Study

Comparative potency of formulations of mometasone furoate in terms of inhibition of 'PIRHR' in the forearm skin of normal human subjects measured with laser doppler velocimetry

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ABSTRACT

Background and aims: Topical glucocorticoid formulations are widely used for effective treatment and control of a variety of dermatoses. Mometasone furoate is a newer corticoid that has high potency but low systemic toxicity. Pharmaceutical factors are known to significantly influence potency and systemic absorption of topically applied glucocorticoids. We studied the potency of "Elocon", a topical formulation of mometasone furoate, compared with two other branded formulations of the same corticoid. **Methods:** Corticoid potency was measured by employing a pharmacodynamic parameter of an inhibitory effect of the corticoid on post-ischemic-reactive-hyperemic–response (PIRHR) in human forearm skin under occlusive dressing. The PIRHR was expressed in terms of % increase in the skin blood flow (SBF) as measured with laser doppler velocimetry (LDV). **Results:** All three active branded formulations of mometasone furoate produced significant inhibition of PIRHR. The $AUC_{(0\text{-}2min)}$ of PIRHR was (Mean ± SEM), Control = 213.52 ± 11.80, Placebo = 209.77 ± 19.31, Formulation A = 119.83 ± 13.71, Formulation C = 53.67 ± 4.85 and Formulation D = 111.46 ± 22.87. Formulation "C" exhibited significantly higher topical anti-inflammatory potency than formulations "A" or "D". **Conclusions:** Thus, branded formulations of the same glucocorticoid, mometasone furoate significantly differed in their topical anti-inflammatory potency. "Elocon" was significantly more potent than the two other branded formulations studied.

Key Words: Mometasone, Topical formulations, Potency, Inhibition of PIRHR, LDV

INTRODUCTION

Topical formulations of glucocorticoids are widely employed by dermatologists for the treatment of a variety of cutaneous inflammatory conditions. However, even a short-term use of a number of glucocorticoids has been shown to result in significant transcutaneous absorption and in turn significant systemic side-effects.[1] A number of glucocorticoids with varying potencies are available and are empirically classified as low, medium and high potency corticoids. The clinicians are therefore required to make a studied choice of a corticoid formulation among a large number of branded and generic formulations of the same and different corticoids available, to suit the specific needs of a given patient. The individual corticoids and their formulations also differ significantly in their "lipophilic" property and in turn transcutaneous absorption and

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liability of causing systemic side-effects.[2] An ideal corticoid for topical use should possess properties that offer high concentration in the skin at the site of application and longer staying ability in the skin, resulting in a lower absorption rate into the systemic circulation. Mometasone furoate, a newer and potent glucocorticoid is one such corticoid agent that has been shown to combine such desirable properties.[3] However, it has also been reported that various pharmaceutical factors like the base, penetration enhancers, additives and excipients, lipophilicity and dilution also influence the topical activity of glucocorticoids.[4,5] Several reports have suggested that different "branded" and "generic" formulations of the same corticoid agent do differ in potency and clinical efficacy.[6-12] It was therefore thought of interest to make an objective assessment of the comparative potencies of three different branded formulations of mometasone furoate in a controlled laboratory study.

In a recent guidance published by the US FDA,[13] it has been suggested that till such time as an acceptable assay procedure is developed, the pharmaceutical manufacturers of such formulations have been advised to follow the "skin blanching" assay described almost 40 years ago, with a refinement— the use of a chromometer to measure the change in the color of the skin. Bisgaard et al[14] have reported that glucocorticoids on topical application, significantly attenuate the post-ischemic-reactive-hyperemic-response (PIRHR) in the human forearm skin and this response can be successfully used to rank the potencies of these agents. The PIRHR in the human forearm skin has been shown to be mediated by local release of vasodilator prostaglandins.[15] We therefore used this experimental model in the forearm skin of normal healthy human subjects to make objective measurement of the topical anti-inflammatory potency of three different branded formulations of mometasone furoate as an attempt to verify the perceived superior clinical efficacy of "Elocon" vis-à-vis the other two brands. The present paper describes these experiments.

METHODS

The study protocol and the methodology followed were as described by Bisgaard et al[14] and in conformity with the recommendations of the Helsinki Declaration for experiments on human subjects. All the human subjects who participated in the study were explained the purpose, exact procedures to be followed, drug treatment involved and the minimal discomfort involved.

Subjects: Ten normal healthy human volunteers (5 males and 5 females), between 20 to 40 years of age, were recruited for the study on informed written consent. None of the subjects had diabetes, hypertension or any other illness requiring any continued medication. None of the subjects were smokers or alcoholics. None had a history of systemic steroid usage less than 6 months prior and topical steroids less than 2 months prior to their inclusion in the study. None had a history of allergic reaction to topical application of mometasone furoate or any other drug. None of the subjects had any drugs, systemic or topical, including NSAIDs in the week prior to the day of study. Subjects with any anatomical abnormality of the skin on the volar aspect of both forearms and inability to keep arms steady for long periods of time were excluded since it would interfere with the steady uninterrupted recording of skin blood flow (SBF) using the laser doppler velocimetry (LDV) technique.

Study design: The study was conducted with a placebo-controlled, randomized, single-blind study design. The laboratory personnel conducting the measurements and analysis of PIRHR were blind to the treatment code of skin sites. All the three brands of mometasone furoate studied and a placebo cream were applied on four sites, on the volar aspect of the skin, (two on each forearm), in a predetermined randomization code. PIRHR was induced and recorded on each of the four skin sites, before and 1 h after a 24-h application, under occlusion, with the applications under study. PIRHR were expressed as Area Under Curve (AUC) of a plot of time (every 10 seconds) against the per cent increase in the SBF over the baseline SBF recorded continuously before and for the first 120 seconds' duration of the hyperemic response.

Study day protocol

Acclimatization: The study subjects reported to the temperature and humidity controlled (23° ± 1°C, 60-

65%) blood flow laboratory at 9:00 am and were rested in supine position on a comfortable foam mattress, with head rested on a 2" thick foam pillow throughout the entire duration of skin blood flow measurements. All measurements were started after a 30-min period of acclimatization. Only one subject was studied per day, with each required to visit the lab on Day 1 for baseline measurements followed by drug applications and on Day 2 for post-treatment measurements.

Induction and record of PIRHR: On acclimatization for 30 min and obtaining a steady record of basal SBF, PIRHR was induced by occluding blood flow to the forearm by inflating a cuff placed around the upper arm to a pressure of 200 mm of Hg, maintaining the occlusion for 4 min and then deflating the cuff instantaneously. SBF was recorded using an angle probe placed in the center of the 20-mm diameter skin site, using double-sided adhesive disc, connected to Periflux P5000 series Laser Doppler Velocimeter (Perimed AB, Stockholm, Sweden). The output from the LDV was recorded on a personal computer using "Perisoft" data acquisition and analysis software. Baseline PIRHR was recorded sequentially, spaced by at least a 15-min interval, at the center of all the four 20-mm diameter skin sites marked, two on each forearm, at least 5 cm apart.

Drug application: 100 μl of the three test formulations of mometasone furoate (MF) and the placebo cream (cream base alone) were placed in the center of the 20-mm diameter marked site on the forearm skin and a 20-mm diameter parafilm disc was placed over it. The medicated skin sites were then occluded with a 3" × 2" "Biocclusive" sterile dressing (Johnson & Johnson). The four treatments were applied sequentially after recording the baseline PIRHR at their assigned sites, following a randomization code. The subjects were then permitted to leave the laboratory and allowed routine indoor activity and were refrained from undertaking any strenuous physical activity.

Post-treatment measurements: At the end of 24 h after drug application, all subjects reported back to the blood flow laboratory. The occlusive dressings were removed and the treated skin sites were washed free of any medication left on the skin, using cotton swabs soaked in soap water. The sites were then dried using soft tissue and were left open for normalization of skin hydration sheath for 1 h. The post-treatment PIRHR was then induced and recorded using the same procedure as described above, at each of the four sites in the same sequence that was followed in the baseline study. Only those subjects who completed the study of the four baseline and four post-treatment PIRHR responses were considered as evaluable.

Analysis of PIRHR: All computerized PIRHR were analyzed to derive the following parameters:

1. **Basal SBF:** mean basal SBF in Perfusion Unit (PU) was derived from a steady record of SBF for approximately 5 min just prior to the induction of the response.
2. **Area under curve ($AUC_{0\text{-}120sec}$):** Using the "Perisoft" data analysis software, percentage increase in SBF at every 10-sec interval, over the basal SBF was calculated for durations of 1 to 120 seconds. The AUC was then calculated using the formula for area of all the trapezoids. The mean of all baseline (pretreatment) PIRHRs recorded at all the 40 sites was designated as the mean baseline pretreatment "control" response. The mean of the 10 post-treatment responses were similarly calculated for each test formulation studied.
3. **Peak % Increase in SBF:** The maximum % increase in SBF attained as determined at the twelve, 10-sec time points of the first 120 seconds of the PIRHR was designated as peak % increase in SBF.

Statistics: All data is presented as Mean ± SEM. The parameters obtained for each treatment group were compared by using analysis of variance (ANOVA) using Excel software.

RESULTS

Effect on basal SBF: None of the active MF formulations studied, and also the placebo cream, on application under occlusion for 24 h, had any significant influence on the basal SBF [Table 1]. Thus it was evident that the MF formulations had no direct effect on the cutaneous blood flow at skin sites treated with these under occlusion for 24 h. This is in conformity with several earlier observations reported, substantiating the fact that the so-called "skin blanching" effect produced by

Table 1: Effect of topical application under occlusion for 24 h of three different formulations of mometasone furoate on AUC of PIRHR, Peak % Increase in SBF during PIRHR and basal SBF studied on the forearm skin of normal human subjects. (Mean ± SEM, n = 10)

Treatment	AUC of PIRHR	Peak % increase in	Basal SBF
Group	(0 – 120 s)	SBF during PIRHR	(PU)
Control	213.52	672.96	7.17
(Pretreatment)	± 11.80	± 26.80	± 0.63
	*	*	NS
Formulation "A"	119.83	431.87	5.43
(Brand of MF) ± 13.71		± 42.77	± 0.78
	NS	NS	NS
Formulation "B"	209.77	621.94	6.66
(Placebo)	± 19.31	± 53.65	± 1.09
	†*	†*	NS
Formulation "C"	53.67	234.47	5.82
("ELOCON")	± 4.85	± 16.89	± 0.83
	*	*	NS
Formulation "D"	111.46	423.76	5.79
(Brand of MF)	± 22.87	± 64.58	± 1.42

*P<0.05 as compared with Control, NS = Not significantly different from control; †P<0.05 as compared with Formulation "A" and "D".
Key: SBF: Skin blood flow, MF: Mometasone furoate, AUC: Area under curve, PIRHR: Post-ischemic reactive hyperemic response

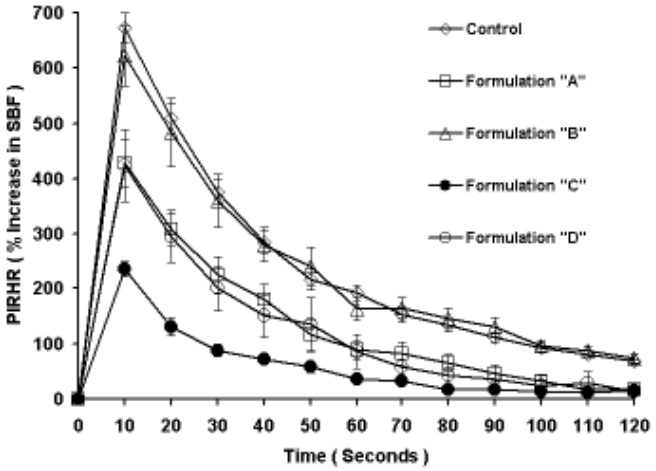

Figure 1: Inhibition of PIRHR in human forearm skin on topical application of the three formulations of Mometasone furoate

glucocorticoids is not as a result of a true "vasoconstrictor" action of the corticoids.

Effect on PIRHR: All the three active MF formulations significantly attenuated the PIRHR recorded at the skin sites treated with these for 24 h under occlusive dressing, as compared with the mean of all the control PIRHR responses recorded at these skin sites prior to drug application. The placebo cream did not exhibit any significant effect on the PIRHR. Table 1 presents the mean AUCs of the PIRHR responses recorded before (Control) and 1 h after 24-h treatment under occlusive dressing with placebo and each of the three MF formulations studied.

The MF formulation of "Elocon" produced significantly higher attenuation of the PIRHR when compared with that produced by the other two brands of MF studied. The mean plots of the PIRHR at control (pretreatment) and skin sites treated with placebo and three formulations of MF studied are presented in Figure 1.

DISCUSSION

The results of the present study clearly indicate that different formulations of the glucocorticoid agent do not exhibit a pharmacodynamic "bioequivalence" when studied by objective assessment of their potency in terms of a measurable pharmacological response. Though two branded formulations "A" and "D" showed a pharmacodynamic bioequivalence, the formulation of "Elococn" exhibited a significantly greater potency in terms of its inhibitory effect on the PIRHR.

The PIRHR in the human forearm skin has been shown to be mediated by local release of vasodilator prostaglandins and is potently inhibited by topical application of glucocorticoids. This response can be easily induced and non-invasively recorded and quantified by employing the technique of LDV in normal healthy human subjects. We propose that the method described here is far more precise, objective than the conventional "skin blanching" assay procedure employed for the assay of the potency of topical formulations of glucocorticoids. Further, the method proposed here is based on the therapeutically relevant pharmacological action of the corticoids, rather than a doubtful "vasoconstrictor" action presumed to cause the "blanching" effect on the human skin. The assay method described here can be employed in research and development studies for assessing the effect of various pharmaceutical factors (cream, ointment or gel bases, excipients, penetration enhancers, agents producing "reservoir" effect, etc.) on the potency as well as duration of action of such topical formulations.

The present study substantiates the clinically perceived superior efficacy of "Elocon" in comparison with other branded formulations of mometasone furoate available

in the market.

Mometasone furoate is a newer potent glucocorticoid agent ideally suited for aggressive topical treatment of severe localized dermatoses like psoriasis. Being more lipophilic, mometasone also may remain topically in the skin in high concentrations with lesser systemic absorption and in turn produce lesser incidence of systemic side effects. The special base and excipients used in the formulation of "Elocon" may also have an enhancing effect on its overall superior clinical efficacy.

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