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Different approaches for improving skin accumulation of topical corticosteroids

Taner Şenyiğit[a,b,*], Cristina Padula[b], Özgen Özer[a], Patrizia Santi[b]

[a] Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 35100, Bornova, Izmir, Turkey
[b] Universita degli Studi di Parma, Dipartimento Farmaceutico, 43100, Parma, Italy

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ABSTRACT

The aim of this paper was to evaluate the effect of vehicle, chemical enhancer and iontophoresis on the skin accumulation of clobetasol propionate (CP) and mometasone furoate (MF). In vitro permeation experiments were performed using pig ear skin as barrier and HPLC as quantification method. The formulations tested were chitosan gels, sodium-deoxycholate gels and commercial creams of CP and MF. The results obtained indicate that Na-DOC gel had an enhancing effect on the skin accumulation of both active agents. This effect was more evident with CP especially in the stratum corneum and epidermis which are the target sites of topical steroidal treatment. Two terpene derivatives (D-limonene and nerolidol) and Transcutol® P were evaluated as chemical penetration enhancers. Nerolidol produced considerable increase in the amount of CP and MF accumulated without any permeation across the skin. The application of electric current (anodal iontophoresis) to the gels improved the accumulation of MF while it did not effect the accumulation of CP. Due to the best accumulation results of nerolidol, the enhancement effect in combination with iontophoresis was also investigated. It was shown that, the combination of anodal iontophoresis and chemical enhancer (nerolidol) produced no further enhancement for both active agents.

1. 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 (Wiedersberg et al., 2008). Most TG are absorbed in quantities that can produce both systemic and topical side effects (Brazzini and Pimpinelli, 2002).

In this study, two topical steroidal drugs were selected as model active agents: clobetasol-17-propionate (CP) and mometasone furoate (MF) (Fig. 1). CP is considered to be the most potent of the currently available corticosteroids. Therefore, the incidence of unfavorable systemic side effects is greater than that of related compounds (Fang et al., 1999). MF is a new derivative, classified as a "potent" synthetic topical steroidal drug which has lower side effects compared with classical TG (Prakash and Benfield, 1998).

Over the years, research has focused on strategies to optimize the potency of steroids while minimizing adverse effects. Several attempts have been made to increase the safety of TG treatment, including new application schedules, special vehicles and new synthesized agents (Schackert et al., 2000).

In topical and transdermal formulations, the selection of a suitable vehicle is of the outermost importance as it can affect both drug release and percutaneous absorption (Kikwai et al., 2002). In this study, chitosan and sodium-deoxycholate (Na-DOC) gel were used as vehicles for CP and MF formulations. Chitosan, a natural polymer obtained by alkaline deacetylation of chitin, is non-toxic, biocompatible and biodegradable (Hejazi and Amiji, 2003). Na-DOC, a naturally occurring bile salt, is a low molecular weight substance which is able to form gels when in contact with excess buffer systems (Valenta et al., 1999).

One of the approaches to reduce the systemic adverse effects of TG is to enhance their permeability so as to reduce the topically applied dose (Fang et al., 1999). Several approaches have been attempted, such as iontophoresis, electroporation or the application of eutectic mixtures (Kaplun-Frischoff and Touitou, 1997; Banga et al., 1999). However, the use of chemical penetration enhancers is the most widely used approach to increase topical and transdermal delivery (Moster et al., 2001). Chemical penetration enhancers have been reviewed by several researchers (Asbill and Michniak, 2000; Sinha and Pal Kaur, 2000; Williams and Barry, 2004; Thong et al., 2007). The authors underline the difficulty to

* Corresponding author at: Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 35100, Bornova, Izmir, Turkey.
Tel.: +90 232 3884000x3985; fax: +90 232 3885258.
E-mail address: taner.senyigit@ege.edu.tr (T. Şenyiğit).

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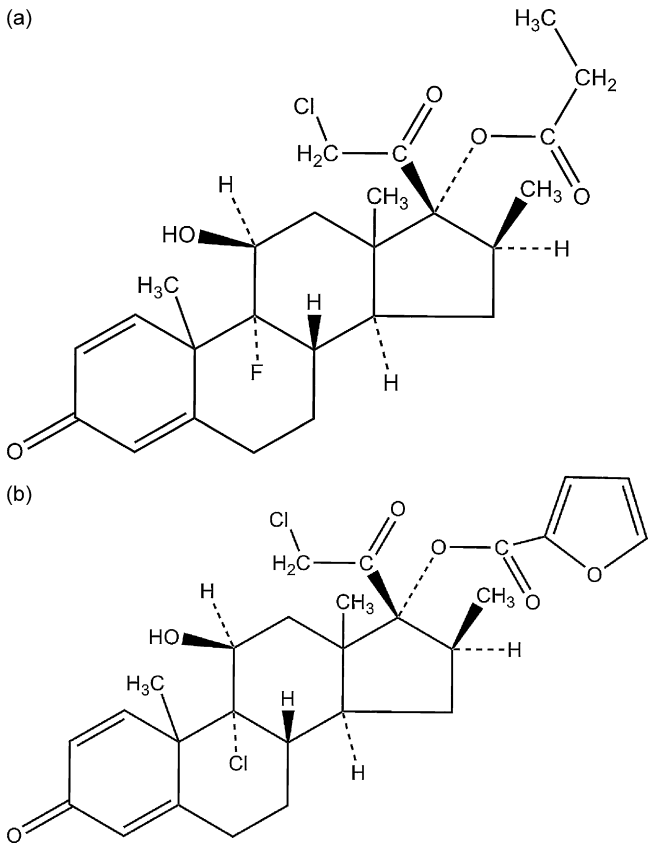

Fig. 1. Structural formula of CP (a) and MF (b).

select rationally a penetration enhancer for a specific permeant. In this study we selected two terpene derivatives (D-limonene and nerolidol) and Transcutol® P as chemical penetration enhancers. Terpenes having low cutaneous irritancy, possess good toxicological profile and appear to be promising candidates for pharmaceutical formulations (El-Kattan et al., 2000). Transcutol (diethylene glycol monoethyl ether) has been recognized as a potential transdermal penetration enhancer, mostly for water insoluble drugs, due to its non-toxicity, biocompatibility with the skin and excellent solubilizing properties (Godwin et al., 2002).

Iontophoresis is a promising technique for enhancing drug penetration across the skin by means of externally applied electric current. Iontophoretic transport promotes drug penetration into the skin by electrorepulsion and electroosmotic flow (Volpato et al., 1998). Although this technique is efficient primarily for ionized molecules, it was shown that it could increase the transport of non-ionized molecules as well (Padula et al., 2005). In fact, one of the transport mechanisms, electroosmosis, is a convective solvent flow, which transports all solutes present, regardless of their charge (Pikal, 1992; Delgado-Charro and Guy, 1994). However, there is no report available in literature on the relative importance of electrorepulsion and electroosmosis on skin retention of non-ionized lipophilic drugs.

A combined approach can also be used between chemical and physical enhancement techniques (Mitragotri, 2000). Several reports documented the synergistic effect between chemical enhancers and iontophoresis in the literature (Bhatia et al., 1997; Oh et al., 1998; Choi et al., 1999).

The aim of this work was to investigate the effect of vehicles (chitosan and Na-DOC gel), chemical enhancers (nerolidol, D-limonene, and Transcutol) and iontophoresis on the skin accumulation and permeation of CP and MF. The goal was to increase skin retention without increasing penetration, to reduce the possible side effects of these drugs. In addition, possible synergic effect between chemical enhancer and iontophoresis was investigated. Commercial cream formulations of CP and MF containing same amount of drug were also used for comparison.

2. Materials and methods

2.1. Materials

Clobetasol propionate and mometasone furoate were kind gifts from GlaxoSmithKline (Turkey) and Orva Drug Company (Turkey), respectively. Sodium-deoxycholate (Na-DOC) was purchased from Fluka (Germany) and medium molecular weight chitosan from Sigma (Germany). Polyethylene glycol 400 (PEG-400) and mannitol were obtained from Merck (Germany). All other chemicals were of analytical grade.

2.2. Preparation of formulations

For the preparation of chitosan gel, 2% chitosan was dissolved in 1.5% (w/v) acetic acid solution. CP or MF 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 or MF was dissolved in 10% PEG-400 and added to Na-DOC gel with continuous stirring until uniformity.

The concentration of CP and MF was 0.05 and 0.1% in all formulations, respectively.

Due to the limited compatibility of the Na-DOC gel with organic substances, chemical enhancers were used only for chitosan gel. The concentration of terpenes and Transcutol® P in chitosan gels was 2 and 20%, respectively.

2.3. HPLC analysis

CP and MF analysis were performed by HPLC (PerkinElmer, Norwalk, CT, USA) using Luna C18 (2) 150 mm × 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. The analytical method was previously validated in accordance with the USP 27.

2.4. In vitro permeation studies

The in vitro permeation experiments were performed using Franz-type diffusion cells (Disa, Milan, I) across pig ear skin. Pig skin was excised after sacrifice from the inner part of pig ears obtained from a local slaughterhouse. When not used immediately, the skin was kept refrigerated (2–5 °C) for up to 3 days. 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. Samples of 300 μl were taken from the receptor compartment at specified time intervals and immediately refilled with the same volume of fresh solution.

At the end of the 6 h of permeation experiments, the excess amount of the formulation was removed and the skin surface was cleaned with dry paper and isopropyl alcohol. SC and epidermis were separated from dermis with heat application (Padula et al., 2005). All samples were weighed and inserted in vials. The drug was extracted from the skin layers under sonication for 15 min using 1 ml acetonitrile:water (60:40) mixture. The amount of CP or MF

in the permeation and accumulation samples was determined by HPLC.

The extraction method was validated in blank experiments and by spiking with a known amount of drug, either CP or MF. In all cases, no interfering peaks derived from the skin samples were detected. The recoveries were above 90% from epidermis and dermis for both of drugs.

CP or MF skin concentration was calculated by normalizing the amount of drug recovered in the SC + epidermis and dermis by the weight of tissues and expressed as μg of drug per mg of tissue.

Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

2.5. Iontophoresis experiments

In the iontophoretic experiments, the current was applied by means of a constant current generator (Iono 1, Cosmic, Pesaro, I), using silver/silver chloride electrodes made from silver wires (diameter 1 mm, purity 99.9%) and silver chloride (Sigma, MO, USA), in accordance with Green et al. (1993). Direct current (0.5 mA/cm^2) was applied for 6 h and anodal iontophoresis was used. In the case of chitosan gel experiments, 0.9% sodium chloride was added to the formulation to guarantee the reversibility of the electrodes.

2.6. Statistical analysis

Each experiment was replicated at least 6 times. Statistical differences were determined using ANOVA (Kaleidagraph® 3.6.2. software on Macintosh Power-Book G4) followed by Dunnet multiple comparison test.

3. Results and discussion

CP and MF in vitro permeation and retention in pig ear skin, after passive diffusion and iontophoresis, were studied using chitosan gel, Na-DOC gel and commercial cream formulations as donor reservoirs. Pig skin was chosen as barrier because previous studies demonstrated that it is a reasonable in vitro model for human skin in passive conditions and in the presence of iontophoresis (Marro et al., 2001; Sekkat et al., 2002). The amount of drug retained in the skin was determined separately in the epidermis (stratum corneum plus viable epidermis) and in the dermis. The data are reported as concentration (μg of drug per mg of tissue).

3.1. Effect of formulation

Firstly, the effect of vehicle type (either gel or commercial cream) on the penetration and accumulation of CP and MF was evaluated. During permeation studies, CP and MF were never found in the receptor medium at the end of 6 h of experiment. The amount of CP or MF accumulated in the skin after 6 h of application are shown in Figs. 2 and 3, for epidermis and dermis respectively.

When the epidermis accumulation data of CP are considered, chitosan gel showed a slightly higher, but not significantly different, amount of drug retained compared to the commercial cream, whereas Na-DOC gel formulation produced a 20-fold higher skin retention. This can be explained by the penetration enhancer property of Na-DOC observed also by Valenta et al. (1999) using rutin as a model active agent across excised rat skin. The thyxotropic property of Na-DOC gel, described in our previous study (Şenyiğit and Özer, 2008), can be considered an additional advantage for an easier application to large skin areas. In the case of dermis accumulation a similar trend was found, with Na-DOC gel producing a skin retention that was twice that obtained with the two other formulations, although the differences in this case were not statistically significant.

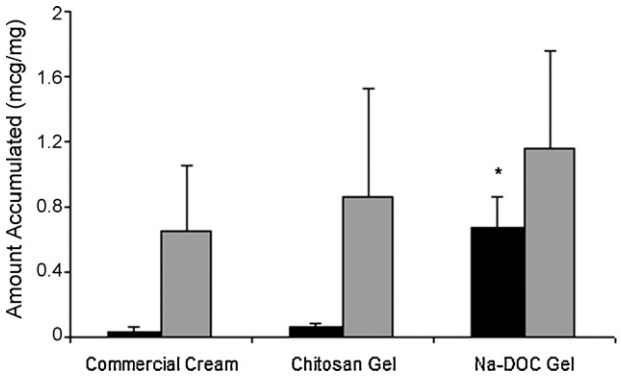

Fig. 2. Amount of CP (dark bars) and MF (light bars) accumulated in the epidermis. *Significantly different from commercial cream ($p < 0.01$). Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

Considering MF retention data, the epidermis accumulation followed the same trend as for CP, although there was no significant difference among the formulations tested. In the case of dermis accumulation of MF, commercial cream formulation showed higher accumulation than other tested formulations, although this difference was not significant due to the very high variability of the experimental data.

As expected, the epidermis accumulation of formulations was found higher than dermis accumulation in all cases.

3.2. Effect of penetration enhancers

Secondly, we investigated the effect of three chemical enhancers, namely nerolidol, D-limonene and Transcutol®. When the chemical enhancers were added to Na-DOC gel, its structure was destroyed and, for this reason, they were tested only in chitosan gel. Figs. 4 and 5 present the effect of chemical enhancers on the accumulation of CP and MF in the epidermis and dermis, respectively. CP and MF were never found in the receptor medium with any of the enhancers at the end of 6 h.

In the case of CP, nerolidol provided a significant enhancement of both epidermis and dermis retention ($p < 0.01$ and $p < 0.05$, respectively). Besides, D-limonene improved only dermis accumulation ($p < 0.01$) while, surprisingly, Transcutol had only a modest, non-significant, effect on epidermis retention.

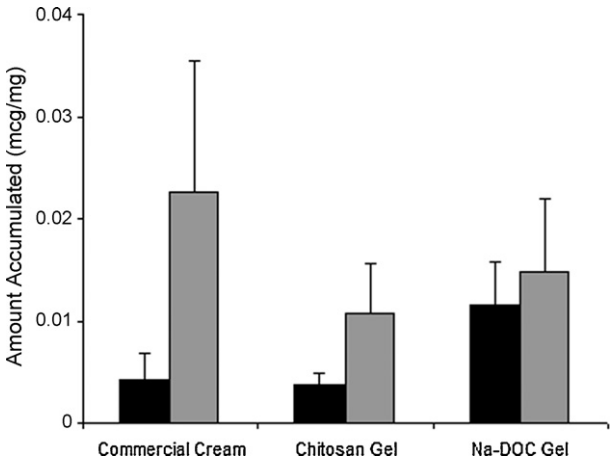

Fig. 3. Amount of CP (dark bars) and MF (light bars) accumulated in the dermis. Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

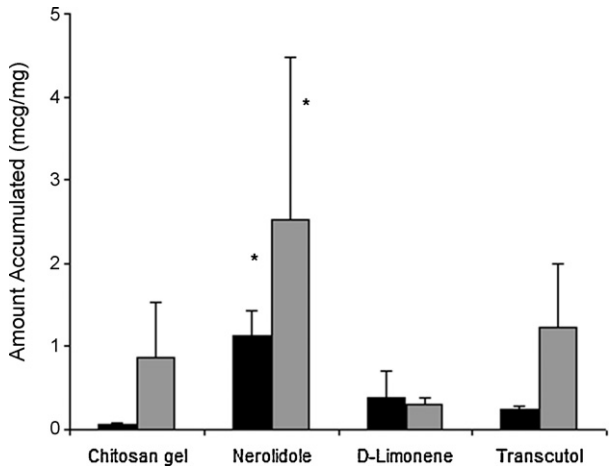

Fig. 4. Effect of enhancers on the accumulation of CP (dark bars) and MF (light bars) in the epidermis. *Significantly different from commercial cream. Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

When the effect of enhancers on MF accumulation data is considered, only nerolidol produced a statistically significant increase at the epidermis level ($p < 0.05$). No significant increment was observed in the dermis accumulation of MF with three penetration enhancers tested.

Overall, nerolidol can be considered the best chemical penetration enhancer, with good accumulation data for both active agents. The mechanism of action of nerolidol as penetration enhancer has been attributed to its amphiphilic structure, that makes the molecule suitable for alignment within the lipid lamellae of the stratum corneum, thus disrupting its highly organized packing (Cornwell and Barry, 1994). However, this interaction with the SC lipids is reversible (El-Kattan et al., 2001). D-Limonene is a lipophilic terpene, that acts mainly on lipophilic drugs, with a mechanism of action linked to lipid extraction from the SC (Aqil et al., 2007). However, D-limonene did not significantly induce the skin penetration and accumulation of CP and MF in this study. Transcutol has been reported to increase the skin accumulation of topically applied compounds by a solubilizing mechanism, without a concomitant increase in transdermal permeation (Ritschel et al., 1991). Surprisingly, Transcutol did not produce skin depot effect for both active

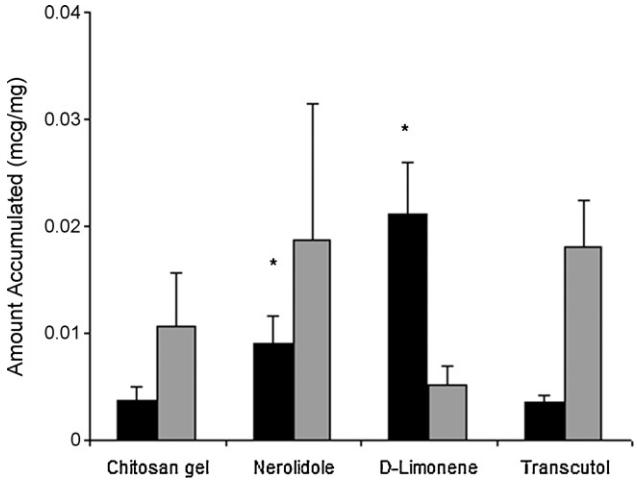

Fig. 5. Effect of enhancers on the accumulation of CP (dark bars) and MF (light bars) in the dermis. *Significantly different from commercial cream. Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

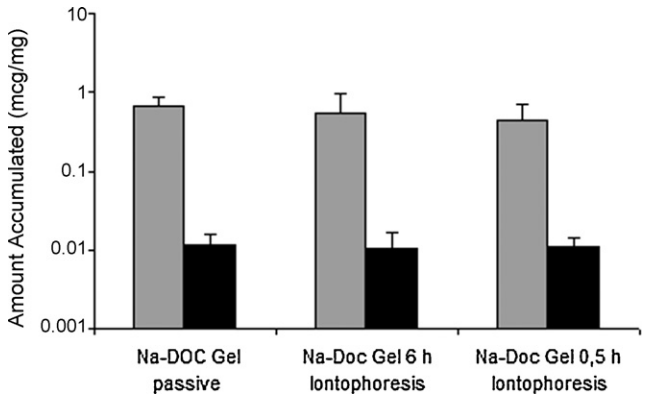

Fig. 6. The effect of 6 h and 0.5 h anodal iontophoresis on the epidermis (light bars) and dermis (dark bars) accumulation of CP from Na-DOC gels. Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

agents. This result was probably due to the inadequate concentration of Transcutol in the formulations tested. Literature data report that Transcutol is active, as penetration enhancer, at concentrations around 50% (Mura et al., 2000; Godwin et al., 2002). However, when Transcutol was present in concentrations above 20% in chitosan gel the viscosity of formulation increased too much.

3.3. Effect of iontophoresis

The effect of iontophoresis, a well known physical enhancement method, on the penetration and accumulation of CP and MF was investigated as well. CP and MF are non-ionized molecules (Moffat, 1986; Prakash and Benfield, 1998), therefore their transport across the skin can be assisted only by electroosmosis. Electroosmosis is a convective transport mechanism occurring when the current is applied, because of net negative charge of skin at physiological pH (Guy et al., 2000). The direction of electroosmosis is the same as counter ion migration, therefore in physiological conditions from the anode (positive electrode) to the cathode (negative electrode). For this reason anodal iontophoresis, 0.5 mA/cm^2, was used. Anodal iontophoresis application for 6 h did not induce drug permeation in the receptor solution.

Fig. 6 reports the amount of CP recovered in the epidermis and dermis layers of the skin after application of anodal iontophoresis from Na-DOC gel. We investigated the effect of 0.5 h anodal iontophoresis (0.5 mA/cm^2) followed by passive diffusion up to 6 h (conditions that are representative of the in vivo application) and of 6 h of the same current applied continuously. No significant dif-

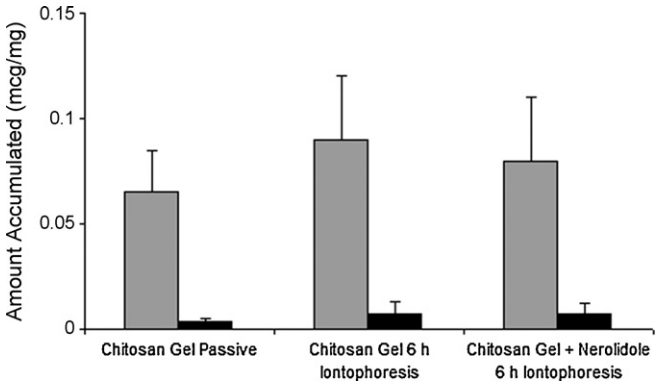

Fig. 7. The effect of 6 h anodal iontophoresis on the epidermis (light bars) and dermis (dark bars) accumulation of CP from chitosan gels. Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

Table 1
Enhancement ratio of the formulations tested.

Formulation	Enhancer	Current application time (h)	CP		MF	
			Epidermis	Dermis	Epidermis	Dermis
Chitosan gel	–	–	2.33	0.90	1.32	0.47
Na-DOC gel	–	–	22.33	2.73	1.78	0.65
Na-DOC gel	–	0.5	14.66	2.57	–	–
Na-DOC gel	–	6	18.33	2.47	3.07	1.43
Chitosan gel	Nerolidol		37.66	2.16	3.87	0.82
Chitosan gel	D-Limonene		12.66	5.02	0.47	0.22
Chitosan gel	Transcutol		8.00	0.85	1.89	0.79
Chitosan gel	–	6	30.00	1.80	2.16	0.80
Chitosan gel	Nerolidol	6	26.66	1.69	1.58	0.73

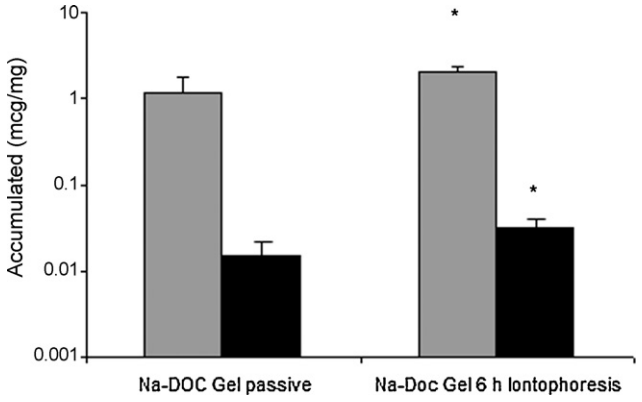

Fig. 8. The effect of 6 h anodal iontophoresis on the epidermis (light bars) and dermis (dark bars) accumulation of MF from Na-DOC gels. *Significantly different compared to passive. Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

ference was found between two different application times, after 6 h, and with the passive diffusion data. The effect of iontophoresis on the accumulation of CP from chitosan gel is presented in Fig. 7. Also in this case, the application of iontophoresis did not increase in a significant way the amount of drug recovered in the epidermis and in the dermis.

Then, we combined the physical and chemical enhancement penetration strategies to evaluate a possible synergistic effect, as reported by Mitragotri (2000). For this aim, we selected nerolidol as chemical enhancer for iontophoresis study, due to the best accumulation data obtained in passive diffusion studies. Fig. 7 shows that the amount accumulated was not significantly different from

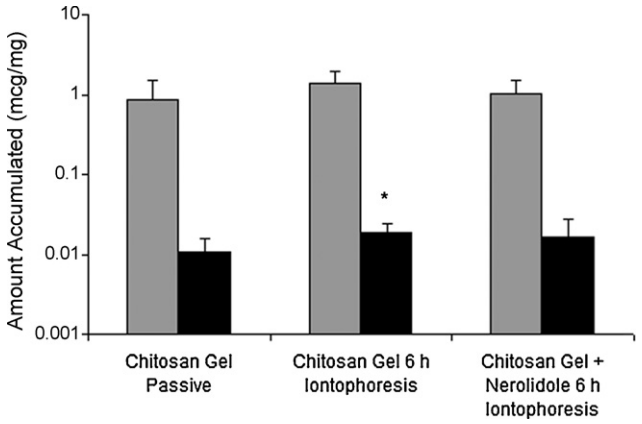

Fig. 9. The effect of 6 h anodal iontophoresis on the epidermis (light bars) and dermis (dark bars) accumulation of MF from chitosan gels. *Significantly different compared to passive. Each experiment was replicated at least 6 times and the values reported are expressed as mean ± standard deviation.

passive or iontophoretic application on chitosan gel with or without enhancer.

Overall both gels, chitosan and Na-DOC, containing CP do not seem to be sensitive to electric current application.

Figs. 8 and 9 illustrate the effect of iontophoresis on the skin accumulation of MF from Na-DOC and chitosan gel formulations, respectively. When we evaluated the effect of 6 h anodal iontophoresis on the accumulation of MF, a significant improvement was found for Na-DOC gels in both epidermis and dermis ($p < 0.05$ and $p < 0.01$, respectively). However, this improvement was evident only in the dermis for chitosan gels ($p < 0.05$). Furthermore, the iontophoretic accumulation from Na-DOC gel was significantly higher than from chitosan gel both in the epidermis and in the dermis ($p < 0.05$ and $p < 0.01$, respectively).

The combined (chemical and physical) enhancement strategy was investigated also on chitosan gel containing MF, and, as shown in Fig. 9, in analogy with the data on CP no enhancement was observed.

The association of anodal iontophoresis and nerolidol produced no further enhancement in steroid skin retention, probably because the main mechanism of CP and MF transport, electroosmosis, is not influenced by the enhancer. In fact, it has been reported that the association of enhancers that fluidize stratum corneum lipids (such as oleic acid) and iontophoresis does not increase the transport of non-ionized molecules such as sucrose, whose iontophoretic transport is determined by electroosmosis (Smith et al., 2002).

Finally, the enhancement ratio (ER) was calculated, to evidentiate the best formulation for both active agents, by dividing the amount accumulated with the test formulation (Q_{test}) by the amount accumulated with the commercial cream (Q_{cream}), i.e.:

$$ER = \frac{Q_{test}}{Q_{cream}}$$

Table 1 summarizes the enhancement ratio of the formulations for both active agents.

It is evident, from the experimental data in Table 1, that the enhancement ratio value of CP accumulated in skin is significantly greater than that of MF. The reason for this difference is probably the structure of the steroid more than the vehicle. In fact, the lipophilicity of the steroid and the duration of action are greatly increased by fluorination of the ring at the C-9 position. CP had a flourine in C-9 position while MF has a chlorine in the same position. The log p values of CP and MF are 4.34 and 3.49, respectively and reflect the skin retention data (Crim et al., 2001; Bos, 2003).

4. Conclusions

From the results obtained in the present work, it can be concluded that Na-DOC gel formulation dramatically improved the amount of CP in the skin without any permeation across the skin. This improvement was more evident in the epidermis, which is the target site of topical steroidal treatment. A similar trend was also

observed for Na-DOC gel of MF, although the differences were not statistically significant. Chitosan gel produced the same skin accumulation as commercial creams, for both active agents. Overall, the Na-DOC gel formulation might be a promising vehicle system for CP and MF for topical application.

Nerolidol resulted to be the best chemical penetration enhancer among the three enhancers tested, with significantly higher accumulation data especially in the epidermis of both active agents. D-Limonene and Transcutol did not increase the skin accumulation of active agents.

The data obtained from iontophoresis experiments indicate that the current application did not produce any further enhancement in the amount of CP in skin. On the contrary, MF skin concentration was clearly improved especially for Na-DOC gel formulation. Additionally, CP and MF permeation across the skin are not sensitive to the application of electric field. Consequently, iontophoresis could be suggested as a suitable method for increasing the skin accumulation of MF. Moreover, the combined approach between nerolidol and iontophoresis did not produce any further improvement on the accumulation of CP and MF.

Finally, the enhancement ratio value of CP and MF was also calculated. The enhancement ratio of CP accumulated in skin is found significantly greater than that of MF and the reason for this difference is probably due to the structure of the steroid more than the vehicle.

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