International Journal of Pharmaceutics 403 (2011) 123–129

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Deoxycholate hydrogels of betamethasone-17-valerate intended for topical use: In vitro and in vivo evaluation

Taner Şenyiğit[a], Işıl Tekmen[b], Ülker Sönmez[b], Patrizia Santi[c], Özgen Özer[a,*]

[a] Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 35100 Bornova, Izmir, Turkey
[b] Dokuz Eylul University, Faculty of Medicine, Department of Histology and Embriology, 35340 Inciraltı, Izmir, Turkey
[c] University of Parma, Department of Pharmacy, 43100 Parma, Italy

ARTICLE INFO

Article history:
Received 15 July 2010
Received in revised form 16 October 2010
Accepted 19 October 2010
Available online 1 November 2010

Keywords:
Betamethasone valerate
Sodium-deoxycholate gel
Texture profile analysis
Skin permeation enhancement
Anti-inflammatory activity

ABSTRACT

The aim of this study was to evaluate the suitability of sodium-deoxycholate (Na-DOC) gels containing betamethasone-17-valerate (BMV) for topical application. The gels were characterized for rheological and textural properties. The in vitro flux of BMV from Na-DOC gels across rat skin was 2.5 (0.05% gel) and 8.5 times (0.1% gel) higher compared to the commercial cream (0.1%), respectively. The pharmacodynamic responses after in vivo topical application in rats were also determined. A significant correlation between anti-inflammatory activity and in vitro permeation of BMV was observed. Na-DOC gels produced significantly higher edema inhibition compared to commercial cream at all time intervals. Finally, according to the results of histology studies, Na-DOC gel has no irritant effect on the skin.

In conclusion, Na-DOC gel formulation could be suggested as a promising alternative system for the topical application of BMV.

1. Introduction

Topical corticosteroids are the most frequently used drugs in dermatological practice. Despite their demonstrated effectiveness for the treatment of psoriasis and atopic dermatitis, topical corticosteroids are associated with various side effects that may limit their use (Del Rosso and Friedlander, 2000; Wiedersberg et al., 2008). Betamethasone-17-valerate (BMV) is the gold standard of these agents and serves as a reference in the clinical studies for the registration of new glucocorticosteroids (Sivaramakrishnan et al., 2004). It is a medium potency glucocorticoid, lacking mineralocorticoid properties, currently available in four topical dosage forms: cream, ointment, lotion and foam all with a strength of 0.1% (w/w) (expressed as betamethasone base) (Franz et al., 1999).

Over the years, research has focused on strategies to optimize the potency of steroids, while minimizing adverse effects. One of the possibilities to reduce the systemic adverse effects of topical steroids is to improve their retention into the skin without augmenting the amount permeated, so as to reduce the applied dose (Fang et al., 2002). Complex approaches, such as iontophoresis, electroporation or eutectic mixtures have been studied as well (Kaplun-Frischoff and Touitou, 1997; Banga et al., 1999). However, it is well known that also the vehicle used in a topical formulation greatly influences the rate and extent of drug permeation, thus modifying the potency of the topical corticosteroid formulation (Stoughton, 1987).

Sodium-deoxycholate (Na-DOC), a naturally occurring bile salt, is a low molecular weight substance (molecular weight: 414.5), widely used as penetration enhancer for mucosal drug delivery, i.e. buccal, ocular and nasal (Chetoni et al., 2003; Zaki et al., 2006; Dhiman et al., 2009). When in contact with excess buffer systems, Na-DOC is able to form stable gels, which has been shown to be useful as drug carrier systems for topical skin application (Valenta et al., 1999). We have recently shown that Na-DOC gels had an enhancing effect on the in vitro skin retention of other topical steroids, namely clobetasol propionate and mometasone furoate, although no skin penetration was observed (Senyigit et al., 2009).

The aim of this study was to prepare and test, both in vitro and in vivo, Na-DOC gels containing BMV at two different strengths, namely 0.05 and 0.1% (w/w). Characterization included rheological and textural properties measurement to assess the applicability of the gel to the skin surface. Then the in vitro permeation characteristics across rat abdominal skin and the in vivo pharmacodynamic effects (carrageenan-induced hind paw edema test on rats) were determined. Although more permeable, rat abdominal skin has been shown to be a reasonable *in vitro* model for human skin in passive conditions (Godin and Touitou, 2007).

A commercial cream and high molecular weight chitosan gel formulation of BMV were used for comparison purpose.

* Corresponding author. Tel.: +90 232 3884000/1357; fax: +90 232 3885258.
E-mail addresses: taner.senyigit@ege.edu.tr (T. Şenyiğit), isil.tekmen@deu.edu.tr (I. Tekmen), patrizia.santi@unipr.it (P. Santi), ozgen.ozer@ege.edu.tr (Ö. Özer).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.10.036

2. Materials and methods

2.1. Materials

Micronized betamethasone-17-valerate (BMV) was a gift from GlaxoSmithKline (Turkey). The commercial product Betnovate® Cream (GlaxoSmithKline) was purchased from a Pharmacy. The product is an aqueous cream containing betamethasone-17-valerate 0.1% (w/w) (expressed as base) as active substance and the following excipients: chlorocresol, cetomacrogol 1000, cetostearyl alcohol, white soft paraffin, liquid paraffin, sodium acid phosphate, phosphoric acid, sodium hydroxide and purified water.

Na-DOC was obtained from Fluka (Germany) and mannitol was purchased from Merck (Germany). High molecular weight chitosan was provided from Sigma (USA). All other chemicals were of analytical grade.

2.2. Preparation of chitosan gel

A blank chitosan gel in water was prepared by dissolving 1.5 g of high molecular weight chitosan in 100 ml of acetic acid 1.5% (w/v) water solution. For preparing the chitosan gel containing BMV, the drug was added to the chitosan gel with continuous stirring until uniformity. The final concentration of BMV was 0.1% (w/w).

2.3. Preparation of Na-DOC gel formulations

The composition and method of preparation of the gel was taken from Valenta et al. (1999). In particular, 0.5% Na-DOC was dissolved in phosphate buffer saline (PBS: 0.1 M phosphate buffer (pH 7.2) containing 0.9% sodium chloride) and 5% of mannitol was added. Finally, BMV was suspended into the formulations in two different amounts [0.05% (F1) and 0.1% (F2)] under continuous stirring.

2.4. Rheological analysis

Rheological properties of gels were studied using a Haake rheometer (Haake Mars Modulars Advanced Rheometer Systems, Germany). The rheometer was based on a thermostatically controlled cone/plate with 60 mm diameter and 1° angle. Throughout the experimental period, the cone/plate temperature was maintained at 5 °C.

In continuous shear analysis, upward and downward flow curves for each formulation were measured over shear rates ranging from 0.001 to 8900 s^{-1}. Shear rate was increased over a period of 150 s, held at the upper limit for 10 s, and then decreased over a period of 150 s. Each measurement was replicated three times.

From oscillating measurements, the shear strain, the stress and the phase angle were determined. The parameters obtained were the complex modulus, G^*, and the phase angle δ. The elastic modulus (G'), the viscous modulus (G'') and the dynamic viscosity ($\dot{\eta}$) were calculated using following equations:

$$G^* = G' + iG'' \quad (1)$$

$$G' = G^* \cos(\delta) \quad (2)$$

$$G'' = G^* \sin(\delta) \quad (3)$$

$$\dot{\eta} = \frac{G''}{\omega} \quad (4)$$

where ω is the angular frequency, which was varied from 0.01 to 100 Hz. In each case, the dynamic rheological properties were determined with at least three replicates. Viscosity measurements were performed at 5 °C for shear rates between 0.001 and 8900 s^{-1}.

2.5. Mechanical characterization of gel formulations

Mechanical properties of the formulations were determined using a Stable Micro System Texture Analyser (Model TA-XT Plus, UK) equipped with 5 kg of load cell in texture profile analysis (TPA) mode. Beakers were filled with the formulation then placed in ultrasonic bath for 20 min before the experiment for avoiding presence of any air bubbles. The analytical probe (Perspex, 10 mm/150 mm; diameter/length) was compressed twice into each sample to a depth of 15 mm at a rate of 2 mm/s. A delay period of 15 s was allowed between the end of the first and the beginning of the second compression. All analysis were performed at least in triplicate at ambient temperature using a fresh sample in each case. Data collection and calculation were performed using the Texture Exponent 3.0.5.0 software package of the instrument. From the resultant force–time plot, mechanical parameters such as hardness, adhesiveness, cohesiveness, compressibility and elasticity were defined (Jones et al., 1996a).

2.6. Stability study

The stability of the Na-DOC gel formulations placed in capped glass vials was studied at 25 °C and 60% relative humidity (RH) for 3 months. Each two weeks the samples were inspected visually and controlled for pH and the amount of the active substance.

2.7. HPLC analysis

BMV analysis was performed by HPLC (Agilent 1100 series, Germany) using ACE 5 C18 250 mm × 4.6 mm column (Scotland) and a mobile phase composed of acetonitrile:water (60:40) at flow rate of 1 ml/min. UV detection at 240 nm was employed. The analytical method was previously validated in accordance with the International Conference on Harmonization guidelines (Validation of Analytical Procedures, 1996).

2.8. In vitro permeation experiments

The in vitro permeation experiments of BMV from Na-DOC gel, chitosan gel and commercial product were performed using Franz-type diffusion cells (Permegear, USA, available diffusion area 0.64 cm^2) across shaved rat abdominal skin.

The experimental protocol was approved by the Local Animal Ethical Committee of Ege University, Faculty of Pharmacy (Approval No. 2007/2-1). Male Wistar rats weighing 180–220 g (6–8 weeks old) were supplied by the Experimental Animal Center of Ege University (Izmir, Turkey). After sacrifice, the skin was excised from the shaved abdominal site. After removing the fat and sub-dermal tissue, the skin was kept at −20 °C and used within 1 week. After thawing to room temperature, the skin was mounted on the diffusion cells with the corneal side facing the donor compartment. The donor compartment contained 80 mg of formulation (125 mg/cm^2), while the receptor compartment, thermostatted at 37 °C to ensure a skin surface temperature of 32 °C, was filled with 5 ml of ethanol–distilled water (1:1) mixture to maintain sink conditions (Senyigit et al., 2009). At predetermined time intervals, 0.5 ml of receptor solution was taken for analysis and replaced with the same volume of fresh solution. The amount of BMV in the samples was determined by HPLC.

All experiments were replicated at least three times. All data are presented as mean ± SD. The flux (μg cm^{-2} h^{-1}) was calculated as the slope of the linear portion of the cumulative permeation curve versus time and the apparent permeability coefficient (cm/h) by dividing drug flux by drug donor concentration.

The permeation enhancing effect of Na-DOC gels were expressed as enhancement ratios (ER_{flux}). ER_{flux} was calculated by dividing the flux of Na-DOC gels by the flux of commercial cream.

2.9. In vivo studies

Male Albino Wistar rats, weighing 180–220 g were used. They were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum. The experimental protocol was approved by the Local Animal Ethical Committee of Ege University, Faculty of Pharmacy (Approval No. 2007/2-1).

2.9.1. Anti-inflammatory activity studies

To determine anti-inflammatory activity of Na-DOC gels, carrageenan-induced hind paw edema test was performed (Garcia Leme et al., 1973; Morteza-Semnani et al., 2004). Twenty-four rats were divided into four groups, one being the control. Prior to administration, paw volume was measured and recorded. Commercial cream, F1 and F2 formulations were applied to the plantar surface of the hind paw of six rats for each treatment, at the dose of 0.125 mg/cm^2, 1 h before induction of inflammation. In order to produce inflammation, 50 μl of a 1% (w/v) solution of λ carrageenan (Sigma Co., USA) in saline was injected into the plantar side of the right hind paw of rats. The contra-lateral paw received the same volume of sterile saline. Rats of the control group received only the carrageenan solution. The increase in paw volume (edema) was measured using digital micrometer (Mitutoyo Corp., Japan) at 1, 2, 3, 4 and 5 h time intervals following carrageenan injection. Edema values were calculated as the difference between the values obtained in both paws and expressed as percentage (%) of increase in the paw volume.

The percentage inhibition of carrageenan-induced paw edema was calculated for each formulation by using the following equation (Jain et al., 2003):

$$\%\text{Inhibition of edema} = \frac{V_{\text{control}} - V_{\text{treated}}}{V_{\text{control}}} \times 100 \quad (5)$$

where $V_{control}$ is the mean edema value of rats in the control group and $V_{treated}$ is the mean edema value of each rat in the test group.

2.9.2. Histological analysis

Animals were sacrificed under anesthesia (urethane 1 g/kg and alfa-chloralose 50 mg/kg) 24 h after treatment. In order to evaluate the surroundings of the site of application, the skin was dissected out, fixed with buffered 10% formalin solution for 48 h. Tissue samples were processed for embedding in Parafin wax by routine protocol. 5 μm thick sections were stained with hematoxylin and eosin (H&E). The slides were examined using a light microscopy (Olympus BH-2, Tokyo, Japan) and the histopathological appearance of tissues in the different groups were compared. Possible structure changes and cell infiltration were evaluated by two analyzers blinded to the treatments.

2.10. Statistical analysis

Statistical differences were determined using Repeated Measures Anova test. Significance was determined by Bonferroni test as a post hoc test. Data were considered significant at $p < 0.05$.

Table 1
Viscosity values of Na-DOC gels (mPa s) at different shear rates.

Formulations	Shear rate (1/s)		
	1420	393	1.106
Drug free gel	0.585	0.849	19.980
F1	6.864	10.960	556.900
F2	10.680	17.670	949.100

3. Results and discussion

3.1. Rheological analysis

The rheological analysis of Na-DOC gels was performed on both the placebo and BMV containing gels, because the presence of the drug can modify the rheological behavior.

The viscosity values of Na-DOC gels containing different concentrations of BMV at varying shear rates were given in Table 1. The viscosity values of gels increased with increasing concentration of BMV in formulations, and there was an expected concentration dependence.

The results of thixotropy test of Na-DOC gel are given in Fig. 1, where it can be seen that gels displayed thixotropic behavior. The thixotropic property of Na-DOC gel can be considered as an additional advantage for easier application to large skin areas (Valenta et al., 1999).

3.2. Mechanical characterization of gel formulations

Mechanical properties of gels for topical delivery are important for the maximum benefit of the patient from the formulation. Texture profile analysis (TPA) defines the mechanical parameters in terms of hardness, adhesiveness, cohesiveness, compressibility and elasticity. The TPA graph and calculated mechanical properties of drug free Na-DOC gel are presented in Fig. 2 and Table 2, respectively.

Hardness is defined as the maximum peak force during the first compression cycle. The hardness of Na-DOC gel, which determines the ease of application on the skin, was 0.026 ± 0.004 N, acceptable

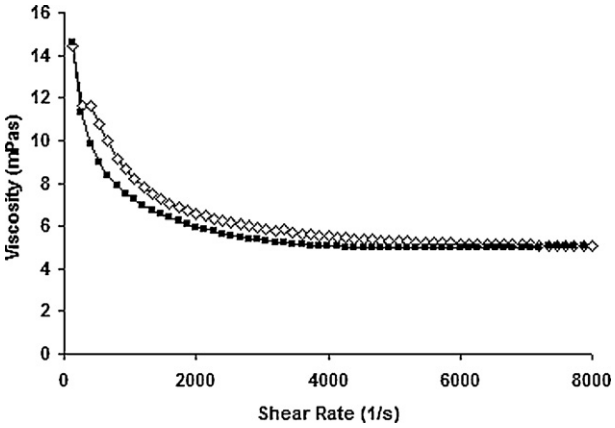

Fig. 1. Thixotrophy graph of Na-DOC gel. Closed symbol represents upcurve and open symbol represents downcurve. Standard deviations have been omitted for clarity.

Table 2
Mechanical properties of drug free Na-DOC gel.

Hardness, (N) ± SD	Adhesiveness, (N mm) ± SD	Cohesiveness, ±SD	Compressibility, (N mm) ± SD	Elasticity, ±SD
0.026 ± 0.004	0.016 ± 0.002	0.861 ± 0.013	0.190 ± 0.006	1.008 ± 0.049

Each experiment was replicated at least three times and the values reported are expressed as mean ± standard deviation (SD).

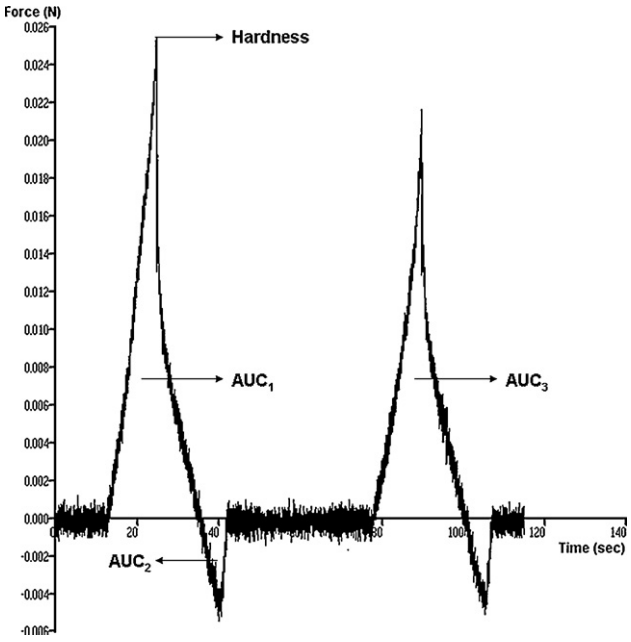

Fig. 2. TPA graph of Na-DOC gel.

for topical gel application and in accordance with previous studies (Bruschi et al., 1997; Jones et al., 1997a). Compressibility defines the work required to deform the product during the first compression of probe and calculated from area under the force–time curve 1 (AUC_1). The compressibility is correlated to the spreadability of the gel on the skin surface (Jones et al., 1997b). Adhesiveness is defined as the negative force area for the first compression cycle and calculated from AUC_2. Adhesiveness is the work required to overcome the attractive forces between the surface of the sample and the surface of the probe and it is related to bioadhesion (Jones et al., 2000). The compressibility and adhesiveness values of Na-DOC gel were 0.190 ± 0.006 and 0.016 ± 0.002 N mm, respectively. These results are consistent with literature findings (Jones et al., 1997b, 2000).

TPA also provides information about cohesiveness. Cohesiveness describes the ratio of the area under the force–time curve produced on the second compression cycle (AUC_3) to that produced on the first compression cycle (AUC_1). The high value of cohesiveness provides full structural recovery following gel application (Jones et al., 1997c). In this study, cohesiveness value of Na-DOC gel was calculated as 0.861 ± 0.013 and this result is enough high for topical application (Karavana et al., 2009). Elasticity is the rate at which the deformed sample returns to its undeformed condition after the removal of the deforming force. It can be calculated from the ratio of the time required to achieve maximum structural deformation on the second compression cycle to that on the first compression cycle. The increase in the quantitative value of elasticity obtained during texture profile analysis shows the decrease in the elasticity of the gel (Jones et al., 1996a,b). The elasticity value of Na-DOC gel was 1.008 ± 0.049. According to this result, Na-DOC gel had an acceptable elasticity value compared with previous literature findings (Jones et al., 1997c; Karavana et al., 2009).

From the results of TPA experiments, it can be concluded that Na-DOC gel has suitable mechanical properties for topical administration.

3.3. Stability study

The stability of Na-DOC gels in terms of drug content and gel stability was investigated during 3 months at ambient temperature. The aspect and pH values were assayed as well (Table 3). The Na-DOC gels remains transparent after 3 months and the drug content did not change. No significant change was observed for the pH values of the formulations ($p > 0.05$). No phase separation or aggregation was detected, which indicated that Na-DOC gels had good physical stability.

3.4. In vitro permeation experiments

In vitro permeation of BMV across shaved rat abdominal skin was studied using Na-DOC gels, chitosan gel and commercial cream formulation as donor reservoirs. Although more permeable, Wistar rat skin has been shown to be a reasonable *in vitro* model for human skin in passive conditions (Harada et al., 1993). Additionally, the carrageenan-induced hind paw edema test on rats is a typical anti-inflammatory test and could be performed on the same animal species.

Due to the low water solubility of BMV (5.9 μg/ml) (Glomme et al., 2005) the drug was incorporated as (micronized) powder in the gels prepared. For the same reason the receptor solution was composed of a mixture of ethanol and water (50:50). Although co-solvents added to the receptor solution can back diffuse and alter the structure of the skin, it is been shown that the penetration of a model compound was the same using, as receptor solution, 50% ethanol or 4% bovine serum albumin, suggesting that ethanol:water does not modify the skin barrier function in a significant way (Magnusson and Koskinen, 2000). On the other hand, Baert et al. suggested the hydroxypropyl-β-cyclodextrine containing media as receptor phase for highly lipophilic testesterone with the higher discrimination factor than ethanol and bovine serum albumin containing media, although ethanol is still most frequently used co-solvent in Franz diffusion cell experiments of lipophilic drugs (Baert et al., 2009, 2010).

Fig. 3 reports the permeation profiles of BMV across excised rat skin from the commercial cream and from the gels prepared. Both Na-DOC gels produced significantly higher skin permeation compared with the commercial cream and chitosan gel ($p < 0.05$), with an evidently shorter lag-time.

The calculated permeation parameters of BMV, namely flux, ER_{flux} and apparent permeability coefficient, are presented in Table 4 together with the cumulative amount permeated at 6 h.

BMV flux from the prepared Na-DOC gels F1 and F2 was 2.5 and 8.5 times higher compared to commercial cream, respectively. Similar trend was also observed for chitosan gel. It is interesting to note that the commercial cream and the gel F2 had the same drug concentration (0.1%, w/w), but the former is a oil in water emulsion

Table 3
Stability test results of Na-DOC gels.

Time (months)[a]	F1		F2		Drug free gel
	pH	Amount of drug (%)	pH	Amount of drug (%)	pH
0	6.67 ± 0.08	99.76 ± 0.14	6.70 ± 0.12	99.56 ± 0.32	6.68 ± 0.08
1	6.70 ± 0.13	99.42 ± 0.19	6.65 ± 0.21	99.38 ± 0.11	6.70 ± 0.11
3	6.82 ± 0.18	99.19 ± 0.28	6.76 ± 0.17	99.24 ± 0.29	6.75 ± 0.16

[a] 25 °C and 60% RH.

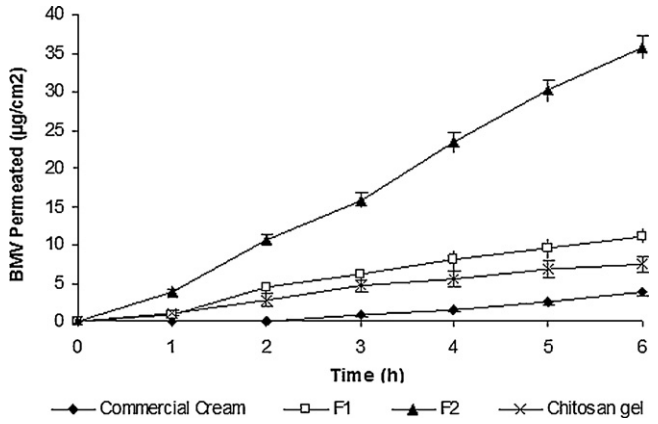

Fig. 3. Permeation profiles of BMV from formulations. Average values ± SD; $n \geq 3$.

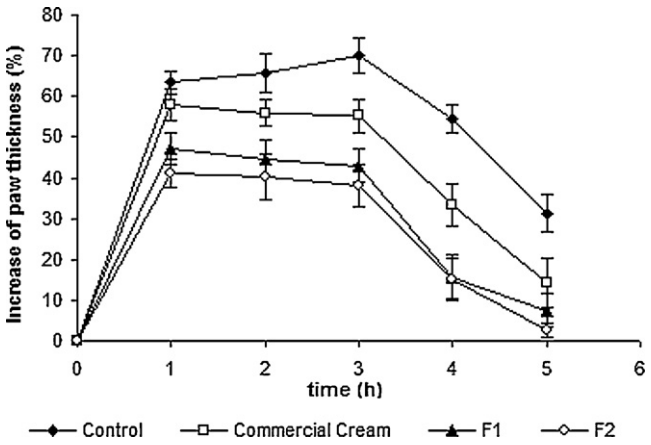

Fig. 4. Comparison of the increase of swelling percentages of the rat paw.

while the latter is a suspension of micronized BMV in an aqueous gel. The high lipophilicity of BMV suggests that the drug is dissolved in the oily phase of the cream, whereas it is probably at saturation in the gel.

This difference in thermodynamic activity of BMV in the two formulations, together with the difference in stratum corneum/vehicle partition coefficient, are probably the reasons for the higher performance of the gel compared to the cream with the same drug concentration. It is also interesting to observe the difference between the two gels: even though the concentration of F2 was twice the concentration of F1, the flux increased three times, suggesting that a possible mechanism was the direct absorption of the micronized drug particles (more concentrated in the F2 gel than in F1) across the skin. The same observation can be made observing the values of apparent partition coefficient, which, assuming passive diffusion as the only mechanism present, should be comparable if the drug was solubilized in both formulations or one twice the other if the drug was suspended in both formulations.

Skin absorption of microparticles is still the subject of debate in the scientific literature, even though there are evidences of the presence of microparticles (in particular of the sunscreen titanium dioxide) inside hair follicles (approximately 1 over 10) (Lademann et al., 1999). Owing to the lipophilic character of sebaceous secretion, which fills the pilosebaceous orifice, BMV particles uptaken into the sebum can dissolve there and the drug can be successively cross the skin. Given the high follicle density of hairy rat skin (approximately 350 per cm^2) (Panchagnula et al., 1997), this penetration route, which in human skin of limited importance, can be more relevant in rats.

3.5. In vivo studies

3.5.1. Anti-inflammatory activity studies

The anti-inflammatory effect of Na-DOC gels after topical administration was evaluated using the model of carrageenan induced acute edema in rat paw and was compared with those of the commercial cream. Fig. 4 shows the comparison of the swelling percentages of the rat paw after carrageenan injection.

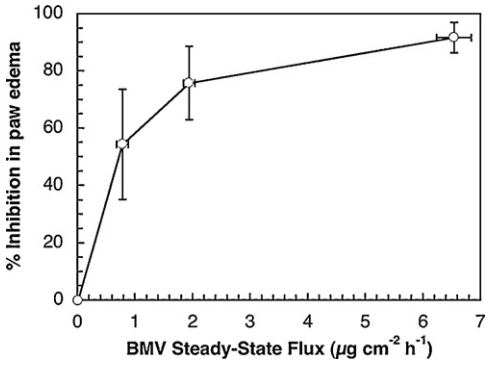

Fig. 5. Relationship between in vivo efficacy (inhibition of paw edema in rats) and in vitro permeation (steady state flux).

Edema inhibition of all formulations are significantly different from control group at all time intervals ($p < 0.05$) except for commercial cream at 1st hour. On the other hand, F1 and F2 formulations produced significantly higher edema inhibition compared to commercial cream at all time intervals even the drug concentration decreased two times in F1 ($p < 0.05$). This result is in accordance with the in vitro permeation data. The relationship between the % inhibition of paw edema and BMV flux is reported in Fig. 5, where it can be observed that the in vivo efficacy increases with in vitro permeation (expressed by steady-state BMV flux).

Overall, according to the results of anti-inflammatory activity studies, F1 and F2 can be considered promising topical formulations with better anti-inflammatory activity than commercial cream for effective therapy.

3.5.2. Evaluation of skin irritation and damage

Morphological changes, such as epidermal liquefaction, edema of collagen fibres and also cell infiltration were the main parameters to evaluate the effects of drug and their permeation enhancers (Narishetty and Panchagnula, 2004). Histological analysis of the rat skin did not reveal morphological tissue changes neither cell infil-

Table 4
Permeation parameters of BMV across excised abdominal rat skin.

Formulation/drug concentration, % (w/w)	Amount permeated at 6 h (µg/cm²)	Flux (µg/cm²/h)	ER_{flux}	Permeability coefficient (cm/h) × 10^3
Cream/0.1	3.75 ± 0.3	0.77 ± 0.1	1.0	0.77 ± 0.1
Chitosan gel/0.1	7.42 ± 1.1	1.26 ± 0.1	1.6	1.26 ± 0.1
F1/0.05	11.01 ± 1.3	1.93 ± 0.1*	2.5	3.86 ± 0.2*
F2/0.1	36.09 ± 0.6	6.53 ± 0.3*	8.5	6.53 ± 0.3*

* Significantly different between them and from commercial cream ($p < 0.05$).

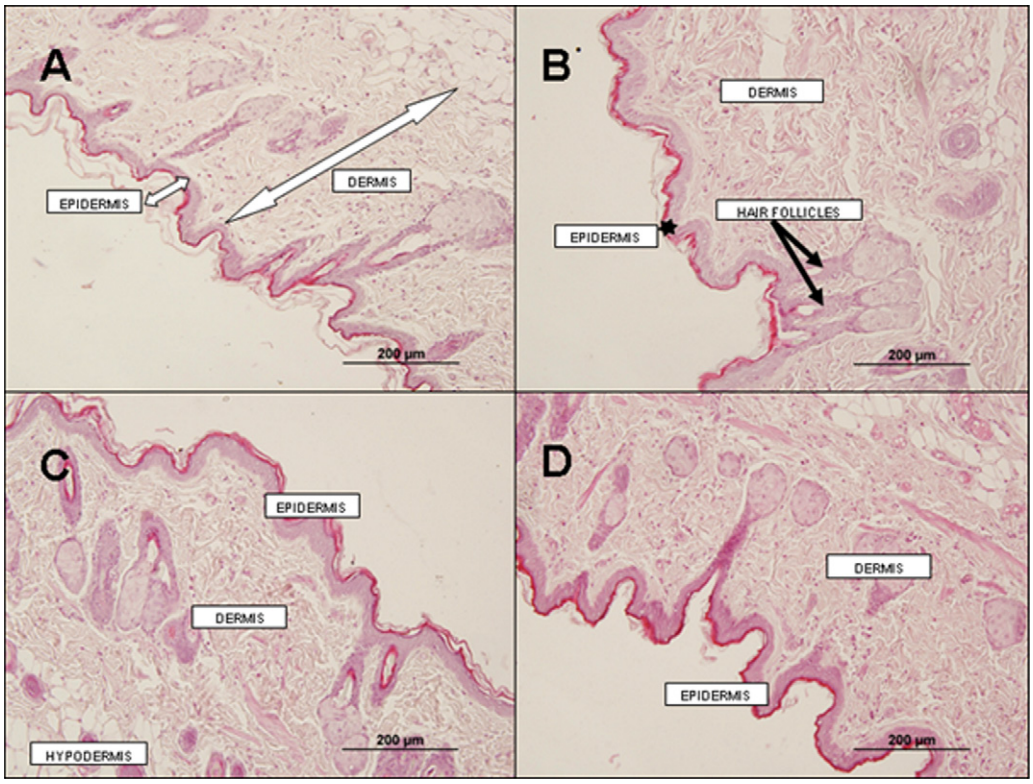

Fig. 6. Histology of the skin samples. (A) Control, (B) commercial cream, (C) formulation F1, and (D) formulation F2.

tration signs after application of the commercial creams or gels, since the structure of the stratum corneum, epidermis and dermis were preserved, as observed in Fig. 6.

4. Conclusions

In this study, two sodium deoxycholate gel formulations containing betamethasone-17-valerate were prepared and characterized regarding their rheological and textural properties. Concerning the in vitro permeation characteristics, betamethasone valerate flux from the prepared sodium deoxycholate gels was 2.5 (0.05% gel) and 8.5 times (0.1% gel) higher compared to the commercial cream (0.1%), respectively. This result indicates that, using sodium deoxycholate gels, the concentration of betamethasone valerate can be reduced and still a higher flux be obtained. This may be a remarkable progress to reduce the dose-dependent side effects and to increase the risk-benefit ratio of betamethasone valerate.

The pharmacodynamic responses after in vivo topical application in rats were also determined. A significant agreement between anti-inflammatory activity and in vitro permeation of betamethasone valerate was observed. Sodium deoxycholate gels produced significantly higher edema inhibition compared to commercial cream at all time intervals.

Finally, according to the results of histology studies, sodium deoxycholate gel has no irritant effect on the skin.

In conclusion, sodium deoxycholate gel formulation could be suggested as a promising alternative system for the topical application of betamethasone valerate.

Acknowledgements

The authors wish to thank Research Foundation of Ege University (19/ECZ/2005) and Novartis Drug Company for financial support given to this study. Also we would like to acknowledge to M.Sc. Alp Alparslan from Ege University Department of Chemical Engineering for rheological analysis.

References

Baert, B., Annavarapu, S., Burvenich, C., De Spiegeleer, B., 2009. Analytical, biopharmaceutical and regulatory evaluation of topical testesterone preparations. Eur. J. Pharm. Biopharm. 72, 275–281.

Baert, B., Bonen, J., Burvenich, C., Roche, N., Stillaert, F., Blondeel, P., Van Bocxlaer, J., De Spiegeleer, B., 2010. A new discriminative criterion fort the development of Franz diffusion tests for transdermal pharmaceuticals. J. Pharm. Pharm. Sci. 13 (2), 218–230.

Banga, A.K., Bose, S., Ghosh, T.K., 1999. Iontophoresis and electroporation: comparisons and contrasts. Int. J. Pharm. 179, 1–19.

Bruschi, M.L., Jones, D.S., Panzeri, H., Gremiao, A.P.D., De Freitas, O., Lara, E.H.G., 1997. Semisolid systems containing propolis for the treatment of periodontal disease: in vitro release kinetics, syringeability, rheological, textural, and mucoadhesive properties. J. Pharm. Sci. 96, 2074–2089.

Chetoni, P., Burgalassi, S., Monti, D., Saettone, M.F., 2003. Ocular toxicity of some corneal penetration enhancers evaluated by electrophysiology measurements on isolated rabbit corneas. Toxicol. In Vitro 17, 497–504.

Del Rosso, J., Friedlander, S.H., 2000. Corticosteroids: options in the era of steroid-sparing therapy. J. Am. Acad. Dermatol. 53, 50–58.

Dhiman, M.K., Dhiman, A., Sawant, K.K., 2009. Transbuccal delivery of 5-fluorouracil: permeation enhancement and pharmacokinetic study. AAPS PharmSciTech. 10, 258–265.

Fang, J.Y., Leu, L.Y., Wang, Y.Y., Tsai, Y.H., 2002. In vitro topical application and in vivo pharmacodynamic evaluation of nonivamide hydrogels using wistar rat as an animal model. Eur. J. Pharm. Sci. 15, 417–423.

Franz, T.J., Parsell, D.A., Halualani, R.M., Hannigan, J.F., Kalbach, J.P., Harkonen, W.S., 1999. Betamethasone valerate foam 0.12%: a novel vehicle with enhanced delivery and efficacy. Int. J. Dermatol. 38, 628–632.

Garcia Leme, J., Hammamura, L., Leite, M.P., Rocha Silva, M., 1973. Pharmacological analysis of the acute inflammatory process induced in the rat's paw by local injection of carrageenan and by heating. Br. J. Pharmacol. 48, 88–96.

Glomme, A., Marz, J., Dressman, J.B., 2005. Comparison of a miniaturized shake-flask solubility method with automated potentiometric acid/base titrations and calculated solubilities. J. Pharm. Sci. 94, 1–16.

Godin, B., Touitou, E., 2007. Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. Adv. Drug Deliv. Rev. 59, 1152–1161.

Harada, K., Murakami, T., Kawasaki, E., Higashi, Y., Yamamoto, S., Yata, N., 1993. In-vitro permeability to salicylic acid of human, rodent, and shed snake skin. J. Pharm. Pharmacol. 45, 414–418.

Jain, S., Jain, P., Umamaheshwari, R.B., Jain, N.K., 2003. Transfersomes—a novel vesicular carrier for enhanced transdermal delivery: development, characterization, and performance evaluation. Drug Dev. Ind. Pharm. 29, 1013–1026.

Jones, D.S., Woolfson, A.D., Brown, A.F., 1997a. Textural analysis and flow rheometry of novel, bioadhesive antimicrobial oral gels. Pharm. Res. 14, 450–457.

Jones, D.S., Woolfson, A.D., Brown, A.F., 1997c. Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers. Int. J. Pharm. 151, 223–233.

Jones, D.S., Woolfson, A.D., Brown, A.F., Coulter, W.A., McClelland, C., Irwin, C.R., 2000. Design, characterisation and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment of periodontal disease. J. Control. Release 67, 357–368.

Jones, D.S., Woolfson, A.D., Brown, A.F., O'Neill, M.J., 1997b. Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: in vitro release kinetics, syringeability, mechanical and mucoadhesive properties. J. Control. Release 49, 71–79.

Jones, D.S., Woolfson, A.D., Djokic, J., 1996a. Texture profile analysis of bioadhesive polymeric semisolids: mechanical characterization and investigation of interactions between formulation components. J. Appl. Polym. Sci. 61, 2229–2234.

Jones, D.S., Woolfson, A.D., Djokic, J., Coulter, W.A., 1996b. Development and mechanical characterization of bioadhesive semi-solid, polymeric systems containing tetracycline for the treatment of periodontal diseases. Pharm. Res. 13, 1734–1738.

Kaplun-Frischoff, Y., Touitou, E., 1997. Testesterone skin permeation enhancement by menthol through formation of eutectic with drug and interaction with skin lipids. J. Pharm. Sci. 86, 1394–1399.

Karavana, S.Y., Güneri, P., Ertan, G., 2009. Benzydamine hydrochloride buccal bioadhesive gels designed for oral ulcers: preparation, textural, mucoadhesive and release properties. Pharm. Dev. Technol. 14, 623–631.

Lademann, J., Weigmann, H., Rickmeyer, C., Barthelmes, H., Schaefer, H., Mueller, G., Sterry, W., 1999. Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol. Appl. Skin Physiol. 12, 247–256.

Magnusson, B.M., Koskinen, L.D., 2000. In vitro percutaneous penetration of topically applied capsaicin in relation to in vivo sensation responses. Int. J. Pharm. 195, 55–62.

Morteza-Semnani, K., Saedi, M., Hamidian, M., 2004. Anti-inflammatory and analgesic activity of the topical preparation of glaucium grandiflorum. Fitoterapia 75, 123–129.

Narishetty, S.T., Panchagnula, R., 2004. Transdermal delivery system for zidovuline: in vitro, ex vivo and in vivo evaluation. Biopharm. Drug Dispos. 25, 9–20.

Panchagnula, R., Stemmer, K., Ritschel, W.A., 1997. Animal models for transdermal drug delivery. Methods Find Exp. Clin. Pharmacol. 19, 335–341.

Senyigit, T., Padula, C., Ozer, O., Santi, P., 2009. Different approaches for improving skin accumulation of topical corticosteroids. Int. J. Pharm. 380, 155–160.

Sivaramakrishnan, R., Nakamura, C., Mehnert, W., Korting, H.C., Kramer, K.D., Schafer-Korting, M., 2004. Glucocorticoid entrapment into lipid carriers-characterisation by paralectric spectroscopy and influence on dermal uptake. J. Control. Release 97, 493–502.

Stoughton, R.B., 1987. Are generic formulations equivalent to trade name topical glucocorticoids? Arch. Dermatol. 123, 1312–1314.

Valenta, C., Nowack, E., Bernkop-Schnurch, A., 1999. Deoxycholate-hydrogels: novel drug carrier systems for topical use. Int. J. Pharm. 185, 103–111.

Validation of Analytical Procedures, 1996. Proceedings of International Conference on Harmonisation (ICH). Commission of the European Communities.

Wiedersberg, S., Leopold, C.S., Guy, R.H., 2008. Bioavailability and bioequivalence of topical glucocorticoids. Eur. J. Pharm. Biopharm. 68, 453–466.

Zaki, N.M., Awad, G.A., Mortada, N.D., Abdelhady, S.S., 2006. Rapid-onset intranasal delivery of metoclopramide hydrochloride. Part I. Influence of formulation variables on drug absorption in anesthetized rats. Int. J. Pharm. 327, 89–96.