

Using Lower Doses of Topical Mometasone Furoate in the Treatment of Atopic Dermatitis by Applying Hyaluronic Acid as a Skin Penetration Enhancer

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Abstract: *The objective of the present study was to investigate the possibility to add hyaluronic acid (HA) as skin penetration enhancers to mometasone furoate (MF) to enhance its skin absorption, and so decrease the dose applied in the treatment of atopic dermatitis and so decrease the side effects. MF was introduced into absorption ointment base in full dose 0.1% and in half dose 0.05% combined with 0.1% HA. An open study of 30 patients with moderate to severe atopic dermatitis was carried out for one week of treatment. The prepared formulations were also evaluated for anti-inflammatory effects in carrageenan-induced oedema in male albino rats also the drug release through a standard cellophane membrane was evaluated. The data obtained from release studies revealed that the total amount of drug released was affected by the nature and the composition of bases. Animal studies showed that the differences in decrease in oedema diameter between the full dose of MF 0.1% and the half and the quarter dose of MF with hyaluronic acid sodium salt 0.1% added were unnoticed and the statistical analysis showed that the difference was insignificant ($p > 0.05$). The clinical study showed that upon the comparison was between the full dose of MF 0.1% and the half dose of MF 0.05% with HA 0.1% added and the statistical studies showed that the difference in improvement of atopic dermatitis cases was statistically insignificant ($p > 0.9999$).*

Keywords: *Hyaluronic acid, mometasone furoate, atopic dermatitis, anti-inflammatory, dose.*

1. INTRODUCTION

Atopic dermatitis is an inflammatory condition of the skin characterized by erythema, pruritus, scaling, lichenification and papulovesicles. Atopic dermatitis is a distinct condition in persons who are genetically predisposed to developing immunoglobulin (Ig) E-mediated hypersensitivity reactions. It is characterized by the itch-scratch cycle. Affected persons have the sensation of itch, followed by scratching and the subsequent creation of a rash. The classic triad of atopy includes eczema, asthma and allergies. A wide range of environmental factors, such as contact allergens, stress, food, skin flora and humidity, play roles in the development and severity of atopic dermatitis [1].

Atopic dermatitis tends to be a chronic relapsing disease. The goals of therapy should be to reduce the number and severity of flares and to increase the number of disease-free periods. The mainstay of treatment for atopic dermatitis is hydrating the skin with the regular use of emollients and suppressing cutaneous inflammation with topical corticosteroids [2]. There is little evidence that the application of topical corticosteroids twice a day is more effective than once-daily applications, and more frequent use may cause more local side effects. A main concern with the use of topical corticosteroids is irreversible skin thinning [3].

Other possible side effects of corticosteroids include facial telangiectasia and glaucoma from periocular use (rarely reported in adults). Secondary adrenal suppression and the suppression of growth resulting from systemic absorption of topical corticosteroids are also concerns, although clinically relevant adrenal suppression is very rare [4].

Mometasone furoate (9α , 21-dichloro-11 β , 17-dihydroxy-16 α -methylpregna-1,4-diene-3,20-dione 17-(2-furoate)) is a synthetic corticosteroid which is non-fluorinated and containing a furoate moiety. Mometasone furoate is used topically to reduce inflammation of the skin or in the

airways. It is a prodrug of the free mometasone. It is used in the treatment of inflammatory skin disorders such as eczema and psoriasis. It is also used in the treatment of allergic rhinitis and asthma [5]. It reduces inflammation by causing several effects such as reversing the activation of inflammatory proteins, activating the secretion of anti-inflammatory proteins, stabilizing cell membranes and decreasing the influx of inflammatory cells.

Mometasone furoate was more effective than betamethasone valerate in the treatment of 53 patients with various dermatoses [6], and also more effective than hydrocortisone 17-butyrate, in 48 patients with childhood atopic dermatitis [7].

In studies comparing mometasone furoate to hydrocortisone 17-butyrate, mometasone furoate applied for 6 weeks resulted in clearing of the skin in 43 patients with atopic dermatitis [8].

Of the various skin layers, it is the stratum corneum that is the rate-limiting barrier to percutaneous drug transport. In fact, the stratum corneum is a remarkably more formidable barrier to drug transport than the epithelial barriers of gastrointestinal, nasal, buccal, vaginal, or rectal delivery routes. Ideally, penetration enhancers reversibly reduce the barrier resistance of the stratum corneum without damaging viable cells [9]. Chemical penetration enhancers were used to increase the skin permeability by reversibly damaging or altering the physicochemical nature of the stratum corneum to reduce its diffusional resistance. One of the problems associated with many chemical penetration enhancers is that they cause irritancy in the skin [10]. Some of the more desirable properties for penetration enhancers have been given such as, being non-toxic, non-irritating and non-allergenic. They would ideally work rapidly; the activity and duration of effect should be both predictable and reproducible. They should have no pharmacological activity within the body.

Hyaluronic acid (HA) has been introduced as a vehicle for topical application of drugs to the skin [11]. It is a naturally occurring polyanionic, polysaccharide that consist of N-acetyl glucosamine and glucuronic acid. It is present in the intercellular matrix of most vertebrate connective tissues especially skin. It is most frequently referred to as hyaluronic acid due to the fact that exists *in vivo* as a polyanion and not in protonated acid form. Commercially produced hyaluronic acid is isolated either from animal sources, within the synovial fluid, umbilical cord, skin, and rooster comb or from bacteria through a process of fermentation or direct isolation. [12]. Generally, HA is thought to act as either a mucoadhesive and retain the drug at its site of absorption or to modify the *in vivo* release rate of the therapeutic agent. Extensive studies on the chemical and physicochemical properties of HA and its physiological role in humans, together with its versatile properties, such as its biocompatibility, non-immunogenicity, biodegradability and viscoelasticity, have proved that it is an ideal biomaterial for cosmetic, medical and pharmaceutical applications [13].

The objective of the present study was to investigate the possibility to add hyaluronic acid to mometasone furoate to enhance its skin absorption, and so decrease the dose applied in the treatment of atopic dermatitis and its side effects. The prepared absorption ointment base formulations were evaluated for anti-inflammatory effects in carrageenan induced oedema in male albino rats. An open study of 30 patients with moderate to severe atopic dermatitis was carried out for one week of treatment. MF was introduced into the base in full dose 0.1% and in half dose 0.05% combined with 0.1% HA

2. MATERIAL AND METHODS

2.1. Materials

Mometasone furoate was kindly supplied by Sigma Pharmaceutical Industries, Egypt. Hyaluronic acid sodium salt from streptococcus, Sigma Aldrich, USA. Dialysis sacks, Sigma Aldrich, USA. Anhydrous lanolin, Elnasr pharmaceutical chemicals, Egypt. All other ingredients were of analytical grade.

2.2. Preparation of Topical Formulations

Mometasone furoate (0.1% w/w) was introduced absorption ointment. It was also introduced into the same bases in half dose 0.05% with addition of 0.1% HA.

Absorption base:

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Hard paraffine was added to anhydrous wool fat and the white soft paraffine, the all were heated up to $70\pm 2^\circ\text{C}$ in a water bath then added to liquid paraffin in which 0.1% MF was levigated at the same temperature then water was added with stirring and cooled down at room temperature (F1). The same base was prepared by the same manner but the dose of MF (0.05%) with the addition of 0.1% HA that was previously dissolved in the water portion of the base (F2). The compositions of the prepared formulations were illustrated in table (1).

Table1. Composition of the Prepared Topical Bases

Component	F1	F2
MF (%)	0.1	0.1
HA (%)	-	0.1
Hard paraffin(g)	22	22
Anhydrous wool fat(g)	10	10
White soft paraffin(g)	8	8
Liquid paraffin(ml)	50	50
Distilled water to(g)	100	100

2.3. In Vitro Drug Release

A sample of 2 grams of each formulation with and without hyaluronic acid added was accurately weighed and placed on a semipermeable standard cellophane membrane previously immersed in distilled water for 24 hours. The loaded membrane was stretched over the lower open end of a glass tube of 3 cm diameter and sealed with a rubber band. The glass cylinder was then immersed in 250 ml beaker containing 150 ml of phosphate buffer (pH 7.4) in such a manner that the membrane was located just below the surface of the sink solution.

The whole dialysis unit was placed in a thermostatically controlled shaker water bath adjusted at $37\pm 0.1^\circ\text{C}$ with a constant stirring at 30 rpm to avoid development of concentration gradient.

Each 15 minutes an aliquot, 2 ml was collected and replaced by equal volume of the buffer at the same temperature to make the volume of the sink solution constant during the 2 hours of the experiment. Samples were then assayed spectrophotometrically. Concentration of MF in each sample was determined from the standard curve previously constructed. Blank samples were carried out to check any interference simultaneously.

2.4. Animal Study

The in-vivo experimental protocol was approved by the ethical committee of faculty of pharmacy, El-Minia University. Male albino rats (120-170 g) were purchased from the animal house of faculty of medicine (Assuit University, Egypt). The animals were maintained under standard environmental conditions and had free access to standard diet and water. Anti-inflammatory activity was measured using carrageenan induced rat paw edema assay.

The animals were maintained under standard environmental conditions and had free access to standard diet and water. Anti-inflammatory activity was measured using carrageenan induced rat paw edema assay.

Rats were randomly classified into 5 groups. Each group contains 5 rats.

- Group 1: the rats were served as untreated group.
- Group 2: the rats were treated topically with absorption ointment base of 0.1% mometasone furoate (F1).
- Group 3: the rats were treated topically with absorption ointment base of 0.05% mometasone furoate (the half dose) combined with 0.1% hyaluronic acid sodium salt (F2b¹).
- Group 4: the rats were treated topically with absorption ointment base of 0.025% mometasone furoate (the quarter dose) combined with 0.1% hyaluronic acid sodium salt (F2c²).
- Group 5: the rats were treated topically with commercial product of mometasone furoate (Elcon, Schering-plough) of 0.1% mometasone furoate.

¹ Fb: the half dose of MF (0.05%) combined with HA (0.1%)

² Fc: the quarter dose of MF (0.025%) combined with HA (0.1%)

After 1 hour, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for 5 hours using paw edema meter (vernier caliper). Anti-inflammatory activity was measured as the reduction in edema diameter when drug was present in full dose or fraction dose combined with hyaluronic acid sodium salt relative to the control group.

2.5. Clinical Evaluation of Anti-Inflammatory Activity of Mometasone Furoate in Atopic Dermatitis

A comparison of efficacy of mometasone furoate 0.1% and mometasone furoate 0.05% combined with 0.1% hyaluronate sodium as a skin penetration enhancer was made in an open study of 30 patients with moderate to severe atopic dermatitis for one week of treatment.

This study was performed on thirty (30) patients with atopic dermatitis. These patients were selected from the attendants of the dermatology outpatient clinic of Minia University Hospital. A right left comparison was made on the 30 patients. Patients were assessed by the investigator before and after starting treatment.

Treatment could be applied by the patients to all lesions, but an individual target area will be preselected for evaluation of change in disease signs and symptoms with both formulations the one with full dose of mometasone furoate and the other with half dose of mometasone furoate combined with HA.

Both formulations were supplied in boxes that were labeled with the site of application. Dosing was twice daily to specified areas for a weekly treatment.

The evaluation were made at the initial visit prior to application of the both formulations, the one with full dose MF and the other with half dose MF combined with 0.1% HA and after one week of treatment. The following scale was used: Cleared=100% except for residual discoloration. Marked improvement=75% but less than 100% clearance of signs/symptoms monitored. Moderate improvement=50% to less than 75% clearance of signs/symptoms monitored. Slight improvement=less than 50% clearance of signs/symptoms monitored. No change=no detectable improvement from baseline condition. Exacerbation=flare of sites being studied [14].

The patients were followed up for evaluation of improvement for one week of treatment. Assessment of efficacy of both formulas was made by the investigator.

Statistical analysis:

All values were expressed as Mean \pm SEM. The statistical analysis was performed using one way analysis of variance (ANOVA). The value of p less than 5% ($p < 0.05$) was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Release of Mometasone Furoate from Absorption Ointment Base

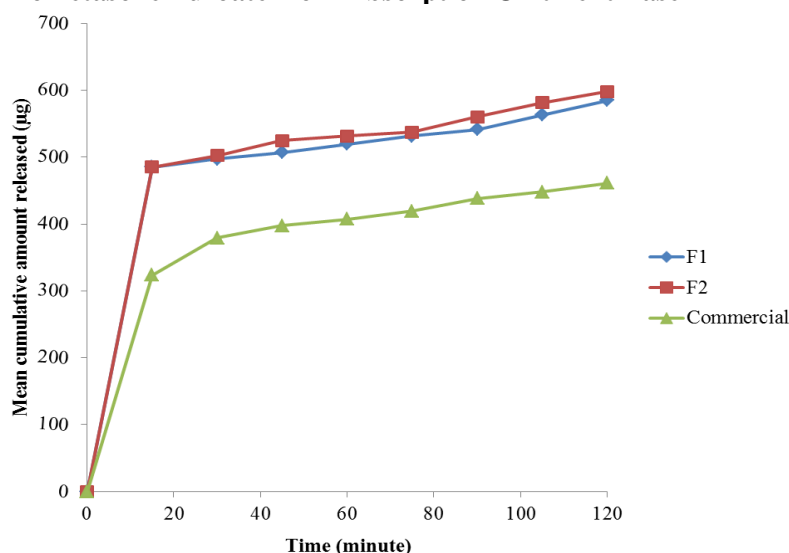


Fig1. Release of MF from absorption ointment base compared to that of commercial cream in phosphate buffer at $37 \pm 0.1^\circ\text{C}$.

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Figure 1, demonstrated that the release of MF from absorption ointment base that contains HA as skin penetration enhancer (F2) was nearly the same as (F1). The statistical studies showed that the difference is insignificant ($P>0.05$). This result demonstrated that HA had no effect on the in vitro release pattern of MF from absorption ointment base.

Anti-inflammatory effect of 0.1% MF and (0.05% and 0.025% MF) combined with 0.1% HA formulated in absorption ointment base on carrageenan induced paw oedema in rats:

Figure 2 showed that after 3 and 5 hours, the reduction in oedema thickness produced by the formulations contain 0.1% of MF (the full dose), was nearly the same as the formulations that contain 0.05% of MF (the half dose) combined with 0.1% HA and those contain 0.025% MF (the quarter dose) combined with 0.1% HA. The statistical analysis showed that no significant difference was produced ($P>0.05$), between the formulations with full dose of MF and the others with half and the quarter dose of MF combined with HA. While the reduction in oedema diameter produced with all formulations was statistically significant when compared to the control group ($*p<0.05$). Results also showed that no significant difference was observed between those formulations and the commercial one.

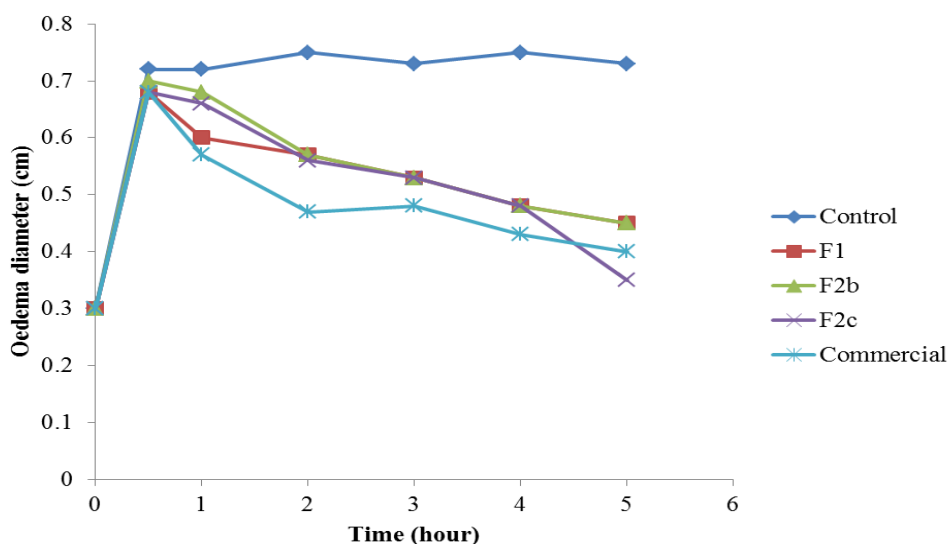


Fig2. Anti-inflammatory effect MF using absorption ointment base (F1, F2b and F2c).

3.2. The Clinical Improvement Evaluation in the Clinical Study

The present study was conducted on 30 patients including patients presenting to the outpatient dermatology clinic of Minia University hospital, 13 (43.3%) males and 17 (56.7%) females table (2).

Table2. Demographic characteristics of patients included in the study

Age range(years)		2-45
Mean±SD		16.85±13.64
Sex	Males	13(43.3%)
	females	17(56.7%)

From table 3, the statistical studies showed that there was a non-significant difference in improvement between the group I with half the dose of MF 0.05% and HA 0.1% and the group II with full dose of MF 0.1% ($P>0.9999$). These results explain the effect of HA when absorbed from the surface of the skin and passes rapidly through epidermis, which may allow associated drugs to be carried in relatively high concentration at least as far as the deeper layers of the dermis. This effect was previously suggested by [11]. Moreover the presence of HA enabled the drug to penetrate the outer skin barrier and then form a reservoir or depot in the epidermis, limiting its systemic absorption, this explanation was previously discussed by [12]. Such localization would be desirable for the topical use of mometasone furoate in lower dose. The permeability of mometasone furoate is enhanced through the powerful hydrating action of HA and retention in the skin is promoted by the unique ability of HA to localize drugs within the

epidermal layers. Such an effect would be especially advantageous for the delivery of mometasone furoate for the treatment of atopic dermatitis or other skin diseases to decrease the incidence of side effects.

The study has been shown that half dose MF0.05% combined with 0.1% HA was an effective treatment for atopic dermatitis when compared to the full dose MF.

Whatever the explanation for its mode of action the fact remains that the inclusion of HA in a topical formulations offers clear and unique potential in the delivery and localization of drugs to the skin.

Table3. Clinical improvement evaluation

Clinical improvement	Group I The side with half the dose of MF and 0.1% HA	Group II The side with full dose of MF	P-value
Cleared	8(26.7%)	0	> 0.9999
Marked improvement	18(60%)	26(86.7%)	
Moderate improvement	0	0	
Slight improvement	0	0	
No change	0	0	
Exacerbation	0	0	
Missed	4(13.3%)	4(13.3%)	

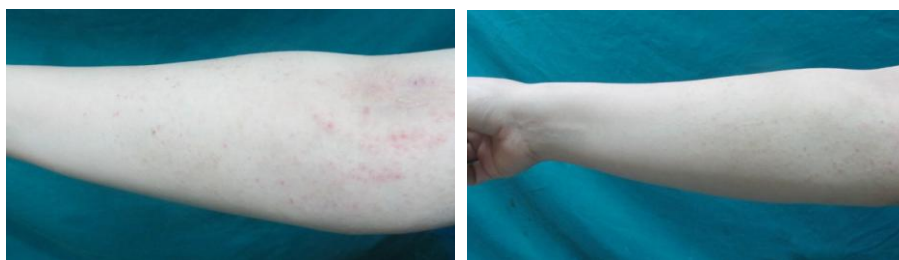


Fig3. Photograph of the right arm of the patient before and after treatment with half dose of MF combined with HA (case I)

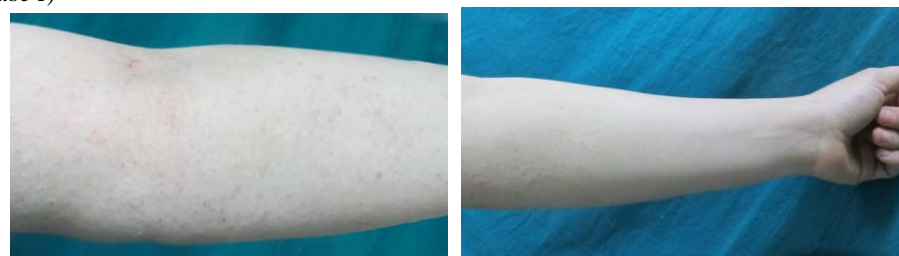


Fig4. Photograph of the left arm of the patient before and after treatment with full dose of MF (case I)

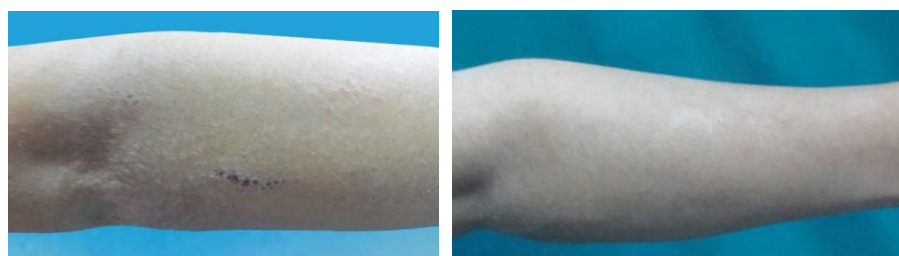


Fig5. Photograph of the right arm of the patient before and after treatment with half dose of MF combined with HA (case II)

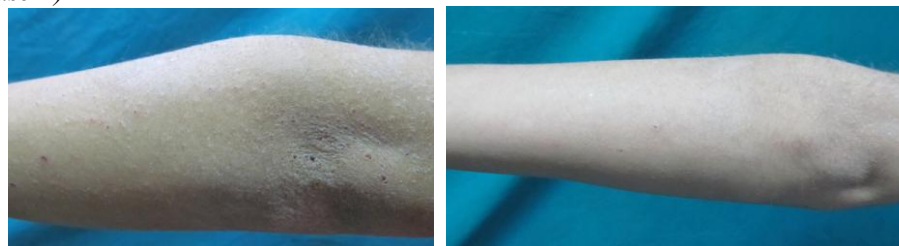


Fig6. Photograph of the left arm of the patient before and after treatment with full dose of MF (case II)

4. CONCLUSION

In conclusion, the diffusion of mometasone furoate from different topical bases through a synthetic cellophane membrane depends on the nature and the composition of the bases. So, the release rate can be altered by changing the nature and the composition.

From the in-vivo anti-inflammatory studies, it could be concluded that the difference in decrease in the oedema diameter in case of using formulation with(full dose) of MF and the same formulation of (half dose) and (quarter dose) MF combined with the skin penetration enhancer 0.1% HA was statistically insignificant ($P>0.05$). These results explain the effect of HA when absorbed from the surface of the skin and passes rapidly through epidermis, which may allow associated drugs to be carried in relatively high concentration at least as far as the deeper layers of the dermis. This effect was previously suggested by [11].

From the clinical study, it could be concluded that the improvement in signs of atopic dermatitis in case of using formulation with(full dose) of MF and the same formulation of (half dose) MF combined with the skin penetration enhancer 0.1% HA was statistically insignificant ($P>0.9999$). That means MF can be used in lower dose in the treatment of atopic dermatitis to lessen the side effects without affecting its therapeutic activity.

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This means MF can be used in lower dose in the treatment of atopic dermatitis to lessen the side effects without affecting its therapeutic activity.

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