EFFECTS OF ESTRADIOL ON IMIQUIMOD-INDUCED MODEL OF PSORIASIS IN MICE

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ABSTRACT:
Psoriasis is a long-lasting autoimmune-related skin disease characterized by red, dry, scaly and abnormal raise of epidermal layer of skin due to hyperproliferation and uncontrolled differentiation of the epidermal keratinocytes with a rapid turnover rate of the epidermal cell. Estradiol is a major female steroid sex hormone which has beneficial effects on skin physiology including delay in skin aging and can modulate epidermal keratinocytes, dermal fibroblasts, hair follicle, the sebaceous gland and melanocytes. The present study aimed to evaluate the potential activity of topical estradiol against imiquimod-induced psoriasiform skin inflammation in mice. A total of 30 Swiss albino mice were shaved on back and allocated randomly into 5 groups (of 6 mice each). Base gel was topically applied on the shaved area of 1st group mice while imiquimod (5%) gel was topically applied on the remaining 4 groups once daily for 6 days. The 3rd, 4th and 5th groups with imiquimod-induced psoriasiform skin inflammation were treated topically with clobetasol ointment (0.05%), estradiol (0.05%) gel, estradiol (0.1%) gel respectively once daily for next 6 days. At the end of the trial period, the mice were sacrificed and skin was taken for measuring certain inflammatory biomarkers levels and for histopathological study. Estradiol could significantly attenuate the severity of psoriatic lesion score, decrease the inflammatory cytokines namely TNF α, IL-17, and IL-23, and elevated the anti-inflammatory cytokines mainly IL-10. In conclusion, the present results suggested that topically applied estradiol gel exhibited significant anti-psoriatic and anti-inflammatory activities in imiquimod-induced psoriasiform skin inflammation in mice.

I. INTRODUCTION:
Psoriasis is a long-lasting autoimmune-related skin disease characterized by red, dry, scaly and abnormal raise of epidermal layer of skin due to hyperproliferation and uncontrolled differentiation of the epidermal keratinocytes with a rapid turnover rate of the epidermal cell. This disease is not curable one and various therapies are used to control symptoms. It can occur at any age and estimated to affect 2-4% of the world population. The various extension and acuteness of psoriasis viewed widely from time to time and among individuals. Psoriasis was thought to be genetic disease that is triggered by environmental factors like infection, trauma, psychological stress, and medication. After some inciting episode, whether it was infectious or traumatic, activation of innate immune cells like plasmacytoid dendritic cells in the epidermis which released proinflammatory cytokines such as IFN-α and TNF α. Then, these cytokines stimulated myeloid dendritic cells to produce IL-23 and IL-12 via activated Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway. In lymphoid tissue, epidermal antigen presenting cells like Langerhans cell presented target antigen and activated Naïve T cells via released IL-12 and IL-23 which were essential to differentiation of T cells into Th1, cytotoxic T cell (Tc1) or Th17 cells. Consequently, activated T-cells migrated to the skin where they stimulate the production of a range of cytokines and factors, which interact to produce changes within the resident epidermal and dermal cells. Topical application of imiquimod (IMQ) is reported as a novel mouse model for psoriasiform skin inflammation by inducing acanthosis, parakeratosis, and a mixed inflammatory infiltrate which are mediated via the IL-23/IL-17 axis. Imiquimod activated IL-23 producing Langerhans cells and thereby activated IL17 and IL22 producing Th17 cells are functionally involved in the pathogenesis of human-like psoriasis. These lesions showed increased epidermal proliferation, abnormal differentiation, epidermal accumulation of inflammatory cells and neoangiogenesis. Estradiol is a major female steroid sex hormone, responsible for regulation of

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menstrual cycle and development of female secondary sexual characteristics and also has many activities on
other organs like bones, fat, skin, liver, and brain. It generally reduced inflammatory markers in non-productive
system by activated ER α especially in adipose tissue and exhibited inhibitory effect against the transcription
of TNFα and IL-6 genes and modulated NFκB trafficking to the nucleus through activation of the PI3K/AKT
pathway 6. Estradiol has beneficial effects on skin physiology including a delay in skin aging and can modulate
epidermal keratinocytes, dermal fibroblasts, hair follicle, the sebaceous gland and melanocytes. Therefore,
topical application of estradiol can improve skin dryness, texture, and elasticity and reduce wrinkles in estrogens
deficiency skin 7. The present study aimed to evaluate the potential activity of topical estradiol gel against
imiquimod-induced psoriasiform skin inflammation in mice.

II. MATERIALS AND METHODS:
Estradiol (Sandrena) 0.1 & 0.05 % gel of Aspen Company and imiquimod(5%) cream (Aldra) of MEDA
Company were supplied by community pharmacies.

A total of 30 apparently healthy male adult Swiss albino mice (24-33 g, 11-15 weeks) were obtained from the
animal house of College of Medicine / Al-Nahrain University and National Center for Drug Control and
Research. The mice were housed in polypropylene cages and fed on a standard pellet diet and water ad libitum
under standard conditions (22 ± 3°C, 50 – 60% relative humidity, and 12 hours light – dark cycles). Prior to start
of the experiments, mice were allowed to acclimatize in laboratory circumstances for period of two weeks. The
Ethics Committee of Higher Education of College of Medicine / Al-Nahrain University approved the experimental
protocol.

Area of about 2cm² on the back of each mouse had been shaved for topical application. The dedulcni mice were
allocated randomly and equally into 5 groups; these groups were as follows: Group 1 (Control group): base
gel was applied once daily on the shaved area for six days. Group 2 (Induction group): 62.5 mg of IMQ cream (5%)
was applied once daily on the shaved area for six days. After psoriasiform skin inflammation being induced (as
what happened in group 2) the inflamed area was treated topically for further six days with once daily
application of either clobetasol ointment (0.05%) (Group 3), estradiol gel (0.05%) (w/v) (Group 4), or estradiol
gel (0.1%) (w/v) ( Group 5). During the experiment, the clinical signs were noticed and scored with the aid of
psoriasis area and severity index (PASI). The skin thickness of the right ear measured in duplicate using a digital
micrometer caliper and scored. Erythema and scaling of mouse niks devahskcab were scored independently on a
scale from 0 to 4 (0: none; 1: slight; 2: moderate; 3: marked; 4: very marked). The level of erythema was scored
using a scoring table with red taints (0: no lesion; 1: slightly pink; 2: pink; 3: red; 4: dark red). The cumulative
score (erythema plus scaling plus thickening) served as a measure of the severity of inflammation (scale 0 –
12) 8. At the end of the experiment, all the mice were euthanized by ether and the skin samples were obtained for
measurement of tissue TNF- alpha, IL 19, IL 17, and IL 23 and for histopathological examination.

Preparation of Tissue Homogenate
Freshly harvested back skin tissue of 1 gm stored in 9 ml of phosphate buffered saline. Tissues were
homogenized by mortar and pestle then centrifuged by cool centrifuged adjusted to 5000 rpm for 10 minutes.
The supernatants were frozen at (−80 °C) for further measurements 9.

Measurement of Biomarkers
The concentration of tissue TNF- alpha, IL 17, and IL 23 were measured by using ELISA technique (shanghai,
china). This assay employs the quantitative sandwich enzyme immunooassay technique 10.

Histopathological examination:
Skin tissues from mice of different groups were preserved in neutral buffer formalin (10%), embedded in paraffin
using standard method. The paraffin tissue blocks underwent serially section to obtain consecutive levels, and
then stained with hematoxylin and eosin 11. They were examined under a light microscope and scored using
barker’s score system to evaluate the pathological alterations on a scale ranging from 0 to 10 as shown in table
(1) 12.

Table (1): The Baker’s scoring system 13.

<table>
<thead>
<tr>
<th>Items</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin</td>
<td></td>
</tr>
<tr>
<td>Munro abscess</td>
<td>2</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th></th>
<th></th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parakeratosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermis</td>
<td>Thinning above papillae</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Lengthening and clubbing of rete ridges</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Acanthosis</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Lack of granular layer</td>
<td>1</td>
</tr>
<tr>
<td><strong>Dermis</strong></td>
<td>Lymphocytic infiltrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Papillary congestion</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS 22). Descriptive statistics for the numerical data were formulated as mean and standard error of the mean (Mean ± SEM) after triplicate sample analysis especially for pharmaceutical assays. Numerical data were analyzed using either independent t test or one-way analysis of variance (ANOVA) test with post hoc Tukey test. The non-descriptive data measured by using Mann-Whitney U test. The P values less than 0.05 were considered statistically significant.

**III. RESULTS:**

**Effects of tested drugs on Scoring severity of psoriasiform skin inflammation**

The induction group (Group 2) showed a significant increase in psoriatic score severity including erythema, scaling, thickening, and cumulative scores when being compared to negative control group (Group 1) (P<0.05) (Figures 1 & 2). Clobetasol ointment (0.05%) significantly reduced the severity of psoriatic lesion scoring in psoriatic induced mice when being compared with induction group (P < 0.05) (Figures 1 & 2). Each of estradiol gels (0.05% & 0.1%) (Groups 4 & 5 respectively) significantly declined severity of psoriatic skin lesion score induced by imiquimod when being compared with induction group (P< 0.05). There were no significant differences among both strengths (0.05% & 0.1%) of estradiol and clobetasol ointment (0.05%) (Group 3) on scoring severity of psoriatic lesion (P < 0.05) (Figures 1 & 2).
Figure (1): Scoring severity of psoriasiform skin inflammation of the experimental groups at end of the experiment. A: Control group (Group 1); B: Induction group (Group 2); C: Clobetasol ointment (0.05%) group (Group 3); D: estradiol gel (0.05 %) (Group 4); E: estradiol gel (0.1 %) (Group 5).
Effects of tested drug on levels of skin tissue biomarkers

Imiquimod significantly elevated tissue levels of TNF α, IL17, and IL23 in comparison with control group \( (P<0.05) \) as shown in table (2) and Figure (3). Clobetasol significantly lowered the levels of tissue biomarkers in comparison with induction group \( (P<0.05) \) as shown in table (2) and Figure (3). Estradiol gels (0.05 & 0.1%) significantly decreased the levels of tissue biomarkers (TNFα, IL17, and IL23) and increased the levels of tissue anti-inflammatory biomarker (IL10) in comparison with induction group \( (P<0.05) \). Estradiol at both strength (0.05 & 0.1%) gels were comparable to clobetasol 0.05% ointment in reducing inflammatory cytokine (TNF α and IL23) \( (P<0.05) \) as shown in table (2) and Figure (3). While estradiol (0.05 & 0.1%) gel groups significantly reduced inflammatory cytokine (IL17) and elevated anti-inflammatory cytokine (IL10) in comparison with clobetasol group \( (P<0.05) \) as shown in table (2) and Figure (3).
Table (2): Effects of tested drugs on levels of skin tissue biomarkers

<table>
<thead>
<tr>
<th>Biomarkers (pg/g)</th>
<th>Groups (Mean ± SEM)</th>
<th>Control</th>
<th>Induction</th>
<th>Clobetasol ointment (0.05%)</th>
<th>Estradiol gel (0.05%)</th>
<th>Estradiol gel (0.1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF α</td>
<td></td>
<td>585.5±38.72</td>
<td>1390.84±59.88</td>
<td>1030.17±47</td>
<td>976.72±59.1</td>
<td>931.26±60.44</td>
</tr>
<tr>
<td>IL10</td>
<td></td>
<td>463.1±7.3</td>
<td>527.11±5.85</td>
<td>442.53±12</td>
<td>783.8±8.54</td>
<td>936.36±9.23</td>
</tr>
<tr>
<td>IL17</td>
<td></td>
<td>122.25±4.85</td>
<td>566.01±13.82</td>
<td>292.56±8.85*</td>
<td>302.29±6.94*</td>
<td>200.47±3.58*</td>
</tr>
<tr>
<td>IL23</td>
<td></td>
<td>73.01±9.4</td>
<td>152.22±5.2</td>
<td>87.1±7.31</td>
<td>92.59±6.22</td>
<td>88.89±4.99*</td>
</tr>
</tbody>
</table>

TNF: Tumor necrosis factor, IL: Interleukin

*: Significant effect (P<0.05) compared to indication group.
#: significant effect (P<0.05) compared to clobetasol group and estradiol 0.05% group.

Figure (3): Effects of Estradiol on the levels of tissue biomarkers.

Effects of the tested drugs on histopathological features of skin

The histological features of control group differentiated by the spread of keratin layer without Munro abscess and no changes in the thickness of epidermis layer, no rete ridges, and absence of granular layer as shown in figure (4). Imiquimod caused significant histopathological changes characterized by Munro abscess, hyperkeratosis, abnormal thickness of epidermis, thinning above papillae, presence of rete ridges, and lack of granular layer when compared with control group (P < 0.05) as shown in figure (5) and table (3). The histopathological features of clobetasol ointment (0.05%) group characterized by thinning of epidermal thickness and mild infiltration of inflammatory cells as shown in figure (6). Clobetasol had significant restorative effect on psoriatic lesion in compared with induction group (P < 0.05) as shown in tables (3). The histopathological features of estradiol (0.05% & 0.1%) groups characterized by intermittent parakeratosis and dermal infiltrates of lymphocytes as shown in figures (7 & 8); besides, there were no significant differences among estradiol (0.05%), estradiol (0.1%), and clobetasol ointment (0.05%) groups (P > 0.05) as shown in tables (3).

Table (3) Barker’s score of all experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Barker’s scores Mean ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Induction</td>
<td>8.58 ± 0.71</td>
</tr>
<tr>
<td>Clobetasol ointment (0.05%)</td>
<td>0.58 ± 0.23</td>
</tr>
<tr>
<td>Estradiol gel (0.05%)</td>
<td>0.42 ± 0.15</td>
</tr>
<tr>
<td>Estradiol gel (0.1%)</td>
<td>0.25 ± 0.11</td>
</tr>
</tbody>
</table>

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*: Significant difference (p < 0.05) when induction group compared to all other groups.

Figure (4): Skin section from mice back skin of control group showing normal spread of keratin, normal thickness of epidermis, and presence of granular layer (H&E (A) 10X & (B) 40X).

Figure (5): Skin section from mice back skin of induction group showing Munro abscess, hyperkeratosis, abnormal thickness of epidermis, thinning above papillae, presence of rete ridges, and lack of granular layer (H&E (A & B) 10X & (C & D) 40X).
Black arrow: epidermis layer

Figure (6): Skin section from mice back skin of clobetasol ointment (0.05%) group showing thinning of epidermis layers (H&E (A) 10X & (B) 40X).

Red arrow: keratin

Figure (7): Skin section from mice back skin of Estradiol gel (0.05%) group showing parakeratosis (H&E (A) 10X & (B) 40X).

Red arrow: keratin; black: epidermis layer; blue: infiltrate lymphocytes

Figure (8): Skin section from mice back skin of Estradiolgel (0.1%) group showing parakeratosis, thinning of epidermis layers, and infiltration of lymphocytes (H&E (A) 10X & (B) 40X).
IV. DISCUSSION

Imiquimod-induced psoriasis in mice is closely similar to those of human beings regarding erythema, scaling, and epidermal thickness within 6-days experimental period since it mediated via the IL-23/IL-17 axis psoriasis-like skin inflammation inducing acanthosis, parakeratosis, and inflammatory cell infiltration. Clobetasol significantly reduced psoriatic scoring severity due to its proofed anti-inflammatory and anti-proliferative effects via suppression activation of immune cells and cytokines production. It possessed many antipsoriatic properties including promotion of the transcription of anti-inflammatory genes over inflammatory genes, reduction of epidermal mitotic rate, shortening of survival of lymphocytes, lowering of blood flow to inflamed site by vasoconstriction, and shifting of adaptive immune response from Th1 to Th2 pathway. Topical application of estradiol significantly improved PASI when being compared with imiquimod induced psoriatic lesion. Several studies referred that estradiol restored and maintained skin barriers in female especially during follicular and ovulatory phases of menstrual cycle where estrogen level is high. It keeps and repairs skin moisture via the enhancing of sebum secretion and raises the levels of mucopolysaccharides and hyaluronic acid in the dermis to maintain the skin hydrated. Other study revealed that estrogen protect skin cells against oxidative damages.

In the present study, IMQ induced mouse psoriatic inflammation via activated Langerhans cells with TLR-7 and mediated activation of the central transcription factor nuclear factor-κB resulted in secretion of cytokines primarily TNF-α, activation of IL-23 producing Langerhans and thereby activation of IL-17 and IL-22 producing Th17 cells. The overexpression of cytokines like TNF-α, IL-17, and IL-23 in skin treated with imiquimod responsible for desquamation, epidermal hyperproliferation, and infiltration of lymphocyte. These changes seemed to be compatible with other experimental studies. TNF-α is responsible for initiated inflammatory psoriatic mechanism and rise in production of pro-inflammatory cytokines (IL-6, IL-17, and vasoactive peptide) and adhesion molecules (Intercellular adhesion molecules-1 (ICAM-1), P-selectin, and E-selectin). High level of TNF-α was detected in hyperproliferation psoriatic lesion. IL-17 is an pro-inflammatory cytokine that present as undetectable amount in normal skin and its level elevated in psoriatic lesion. It is responsible for expression of other inflammatory cytokines, colony stimulating factors and chemokines from dendritic cells, neutrophil, macrophage, and epithelial cells. IL-23 is an inflammatory cytokine that induced the expansion and maintenance of psoriatic lesion by activation Th17 cells and then enhanced keratinocyte proliferation. In the present study, estradiolsignificantly reduced the levels of inflammatory cytokines (TNF-α, IL-17, and IL-23). Lin et al., 2016 observation that improvement of psoriasis during pregnancy related to estrogen level. Estrogens reduce the psoriatic inflammation by many effects including: they reduce the neutrophil’s account in blood and keratinocytes which responsible for expression of some macrophage-chemotaxis cytokines, they stimulated B lymphocytes and dendritic cells to release IL-10 which is considered anti-inflammatory cytokine. IL-10 mediates many therapeutic benefits including suppression of cytokines and chemokine including IL-12 and TNF-α producing macrophage, inhibit T-cell activation indirectly mediated via down regulation of monocyctic antigen presentation, and suppression of IFN-γ production of lymphocytes by inhibition of IL-12 synthesis in accessory cells. The activity of matrix metalloproteinase in fibroblasts also decreases by estrogens which minimize the extracellular matrix destruction and the release of growth factors, this considers other contributor psoriatic link.

IMQ caused significant histopathological changes characterized by Munro abscess, hyperkeratosis, abnormal thickness of epidermis, thinning above papillae, presence of rete ridges, and lack of granular layer; such histopathological features in compatible with other studies. Clobetasol had a restorative effect on histological feature of imiquimod-induced psoriatic lesion characterized by normal spread of keratin, very thin epidermal layer and presence of granular layer which mediated anti-inflammatory and immunosuppression effects. Clobetasol is considered as a standard topical therapy of psoriasis and frequently used as positive control group for investigation efficacy of other tested agent. Estradiol showed an effective therapeutic effect on IMQ-induced psoriatic lesion via improvement of histopathological features by retaining skin appearance similar to normal skin probably via estrogen-mediated ER-dependent pathways including inhibit production of macrophage, reduce expression of chemokine (CXCL-8) which known to cause allergic reaction and profound inflammation, reduce the neutrophil adhesion molecule L-selectin leading to decrease accumulation of neutrophils at site of inflammation, and through activation of ERβ lead to a beneficial changing in the pattern of differentiation and the proliferation activity of keratinocytes.
V. CONCLUSION:

In the present study, the topically applied estradiol gel, at its both concentrations (0.05 & 0.1%), exhibited a potentially anti-psoriatic and anti-inflammatory activities via its ability in reduction of inflammatory cytokines and improvement of histopathological features of skin.

REFERENCES:


