DIFFERENT APPROACHES FOR IMPROVING SKIN ACCUMULATION OF CLOBETASOL-17-PROPIONATE

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INTRODUCTION

Topical glucocorticoids (TG) are the most frequently prescribed drugs by dermatologists. Their clinical effectiveness in the treatment of psoriasis and atopic dermatitis is related to their vasoconstrictive, anti-inflammatory, immunosuppressive and anti-proliferative effects. Despite their benefit in the therapy of inflammatory diseases, TG are associated number of side effects that limit their use (1). Clobetasol-17-propionate (CP) is widely considered to be the most potent of the currently available corticosteroids. Therefore, incidences of unfavorable side-effects are greater than those of related compounds (2). Over the years, research has focused on strategies to optimize potency of steroids while minimizing adverse effects. Several attempts have been made to increase the safety of TGs treatment, including new application schedules, special vehicles and new synthesized agents (3). The aim of this study was to evaluate the effect of vehicle, chemical enhancer and iontophoresis on the skin permeation and accumulation of CP for obtaining enhanced topical delivery. The synergic effect between chemical enhancer and iontophoresis was also investigated. Commercial cream formulation of CP containing the same amount of drug were used for comparison.

MATERIALS AND METHODS

Materials

CP was a kind gift from GlaxoSmithKline. Na-DOC was purchased from Fluka and Medium Molecular Weight Chitosan from Sigma. Polyethylene glycol 400 (PEG-400) and Mannitol were obtained from Merck. All other chemicals were of analytical grade.

Preparation of Formulations

For the preparation of chitosan gel, 2% chitosan was dissolved in 1.5% (w/v) acetic acid solution. CP was dissolved in 10% PEG-400 and added to the chitosan solution with continuous stirring until uniformity. In the case of Na-DOC gel, 0.5% Na-DOC was dissolved in phosphate buffered saline (PBS). PBS consisted of phosphate buffer (pH=7.2) and 0.9% sodium chloride. 5% mannitol was added to this solution. Finally, CP was dissolved in 10% PEG-400 and added to Na-DOC gel with continuous stirring until uniformity. The concentration of CP was 0.05 % in all formulations. Due to the sensitive structure of the Na-DOC gel, chemical enhancers were used only for chitosan gel. The concentration of Terpenes (Nerolidol, D-Limonene) and Transcutol P in chitosan gels was 2 and 20 %, respectively.

***In Vitro* Permeation Studies**

The *in vitro* permeation experiments were performed using Franz-type diffusion cells across pig ear skin. The available diffusion area was 0.6 cm^2 and the volume of receptor compartment, containing ethyl alcohol-PBS (3:7) mixture thermostatted at 37°C, was about 4 ml. Infinite dose regimen was applied in all experiments.

In the iontophoretic experiments, anodal iontophoresis (direct current, 0.5 mA/cm^2) was applied for 6h. In the case of chitosan gel experiments, 0.9% sodium chloride was added to the formulation to guarantee the reversibility of the Ag/AgCl electrodes.

At the end of the 6 h permeation experiments, the excess amount of the formulation was removed and stratum corneum (SC) and epidermis were separated from dermis with heat application. The drug was extracted using acetonitrile:water (60:40) mixture. The amount of CP in the permeation and accumulation samples was determined by HPLC using Luna C18 (2) 150 mm x 3 mm column (Phenomenex, USA) and a mobile phase composed of acetonitrile/water (55:45) at 1 ml/min. UV detection at 240 nm was employed.

Statistical Analysis

All experiments were replicated six times. Statistical differences were determined using ANOVA followed by Dunnet multiple comparison test.

RESULTS AND DISCUSSION

In vitro permeation and accumulation of CP in pig ear skin layers after passive diffusion and iontophoresis was studied using chitosan, Na-DOC gel and a commercial cream as donor reservoir. Previous studies demonstrated that pig skin is a reasonable model for the human barrier in passive condition and iontophoresis studies (4,5). During the all passive or iontophoretic permeation studies, CP was never found in the receptor medium at the end of 6 h.

The amounts of CP accumulated in the skin layers were normalized by the weight of tissue and expressed as μg of CP per mg of tissue. The accumulated amount of CP from the formulations after 6h of application in SC+epidermis and dermis were shown in Figure 1.

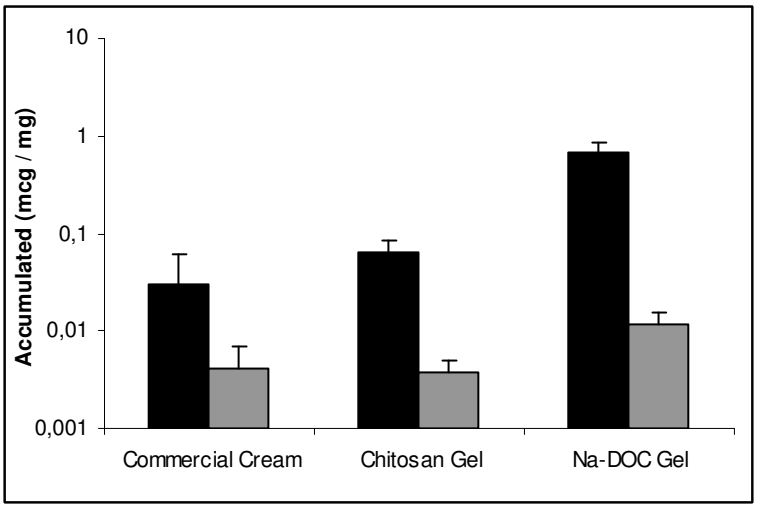

Figure 1: The accumulated amount of CP from the formulations after 6h of application in SC+epidermis (dark bars) and dermis (light bars).

Na-DOC gel formulation dramatically improved the amount of CP in skin layers. This improvement was more evident in SC+epidermis where the inflammatory reactions take place when compared to commercial product of CP ($p < 0.01$).

Figure 2 present the effect of chemical enhancers on the accumulation of CP. Nerolidol was selected to be the best chemical penetration enhancer with significantly higher accumulation data for chitosan gels.

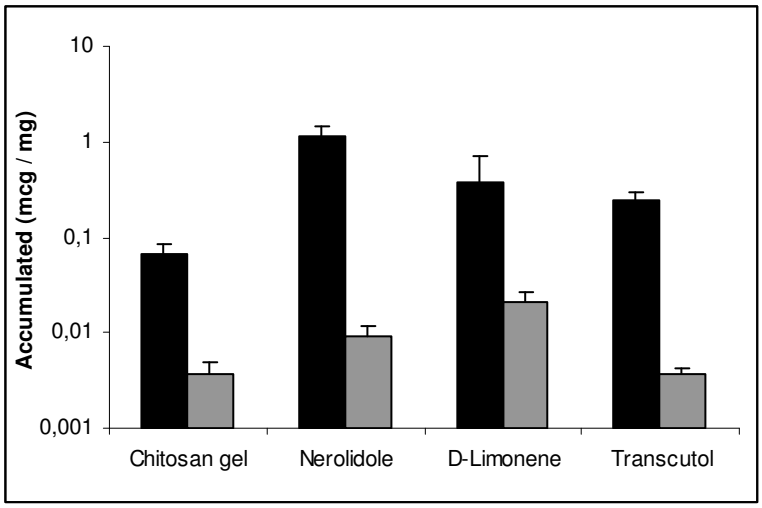

Figure 2: The effect of chemical enhancers on the accumulation of CP in SC+epidermis (dark bars) and dermis (light bars).

The data obtained from iontophoresis experiments indicate that the current application did not produce any further enhancement in the amount of CP in skin for both formulations. Due to the best accumulation results of Nerolidol, the enhancement effect in combination with iontophoresis was also investigated and no further enhancement was found.

Table 1 shows the accumulation of CP after 6h of application in SC+epidermis and dermis for all tested formulations.

Table 1: Accumulation of CP after 6h of application in SC+epidermis and dermis for all tested formulations

Formulations	CP	
	SC+epidermis (µg / mg)	Dermis (µg / mg)
Commercial Cream	0.03 ± 0.02	0.0042 ± 0.0027
Chitosan Gel	0.07 ± 0.02	0.0038 ± 0.0012
Na-DOC Gel	0.67 ± 0.19	0.0115 ± 0.0043
Nerolidol	1.13± 0.30	0.0091 ± 0.0025
D-Limonene	0.38 ± 0.33	0.0211 ± 0.0049
Transcutol	0.24 ± 0.05	0.0036 ± 0.0006

6 h ionto. (Na-DOC gel)	0.55 ± 0.39	0.0104 ± 0.0066
6 h ionto. (Chitosan gel)	0.09 ± 0.03	0.076 ± 0.0058
Nerolidol + 6 h ionto. (Chitosan gel)	0.08 ± 0.03	0.071 ± 0.0055

CONCLUSION

On the basis of above observations, Na-DOC gel and chitosan gel containing Nerolidol could be suggested as promising vehicles for topical application of CP.

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