis and acanthosis (5 cells) of the epidermis with heavy inflammatory cells in the upper dermis as in Figure 6 B. Also, paquinimod pretreatment produce a section of back area skin that showed the normal-looking appearance of skin tissues as in Figure 6 C. In the dexamethasone suspension pretreated mice, the section of the back area showed a thin epidermis layer, there is still hyperkeratosis with mild acanthosis and a rete ridge with mild inflammatory cells as in Figure 6 D. In the group (Paquinimod oint only) group, the histological sections back area showed a normal-looking appearance as in Figure 6 E.

Figure 5. Effect of oral administration of paquinimod on gene expression of IL-1B, TNF-α, NF-κB, and IL-17 in imiquimod-induced psoriasis-like inflammation in mice.

Each value represents mean ± standard deviation (SD). * significantly different (P<0.05) concerning the negative control group. -# significant compared to the vehicle susp+ IMQ group.
Figure 6. Effect of paquinimod on IMQ-induced pathological changes in psoriasis on the skin of the back area. Staining: Hematoxylin and eosin (H&E). Black arrows: Sign of the thickness of the epidermal layer of the right ear section. A: control group; B: Vehicle susp+IMQ group; C: Paq susp+IMQ group; D: Dexamethasone suspension+IMQ group; E: Paq susp only group; F: Histology scoring analysis. Each value represents mean ± SD. * Significantly different compared to the control group (P<0.05). # Significantly different compared with the Vehicle+IMQ group (P<0.05).

Discussion

Psoriasis is a chronic, inflammatory, immune-mediated disease of the epidermis with systemic involvement. Psoriatic lesions appear as itchy, reddish raised plaques covered with silvery scales\(^{16}\). In this study, imiquimod has been used to induce psoriasis-like skin inflammation by topical application on the back area for 14 consecutive days which have been led to skin inflammation as monitored by increased skin thickness when measured at sacrificing time, and histopathological examination also confirmed that skin of the back area was inflamed, acanthosis, hyperkeratosis, and elongation of the rete-like ridge as shown in the model group. The quinoline-3-carboxamides (Q compounds) are small molecule compounds that have exhibited beneficial effects in several mouse models of inflammatory disease. In this study, Paquinimod is prepared as a suspension and has been orally given to mice before imiquimod application. Importantly, the present study showed that paquinimod ameliorated the psoriasis like inflammation induced by IMQ, evidenced by the significant decrease in the serum levels of TNF-α, IL-17, IL-23, coupled with a significant decrease in PASI score, skin thickness and spleen index. Greater than that serum IL-17, IL-23 level was significantly reduced in (Paquinimod susp+IMQ) group as compared with the control group which indicates that paquinimod suspension act as an anti-inflammatory agent by decreasing the serum level of these interleukins. IL-23, mainly produced by dendritic cells, macrophages, and keratinocytes acts on numerous target cells via either an IL-17-dependent or an IL-17-independent mechanism. In the first, IL-23 stimulates Th17 cells via IL-23R and induces the release of molecules such as IL-17 or IL-22\(^{16}\). These, by binding their cognate receptors IL-17R or IL-22R, eventually activate the “effector cells” keratinocytes, B cells, osteoclast precursors, macrophages, and FLS. Alternatively, the same subset of target cells can be directly challenged by the IL-23 in an IL-17-independent manner\(^{16}\). The overall effect of the activation of the IL-23 pathway consists of the recruitment of inflammatory cells within the inflamed tissue. The pro-psoriatic activity of IL-23 has been suggested by the occurrence of psoriatic-like skin lesions in a mouse model of IL-23 administration intradermal\(^{16}\). IL-23 can indeed act both independently of IL-17 by promoting the epidermal hyperplasia and activating the keratinocyte proliferation via the increase of Keratin 16 (K16) expression, and in association with IL-17 by enhancing dermal acanthosis, neutrophil recruitment and infiltration of IL-22 and IL-17-producing cells into the lesional skin\(^ {17}\). Skin-resident cells such as keratinocytes, fibroblasts, and endothelial cells, which express the receptor machinery for responding to IL-17 and IL-22 stimulation, react by up-regulating the expression of pro-inflammatory cytokines, chemokines (e.g., CXCL1 and CCL20), and anti-microbial peptides (such as LL-37 or β-defensins)\(^ {16}\). Overall, IL-17 stimulation can increase keratinocytes proliferation, neo-angiogenesis, recruitment, and activation of mast cells, neutrophils, and macrophages, while reducing the expression of adhesion molecules thus favoring the disruption of the skin barrier\(^ {16}\). Findings regarding gene expression of IL-1B, TNF-α, NF-κB, and IL-17 showed that in mice treated with IMQ was highly significant expressed. These results are consistent with the previous researches that reported the Potent disease-inhibitory effects in experimental models of autoimmune diseases\(^ {16}\). A study by Martin Stenström\(^ {16}\) revealed that Paquinimod reduces skin fibrosis in tight skin mice, an experimental model of systemic sclerosis they found that Paquinimod reduces skin fibrosis in an experimental model of SSc, and this effect correlates with local and systemic effects on the immune system\(^ {16}\). Another study by Nina Franse’n Pettersson(2018) showed that The immunomodulatory quinoline-3-carboxamide paquinimod reverses established fibrosis in a novel mouse model for liver fibrosis\(^ {18}\). The anti-inflammatory effect for paquinimod revealed a significant reduction in the expression of paquinimod treated group in comparison to the model group, indicating the strong anti-inflammatory effect of paquinimod against psoriasis induced by imiquimod and this can be proved by histopathological examination of mice skin section which has been revealed that paquinimod reduced skin inflammation, scaling, thickness, parakeratosis and epidermal infiltration that have been induced by imiquimod.
Conclusion
In conclusion, the present study revealed that the treatment of mice with paquinimod have ameliorative effects, that are comparable to those of dexamethasone, against imiquimod-induced psoriasis through reduction in TNF-α IL-17, IL-23 level, reducing (PASI, skin thickness, spleen index) and a significant reduction in the gene expression with paquinimod being more effective than dexamethasone. All the findings indicated the strong ameliorative effect of paquinimod and it is a promising intervention for psoriasis treatment, which may explain its anti-inflammatory activity.

Acknowledgment
The data of this article were abstracted from the Ph.D. thesis submitted to the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. The authors are extremely grateful to the College of Pharmacy/University of Baghdad for supporting this work.

References
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