Possible Protective Effect of Ozone Therapy in Imiquimod Induced Psoriasis-Like Inflammation in Mice

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Abstract

Psoriasis is a common chronic skin condition characterized by the infiltration of inflammatory cells into the epidermis and altered keratinocyte differentiation. This study was designed to investigate whether ozone act as an anti-psoriatic agent in psoriasis induced by imiquimod in mice and compared this effect with olive oil. For this 21 Albino, BALB/c mice male, 8 weeks of age weighing between 25 to 40 gm were used. Psoriasis was introduced in them by treating them with imiquimod. Topical ozone treatment and treatment with olive oil were done to compare the efficacy of the ozonated oil in the experimental animals. In this study albino mice were allocated into Three groups and treated as follows: 1. The first group was named negative control olive oil group which receive 62.5mg, 5mg of olive oil topically on both back area and right ear after imiquimod application for 14 consecutive days.

2. The second group named ozone-treated group in which animals receive 62.5mg, 5mg of ozone oil on both back area and right ear after imiquimod application for 14 consecutive days.

3. The third group named control normal group in which animals receive only olive oil 62.5mg,5mg of olive oil topically on both back area and right ear after application of imiquimod cream base for 14 consecutive days.

The study result showed that ozonated oil can effectively reduce psoriasis-like skin inflammation in the experimental mice model. The level of inflammatory cytokines such as IL-17 and IL-23 were also reduced significantly in the ozone treatment group compared with the olive oil-treated one. Further, it was also shown that the spleen index and the PASI score also decreased significantly in the ozone-treated group. The NF-κB signaling pathway was also inhibited after the ozone treatment was done. Thus this study concluded that ozonated oil is a safe and effective mode of treatment in IMQ-induced psoriasis when compared with olive oil.

Keyword: Ozonated oil, psoriasis, imiquimod induced psoriasis, inflammation

1. Introduction

Psoriasis is a chronic skin condition commonly found in all countries across the globe. Approximately 1% to 2% of the world's population comprises of psoriasis population and has a significant impact on the quality of life of the patients suffering this condition [1]. This condition usually develops late in the adolescent period and is a difficult condition to be cured completely. This condition is characterized by scaly and plaque-like skin damage which is accompanied by pain and itching. This is an inflammatory condition where different pro-inflammatory cytokines are secreted. The activation of adaptive as well as innate immune system cause damage of the multiple tissues and organs. The characterized proliferation of keratinocytes in the dermis and epidermis layer of the skin results from the activation of innate and adaptive immune systems. Dendritic cells and T cells play a significant role in this process [2].

The clear mechanism of pathogenesis of psoriasis is still not clearly understood; hence the detailed treatment planning of the same is not uniform. Recently, several studies have addressed the treatment options of psoriasis in animal models [3]. However, owing to the complicated mechanism of the condition none of the treatments were shown to cure this condition.

For this purpose Imiquimod, an isocyclic imidazoquinoline amine was used for developing psoriasis-like skin conditions in experimental animals. Studies have shown that the application of 5% imiquimod cream to mouse skin induces psoriasis-like conditions. In addition, it also increases the levels of immune cell infiltration in the affected area [4].

Therefore, this is an ideal model for the evaluation of an effective treatment regimen in psoriasis. For more than a century, ozone has been known to be an excellent disinfectant that nevertheless had to be used with caution for its oxidizing properties. Only during the last decade, it has have been learned how to tame its great reactivity by precisely dosing its concentration and permanently incorporating the gas into triglycerides where gaseous ozone chemically reacts with unsaturated substrates

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leading to therapeutically active ozonated derivatives. However, a detailed analysis of all these products is yet not done. Hence, the present paper aims to analyze these derivatives in psoriasis induced mice so that the strategy to obtain products with the best characteristics can be achieved.

2. Materials and methods

Chemicals and kits

The chemicals that were used in this work include formaline (merk chemicals, Germany), diethyl ether (BDH chemicals, India), natural olive oil.The kits used in this study include (Interleukin-23, Interleukin-17, Tumor necrosis factor alfa,nuclear factor kappa B)all of them from (Elab.science, China).

Methods

For animal treatment total of 21Albino, BALB/c mice male, 8weeks of age weighing between 25 to 40 gm were purchased from the department of drug control and divided into 3 groups each.

1. The first group named negative control olive oil group which receive 62.5mg,5mg of olive oil topically on both back area and right ear after imiquimod application for 14 consecutive days.
2. The second group named ozone-treated group in which animals receive 62.5mg, 5mg of ozone oil on both back area and right ear after imiquimod application for 14 consecutive days.
3. The third group named control normal group in which animals receive only olive oil 62.5mg,5mg of olive oil topically on both back area and right ear after application of imiquimod cream base for 14 consecutive days.

They were kept in the animal house of the college of pharmacy/University of Baghdad under specific pathogen-free conditions and provided with water and food ad libitum under a 12-hr light-dark cycle and maintained conventionally during the study with regulated air temperature (15-21˚C).

Preparation of ozone oil

Unsaturated lipid substrates react with insufflated gaseousO2/O3 mixture leading to therapeutically active ozonated derivatives. Briefly, the postulated mechanism known as the Criegeereaction provides that ozone combines with an unsaturated bond to form an initial, unstable primary ozonide which readily decomposes to form a zwitterions and a carbonyl fragment. In an anhydrous environment, these substrates combine to give the typical cyclic trioxolane derivative.

Scoring the severity of skin inflammation

To score the severity of inflammation of the back skin, the Psoriasis Area, and Severity Index (PASI) score for back skin was determined by a professional dermatologist. Erythema, scaling, and thickening was scored independently on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. The cumulative score (erythema plus scaling plus thickening) served as a measure of the severity of inflammation (scale 0–12)

Measurements of spleen index

At the end of the experiment (day 14), the body weight and spleen weight for all mice was measured and the spleen index was calculated by dividing spleen weight in mg by the bodyweight that was measured during sacrificing time in gm.

Measurements of skin thickness

After sacrificing our mice, back skin thickness was measured in mm via digital vernier caliper and comparing the measured thickness among groups.

Preparation of serum sample

After euthanization of the animal by anesthetic diethyl ether, blood was collected by heart puncture and put in an Eppendorf tube and centrifuged at 3500rpm for 15 minutes to obtain serum, which was separated and stored at -20 C until the day of analysis. Serum was utilized for estimation of IL-23 and IL-17.

Preparation of skin tissue homogenate

At the end of the experiment (day 14), the skin from the back area was taken by incision and washed with phosphate buffer pH (7-7.2). Ten percent (10%) of back skin tissue homogenate was prepared and centrifuged at 5000rpm for 15 minutes, and the supernatant is stored at -20 ˚C until the day of analysis. Skin homogenate was utilized to determine the TNF-α and NF-Kb level in the tissue.

Histological examination

Skin from the back area and right ear utilized in this study were prepared for histological examination according to the method of Junqueira et al at 1995 using paraffin section methods.

Statistical analysis

The significance of differences between the mean values was calculated using the paired and unpaired student t-test. A P-value of less than 0.05 was considered significant for all data shown in this respect.

3. Result and Interpretation

1. An imiquimod-induced psoriasis-like dermatitis model preparation

Psoriasis like dermatitis model was prepared by using 5% imiquimod cream for 14 days on the backside and the ear part of the mice model. The skin of the mice started showing psoriasis-like mild erythema on the 5 days onwards. The PASI score increased in the imiquimod treated groupcompared to the control group. Histopathology showed that presence of psoriases like dermatitis features in imiquimod treated groups including Munro's microabscesses and extended rete ridges (Figure 1).

2. Ozone treatment inhibits phenotypes of imiquimod-induced (IMQ) psoriasis-like dermatitis

To further evaluate the therapeutic effectivity of the topical ozone treatment on imiquimod-induced psoriasis-like dermatitis the PASI score and spleen size was also measured. The PASI score in the ozone-treated group showed significant decreases in value compared with the IMQ+Ozone treated group (Figure 3). In previous studies, it was reported that spleen enlargement is a common

phenomenon in IMQ-induced psoriasis in mice. We found that in the ozone-treated group the spleen weight was significantly reduced compared to the control group or IMQ+olive oil group (Figure 5).

3. Topical ozone treatment inhibits IMQ-induced psoriasis-like inflammation

The mechanism behind the inhibition of psoriasis-like dermatitis in IMQ treated mice further the neuro-inflammatory protein concentration was measured. It was found that IMQ-induced activation of different inflammatory proteins gets significantly reduced in ozone-treated mice compared to control groups and also in the IMQ+olive oil group. The level of NF-κB, IL-17, and IL-23 was found to be reduced in the ozone-treated group. These results indicate that ozone treatment treats psoriasis lesions by inhibiting the local inflammatory processes.

4. Discussion

Psoriasis is a chronic inflammatory condition that is not completely curable. Several studies have recommended different treatment strategies for psoriasis but to date, no such definitive treatment regimen is derived [5]. Ozone in high concentration can cause cell damage, skin irritation, and other respiratory diseases. It was reported that in lower concentration ozone can be effectively quenched by the powerful ant oxidative system of the human body. In these concentrations, ranges ozone has been used as sterilizing agents and regulation of immunity functions. They have also been shown to promote wound healing [6].

In contact with the skin tissues, they are shown to produce reactive oxygen species and aldehydes. It was reported to play a therapeutic role in many inflammatory conditions for this antioxidant property. In experimental rats, the endometrial injury caused by inflammatory diseases was shown to be cured with ozone therapy [7]. Recently an extensive body of evidence suggests that ozone therapy can reduce the use of antibiotics and hence decreasing the risk of producing antibiotic resistance and other side effects arising from long-term use of antibiotics [5].

In this paper, we have shown that ozone therapy can control the progression of IMQ-induced psoriasis in an experimental mice model by inhibiting the inflammatory responses. In the past studies have shown that ozonated oil is capable of delivering reactive oxygen species to the infected section and also maintains the concentration of such ozonated derivates in the part for a longer period [7]. Tan et al (2018) have shown that ozonated oil can effectively reduce the stable psoriasis Vulgaris and have similar efficacy as the topical application of glucocorticoid derivatives. Moreover, this study also reported that in the ozonated oil-treated group the infiltration of inflammatory cells was significantly reduced compared with the control group [8].

In the present study, we have also shown that the psoriasis skin of the infected animal is mitigated significantly with the inflammatory biomarkers including IL-17, IL-23, and NF-κB. It was also shown that ozone therapy was effective in reducing the number of all these biomarkers compared with the control group. This data provides an important insight into the underlying therapeutic mechanism of ozone treatment on IMQ-induced psoriatic mice.

In psoriatic mice, the NF-κB signaling pathway activation happens because of the elevated level of the TLRs [9]. In the present study, we have shown that this TLR activated NF-κB pathway is inhibited with ozone therapy. Apart from ozonated oil, another treatment regimen with ozone has also been reported. Ozone has also shown to be effective when used in combination with other agents to increase efficacy.

Despite several strengths, this study also has a few limitations. Firstly, this study hasn't reported the long term efficacy of the treatment and there is also no data available for recurrent psoriasis. Moreover, whether ozone treatment plays a regulatory role in the differentiation of the keratinocytes is beyond the scope of this study and has nor answered.

5. Conclusion

This study reported that ozone treatment can significantly reduce IMQ-induced psoriasis in mice by inhibiting the inflammatory pathways by reducing the level of inflammatory cytokines via modulation of NF-κB signaling pathways.

What is already know on this topic

Psoriasis is a chronic skin condition commonly found in all countries across the globe.

Psoriasis is a chronic inflammatory condition that is not completely curable.

What this study adds

Ozone treatment can significantly reduce IMQ-induced psoriasis in mice by inhibiting the inflammatory pathways by reducing the level of inflammatory cytokines via modulation of NF-κB signaling pathways.

Ozonated oil is a safe and effective mode of treatment in IMQ-induced psoriasis and can be approved for usage in future.

Competing interests

The authors declare no competing interest.

Authors' contributions

All authors conceived and designed the study.

Raghad Abdulsalam conducted the experiments and wrote the paper,Munaf Hashim analyze the data, Wrood Salim check statistics carefully, Yasir Wael determine PASI score by physical examination,Abdullah Mohammad prepare ozonated oil.All authors contributed to the manuscript revisions.All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

6. Acknowledgements

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Tables and Figures

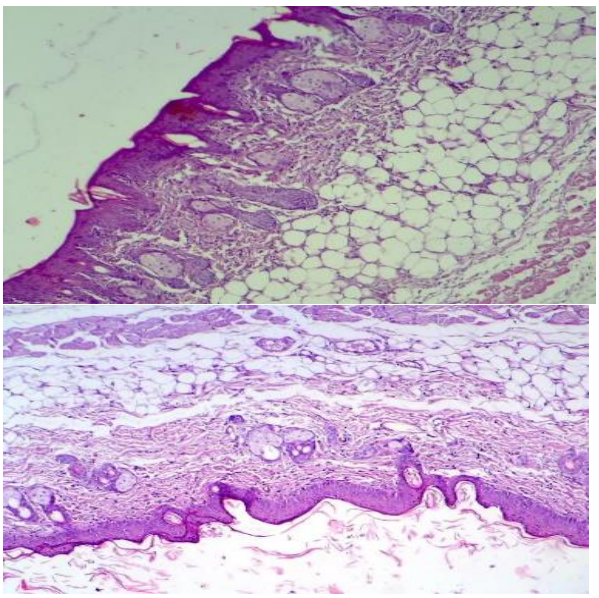


Figure 1: Histopathological experiments. Above diagram: Control group treated with olive oil. Below: mice treated with ozonated oil

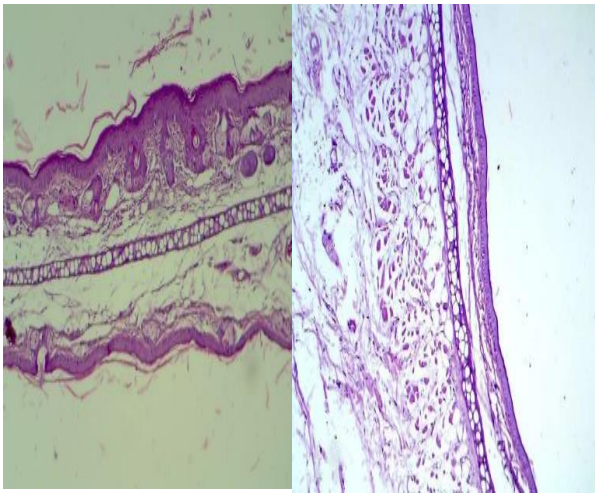
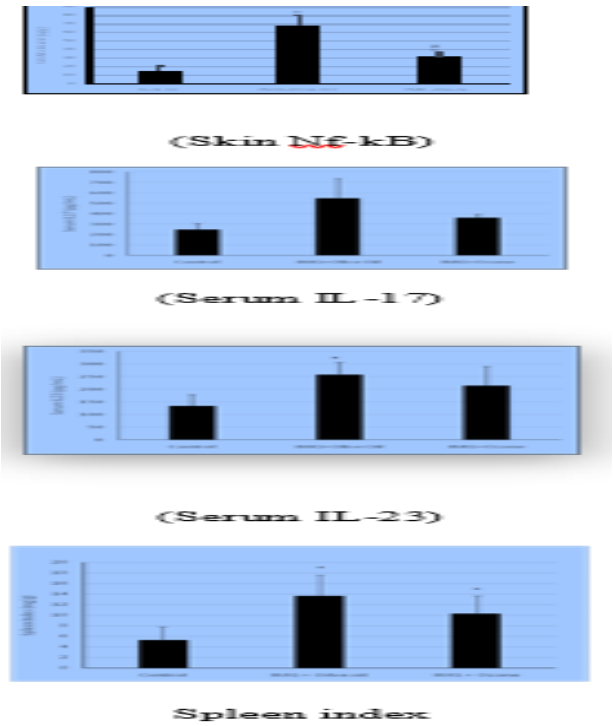


Figure 2: Histopathological experiments. Above diagram: (Right ear)Control group treated with olive oil. Below: mice treated with ozonated oil

Figure 3: Ozonated Oil treatment decreases the level of immune-inflammatory cell concentration. The concentration of NF□B, IL-17, IL-23, and spleen index value decreases in IMQ+ozone treated mice



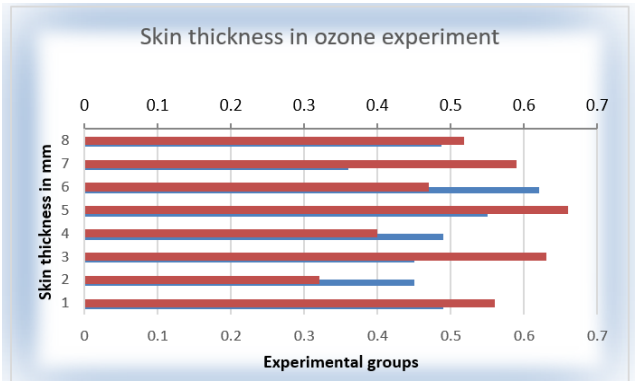


Figure 4: The thickness of skin after ozone treatment

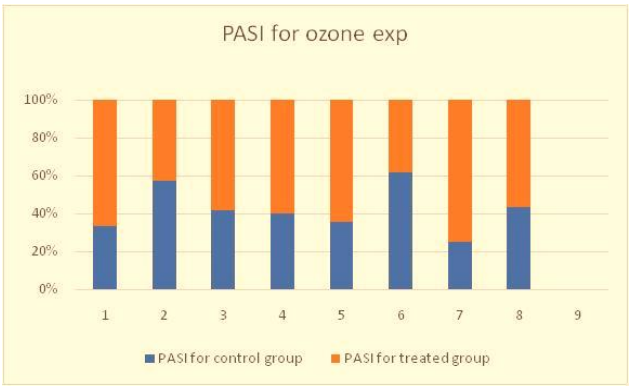


Figure 5: PASI score for ozone-treated and control group

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