DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 10, pp. 1095–1102, 2004

Development of a New Formulation Combining Calcipotriol and Betamethasone Dipropionate in an Ointment Vehicle

Lene Simonsen,* Gert Høy, Erik Didriksen, Jette Persson, Nina Melchior, and Jens Hansen

Pharmaceutical Development, LEO Pharma, Ballerup, Denmark

ABSTRACT

Calcipotriol and betamethasone dipropionate are widely used effective treatments for psoriasis. Combined therapy is known to be superior to monotherapy, but current formulations do not permit simultaneous application as the drug substances will degrade when mixed. The purpose of the study was to develop a formulation which combines calcipotriol and betamethasone dipropionate in a single vehicle hereby achieving optimal delivery of both substances into the skin. As the two substances are incompatible in aqueous and alcoholic medias, different non-aqueous formulations were prepared. Skin permeation studies were investigated using Franz-type diffusion cells. Formulations based on isopropyl myristate were found to decrease the permeation rate (25–35%) as compared with marketed monotherapy products (set to 100%). Lanolin had no overall effect on skin permeability. However, polyoxypropylene-15 stearyl ether (PSE) had a marked effect. A 5% PSE formulation resulted in a permeation rate comparable to the marketed products. Thus, by using PSE as solvent, it was possible to combine calcipotriol and betamethasone dipropionate in a single formulation while optimal skin permeability was attained. Recently, the efficiency of this formulation (Daivobet®) has been verified in clinical studies showing an improved efficacy in the treatment of psoriatic patients.

Key Words: Calcipotriol; Betamethasone dipropionate; Daivobet®; Skin permeation; Formulation.

*Correspondence: Dr. Lene Simonsen, Pharmaceutical Formulation, LEO Pharma, 55, Industriparken, DK-2750 Ballerup, Denmark; Fax: +45-72263556; E-mail: lene.simonsen@leo-pharma.com.

DOI: 10.1081/DDC-200040297
0363-9045 (Print); 1520-5762 (Online)

INTRODUCTION

Psoriasis is a chronic, inflammatory skin disease affecting 1–3% of the world's population.[1] Despite decades of active research, there is currently no cure and different treatment strategies focus on relieving the symptoms and restricting its severity.

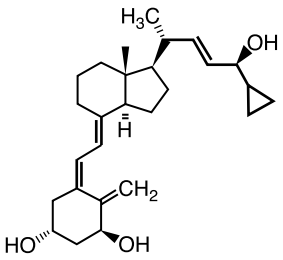
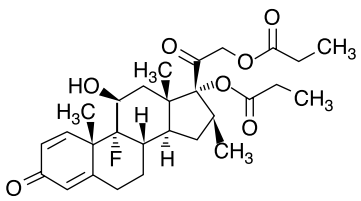
Topical corticosteroids and the vitamin D analogue, calcipotriol, have become mainstays in psoriatic treatment,[2] each having different mechanisms of action.[3,4] Several studies have shown that combined therapy with products containing these two active substances is more effective than monotherapy, and that additive clinical effects and reduced skin irritation may be achieved.[5–8] However, current corticosteroid products cannot be applied together with a calcipotriol product as the drugs are incompatible and will degrade when mixed.[9,10] Consequently, patients have been applying one product in the morning and the other in the evening. To explore the effect of simultaneous treatment and to improve patient compliance alleviating the inconvenience of separate applications, it is desirable to have a single product in which both substances are stable and can be applied simultaneously.

Due to differences in physico-chemical properties and stabilities it has not previously been possible to successfully combine the two drug substances in a single formulation. Calcipotriol is sensitive to oxidising agents and acidic residues; it reacts with alcohols, and easily undergoes epimerisation processes. Contrary to this, corticosteroids like betamethasone dipropionate are stable under acidic conditions, but sensitive to alkaline residues and oxidising agents. If water is present in the formulation, calcipotriol requires a pH value above 8 for maximum stability, whereas betamethasone dipropionate requires pH-values in the range of 4–6 (Table 1).

An optimal vehicle combining the two drug substances must therefore be non-aqueous in order to avoid pH-dependent degradation. In addition, the choice of the pharmaceutical excipients is limited as many have acidic or alkaline residues, or oxidising abilities. Furthermore, as calcipotriol and betamethasone dipropionate are very potent drugs and only a small amount is needed in a formulation (0.005% and 0.05%, respectively), a homogeneous distribution of the drugs must be ensured. Betamethasone dipropionate can be suspended homogeneously as it is available as micronised particles. However, calcipotriol is present in a much lower concentration and needs to be dissolved in a carefully selected vehicle component to ensure an even distribution.

The aim of the present study was to combine the two drug substances in a single formulation, and in addition achieve a skin delivery similar to the marketed monotherapy products; Daivonex® ointment and Diproderm® ointment. In particular, we wished to investigate the effect of different vehicle components

Table 1. Physico-chemical properties of the drug substances.

Drug substances		Mw (g/mol)	Log P[a]	Water solubility (mg/L) 25°C	Maximum stability at
Calcipotriol		412.6	5.43	1.3±0.7 at pH 10[b]	pH>8
Betamethasone dipropionate		504.6	4.23	1.2 at pH 7.4	pH 4–6

[a]Octanol/water partition coefficient.
[b]Data obtained from Ref. [11].

on the skin permeability of both substances. It is the overall goal that a formulation combining calcipotriol and betamethasone dipropionate will increase treatment efficacy and patient satisfaction and thus become a valuable new approach to the treatment of psoriasis.

MATERIALS AND METHODS

Chemicals

Calcipotriol hydrate was supplied by LEO Pharma (Ballerup, Denmark) and betamethasone dipropionate (BDP) was from Sicor (Milan, Italy). Isopropyl myristate (IPM) and polyoxypropylene-15 stearyl ether (PSE) were obtained from Uniqema (Gouda, Netherlands), propylene glycol (PG) from Lyondell (Fos-sur-Mer, France), and lanolin from Croda/Westbrook (Rawcliffe Bridge, UK). Liquid paraffin and white soft paraffin were purchased from Witco (Middlebury CT, US). Diammonium hydrogen phosphate and 2-propanol were of analytical grade and obtained from Merck (Darmstadt, Germany). For physico-chemical properties of the drug substances, see Table 1.

Test Formulations

The ointments were prepared by dissolving calcipotriol in the test solvents (IPM, PG, or PSE) and then adding melted white soft paraffin and the test component lanolin (both phases were 70°C). Betamethasone dipropionate was suspended as micronised particles in liquid paraffin and added to the mixture. The ointments were continuously stirred while cooling. It was ensured that both drug substances were homogeneously distributed in the intended concentrations of 0.05 mg/g for calcipotriol (as hydrate) and 0.5 mg/g for betamethasone (as dipropionate). The test formulations were based on the following solvent variations; 5% and 10% IPM (oint. 1 and 2); 5% PSE and 5% lanolin (oint. 4); and 2.5% PSE and 5% lanolin (oint. 6); 10%, 5%, and 2.5% PSE (oint. 3, 5 and 7); 10% PG and 5% IPM (oint. 8). The amount of the inert ointment base, white soft paraffin, was varied accordingly. All percentages are weight to weight-ratios (w/w). The test ointment based on 5% PSE (oint. 5) is identical to the recently marketed Dovobet® (Daivobet®) ointment (LEO Pharma). As reference products, Dovonex® (Daivonex®) ointment (calcipotriol 0.05 mg/g; LEO Pharma) and Diprosone® (Diproderm®) ointment (betamethasone (as dipropionate) 0.5 mg/g; Schering-Plough) were used.

In Vitro Skin Permeation Studies

Full-thickness skin was removed from the back of pig ears obtained from a local abattoir. The excised skin was cut into appropriate pieces after careful removal of the subcutaneous tissue. The skin samples were mounted in open two-chamber Franz-type diffusion cells with an available diffusion area of 3.14 cm^2 and a recipient volume of 10 ml. The diffusion cells were placed in a thermostated water bath resulting in a temperature of 32°C on the skin surface. A mixture of 0.04 M isotonic phosphate buffer pH 7.4 and 2-propanol (70:30) was used as recipient phase. After a 2 hour equilibrium period, the test formulation (5 mg/cm^2) was applied to the stratum corneum side of the skin and spread with a tared glass spatula. At appropriate time intervals an exact amount of the recipient phase was withdrawn and replaced by thermostated fluid. Throughout the study, the formulations were reapplied corresponding to a twice daily dosage regime. The permeation was followed for 72 h and it was ensured that sink conditions were present. As a minimum, all permeation experiments were carried out in triplicates. The samples were kept at 4°C for a maximum of two days until HPLC analysis, see below.

HPLC Analysis of the Drug Substances

A high-performance liquid chromatographic (HPLC) method was developed for the analysis of calcipotriol and betamethasone dipropionate in the recipient phase samples. The concentrations were quantified using a Merck-Hitachi HPLC system (L-7250 Programmable Autosampler, L-7300 Column Oven, L-7400 UV Detector, L-7100 Pump and D7000 Interface). The substances were separated on a LiChrospher 60 RP-select B (125 mm × 4 mm, 5 μm) from Merck (Darmstadt, Germany) using a column temperature of 40°C, a detector wave length of 264 nm, an injection volume of 1000 μl, and a flow rate of 1.0 ml/min. The mobile phase consisted of 2-propanol and 0.005 M ammonia phosphate buffer pH 7.4 in a step gradient mixture of 26:74 (2-propanol:buffer) at an interval of 0 to 1 min, and 36:64 from 1 to 16 min. The retention time was 10 min. for betamethasone dipropionate and 12 min. for calcipotriol. The detection limit was 10 ng/ml for both calcipotriol and betamethasone dipropionate, and the quantitation limit was 25 ng/ml for both substances. Calibration curves were linear over a concentration range of 25–1000 ng/ml (R^2>0,9990) and validated for intra- and inter-day variation (CV_{intra}: 2%, CV_{inter}: 4%). The recovery

was 92% for calcipotriol and 100% for betamethasone dipropionate.

Data Analysis

Based on the obtained concentrations and the amount of recipient phase withdrawn at each specific point of time, the permeated cumulated amount of drug was calculated for each diffusion cell (ng/cm^2) and plotted as a function of time (h). For all individual cells, steady state flux was determined from the slope of the linear part of the curve, and lag time as the x-intercept using linear regression analysis. Post-study data analysis was used to check the integrity of the skin comparing the individual cell data with the mean data for the other cells in the specific group (ANOVA). No damaged skin preparations were identified.

For statistical comparison of the formulations, a two-way ANOVA model was applied. The observations were logarithmically transformed to fulfil the assumption about normality and variance homogeneity. Pairwise comparisons of the ointments were performed using the Students' t-test within the ANOVA. P-values below 0.05 are considered significant.

RESULTS

The in vitro skin permeation profiles of calcipotriol and betamethasone dipropionate in the different test formulations are shown in Fig. 1A and 1B. It is seen that the different solvent systems had a marked effect on the cumulated permeated amount of both drug substances. Based on these permeation profiles, the steady state fluxes and lag times were calculated (Table 2). In order to make a direct comparison of the different formulations with the reference products, all flux values were normalised by dividing the individual

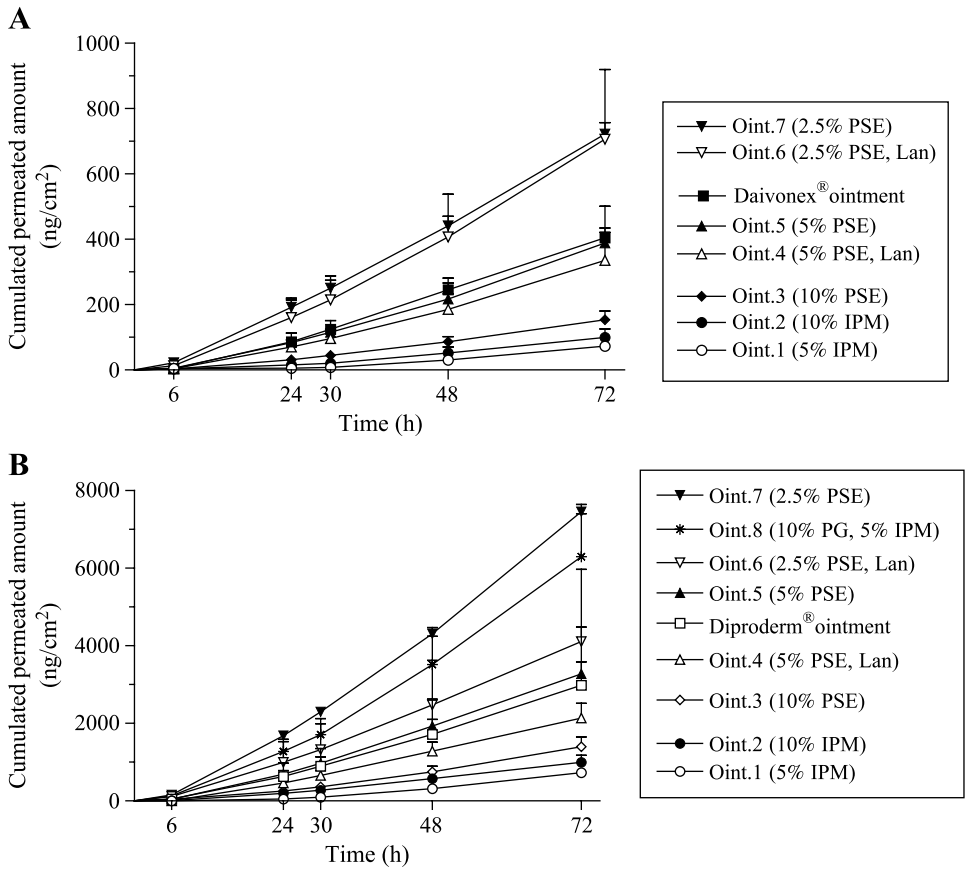


Figure 1. In vitro skin permeation profiles of calcipotriol (A), and betamethasone dipropionate (B) from the test formulations based on different vehicle components (Lan: lanolin; IPM: isopropyl myristate; PG: propylene glycol, PSE: polyoxypropylene-15 stearyl ether) expressed as mean values + SE (n = 3–7 cells per formulation).

Table 2. In vitro skin permeation results (mean values±SE; n=3–7 cells).

Test formulations	Steady state flux ($ng/cm^2 \cdot h^{-1}$)		Lag time (h)	
	Calcipotriol	BDP	Calcipotriol	BDP
Ointment 1 (5% IPM)	1.6±0.1	15±1	26±3	24±6
Ointment 2 (10% IPM)	1.9±0.4	17±3	21±4	15±2
Ointment 3 (10% PSE)	2.6±0.5	25±5	13±3	15±4
Ointment 4 (5% PSE, Lan)	6±1	36±6	14±3	12±3
Ointment 5 (5% PSE)	7±2	52±17	13.6±0.6	13±1
Ointment 6 (2.5% PSE, Lan)	12±3	66±28	13±2	11±2
Ointment 7 (2.5% PSE)	11.3±0.5	123±2	8±2	12±1
Ointment 8 (10% PG, 5% IPM)	n.a.	110±17	n.a.	16±2
Daivonex® ointment	6.7±0.4	–	12±1	–
Diproderm® ointment	–	50±9	–	13±2

n.a.: Data not available due to degradation (see results).

fluxes with the mean flux of the reference product (Fig. 2).

The otherwise well known skin penetration enhancer IPM (oint. 1 and 2) was found to have no enhancing effect itself on either of the drug substances compared with the reference products as the total cumulated permeated amount was significantly lower (t-test; $p<0.05$). As seen from Table 2, the fluxes were significantly lower and the lag times longer than for the references. From Fig. 2 it is seen that the permeability of both drug substances ranged 25–35% of that of the reference products (set to 100%). There was a tendency to when increasing the amount of IPM from 5% to 10%, the permeability increased slightly but no statistically significant difference was found ($p>0.21$).

PG was added as a co-solvent to a formulation containing 5% IPM (oint. 8). This resulted in a significantly increased skin permeability for betamethasone dipropionate (Fig. 1B, Table 2), and a normalised flux ratio of 219% compared with the reference. No data is shown for calcipotriol as the drug substance was found to be very unstable in the formulation and rapidly transformed into degradation substances.[9]

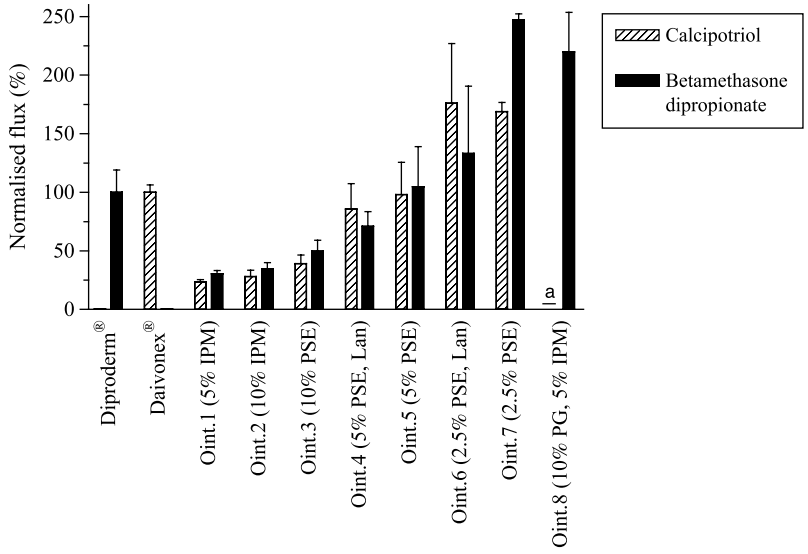


Figure 2. Normalised flux ratios for calcipotriol and betamethasone dipropionate in the test formulations based on different vehicle components (Lan: lanolin; IPM: isopropyl myristate; PG: propylene glycol, PSE: polyoxypropylene-15 stearyl ether). Normalisation was performed by dividing the fluxes with the mean flux of the reference products; Daivonex® ointment and Diproderm® ointment (mean values+SE). [a]Data on calcipotriol was not available due to degradation (see results).

In contrast to IPM, the solvent PSE (oint. 3–7) proved to increase the skin permeability of both drug substances. Within the tested range, an inverse relationship was found between the amount of PSE in the formulation and the skin permeability. When comparing the total cumulated permeated amount of both drug substances, there was a statistically significant difference between the formulations containing 2.5%, 5%, and 10% PSE (two-way ANOVA; $p<0.0001$). As seen from Table 2, the fluxes decreased as the content of PSE increased. In general, the lag times were lower than those of the IPM formulations, and within the range of the reference products. However, within the group, the varying amount of PSE had no significant effect on the lag times (two-way ANOVA; $p>0.41$). The formulations containing 5% PSE (oint. 4 and 5) had fluxes comparable to those of the reference products (t-test; $p>0.29$) corresponding to 100% for both drug substances (Fig. 2). Increasing the amount of PSE in the formulation to 10% (oint. 3), caused a reduction in the normalised flux ratio to 39% for calcipotriol and to 49% for betamethasone dipropionate. When the amount of PSE in the formulation was reduced to 2.5% (oint. 7), the flux ratio increased to 170% and 247%, respectively.

The skin emollient lanolin was added to ointment 4 and 6, and by pairwise comparison to ointment 5 and 7, respectively, no significant effect of lanolin was found for calcipotriol ($p>0.72$). However, for betamethasone dipropionate, the formulation containing 2.5% PSE had a statistically significant lower flux when lanolin was present (t-test: $p = 0.009$). This is also reflected in Fig. 2 showing that normalised flux ratio decreased from 247 to 133%, when lanolin was added to the formulation.

DISCUSSION

The present study demonstrated that it was possible to combine calcipotriol and betamethasone dipropionate in a formulation and by careful selection of the vehicle components also attain a skin delivery similar to the marketed monotherapy products.

Several factors had to be considered when combining the two otherwise incompatible drug substances. The vehicle had to be non-aqueous, consisting of compatible components which ensured stability of both substances, and at the same time delivered both drug substances into the skin at a rate comparable to the marketed reference products. For this purpose, the applied in vitro set-up with skin samples mounted in Franz-type diffusion cells proved to be a very valuable tool for testing the effect of the different vehicle components on the skin permeability.

IPM is a well known solvent generally acting as a skin permeation enhancer. However, in the present study it had no increasing effect as it only lead to a skin permeation rate of about 25–35% compared with the references. IPM facilitates the passage of the drugs through the skin barrier often in conjunction with a cosolvent serving as a drug carrier. In the investigated formulations, IPM had no effect presumably because none of the other components in the formulation served as a carrier. To test this, we added PG to the formulation and found a significantly increased skin permeability of betamethasone dipropionate (oint. 8, Table 2). Unfortunately, this led to a rapid degradation of calcipotriol.[9]

The solvent PG is successfully used in some of the marketed calcipotriol products; Daivonex® ointment and Daivonex® solution, and was also initially considered in the present study as described above. PG may, however, contain some acidic impurities, which will catalyse an epimerisation process of calcipotriol and lead to the formation of mainly calcipotriol propylene glycol ethers. To avoid this, PG has to be buffered to a pH above 8. In a combination product this would lead to hydrolysis of the ester side chain of betamethasone dipropionate and further degradation of the acetone–alcohol group. Therefore, it was not possible to use PG in the formulation.

PSE is used as an emollient in cosmetic products and also as a drug solvent in pharmaceuticals. It was found to be a very effective solvent for both drug substances having a significant effect on the skin permeability. PSE is a lipophilic component and thus, when a high amount is used in the vehicle, the skin-vehicle partition coefficient is expected to decrease. This was also reflected in the inverse relationship between the amount of PSE in the formulation and the total cumulated permeated amount (Fig. 1) and the flux values (Table 2).

Lanolin was added to the ointment base (oint. 4 and 6) due to its emulsifying and skin emollient properties. When an extra component is added to a dermatological vehicle, it may affect the skin permeability owing to a change in the partition coefficient. In the present study, lanolin was found to have no effect on the skin permeation of calcipotriol and actually decreased the skin permeation of betamethasone dipropionate. As lanolin contains lanosterols, which have a chemical structure similar to betamethasone dipropionate (Table 1), it may be speculated that betamethasone dipropionate has a special affinity to lanolin. This may have caused a reduction of the skin-vehicle partition

coefficient and may explain the decreased flux ratios (Fig. 2). As the primary objective is to deliver the optimal amount of drug substance to the skin, the intention of adding a supplementary emollient to the ointment base was abandoned.

High skin permeabilities are not necessarily desirable for a corticosteroid product due to the possibility of local side effects such as skin atrophy, stria, telangiectasia, or the more rare side effect; suppression of the hypothalamic-pituitary-adrenal axis.[8] For calcipotriol, the side effects are fewer and restricted to local skin irritation.[5] The two marketed monotherapy products were therefore used as references serving as valuable bioequivalence markers to attain comparable skin permeabilities. The formulation containing 5% PSE (oint. 5) had fluxes and lag time values similar to both references (Table 2). Furthermore, excellent stability of both drug substances has been documented,[9] and this formulation has now become available as Daivobet® ointment.

The atrophogenic potential of this new formulation has recently been investigated in a human study.[12] The clinical results support the present skin permeation results as no difference in skin atrophy was found between the new combination product and the reference product; Diproderm® ointment. In addition, skin atrophy was similar for the two products following twice daily application over a 4-week treatment period.

It is emphasized that the present permeation results are based on in vitro experiments performed on excised skin from pig ears. In vitro studies using skin samples mounted in diffusion cells are, however, a well-established method to predict skin permeability.[13,14] Moreover, skin from pig ear has proved to have properties similar to those of human skin and has been suggested a good model for human skin permeability.[15] Nevertheless, we repeated parts of the present study (oint. 3–7) using excised human abdominal skin (data not shown) and found the exact same rank order of the formulations, and flux values within the same range as the present results. This shows that the present set-up is very predictive for human skin absorption as also demonstrated by the correlating results on skin atrophy (see above). In addition, the new combination product has recently been tested in large clinical trials showing an improved efficacy after application once daily for the treatment of psoriasis compared to either drug used alone, and also showing that the treatment was well tolerated by the patients.[16–19] These results clearly support the rationale for developing a combined formulation and that the permeability of the drug substances from this new vehicle was as intended.

CONCLUSION

The study proved it possible to develop a formulation which combined calcipotriol and betamethasone dipropionate in a single vehicle achieving optimal delivery of both substances into the skin. The results demonstrated that the choice and the amount of the solvent had a significant influence on the skin permeability. Especially, the solvent PSE had a marked influence. The formulation containing 5% PSE resulted in a skin permeation rate similar to that of the marketed reference products; Daivonex® and Diproderm® ointment. This new combination product has recently been tested in clinical studies showing an improved efficacy in the treatment of psoriasis and has now been marketed as Daivobet® ointment.

REFERENCES

1. Greaves, M.W.; Weinstein, G.D. Treatment of psoriasis. N. Engl. J. Med. **1995**, *332* (9), 581–588.
2. van de Kerkhof, P.C.; Vissers, W.H. The topical treatment of psoriasis. Skin Pharmacol. Appl. Skin Physiol. **2003**, *16* (2), 69–83.
3. Cunliffe, W.J.; Berth-Jones, J.; Claudy, A.; Fairiss, G.; Goldin, D.; Gratton, D.; Henderson, C.A.; Holden, C.A.; Maddin, W.S.; Ortonne, J.P. Comparative study of calcipotriol (MC 903) ointment and betamethasone 17-valerate ointment in patients with psoriasis vulgaris. J. Am. Acad. Dermatol. **1992**, *26* (5), 736–743.
4. Kragballe, K.; Gjertsen, B.T.; De Hoop, D.; Karlsmark, T.; van de Kerkhof, P.C.; Larko, O.; Nieboer, C.; Roed-Petersen, J.; Strand, A.; Tikjob, G. Double-blind, right/left comparison of calcipotriol and betamethasone valerate in treatment of psoriasis vulgaris. Lancet **1991**, *337* (8735), 193–196.
5. Kragballe, K.; Barnes, L.; Hamberg, K.J.; Hutchinson, P.; Murphy, F.; Moller, S.; Ruzicka, T.; van de Kerkhof, P.C. Calcipotriol cream with or without concurrent topical corticosteroid in psoriasis: tolerability and efficacy. Br. J. Dermatol. **1998**, *139* (4), 649–654.
6. Lebwohl, M.; Siskin, S.; Epinette, W.; Breneman, D.; Funicella, T.; Kalb, R.; Moore, J. A multicenter trial of calcipotriene ointment and halobetasol ointment compared with either agent alone for the treatment of psoriasis. J. Am. Acad. Dermatol. **1996**, *35* (2), 268–269.
7. Ruzicka, T.; Lorenz, B. Comparison of calcipotriol monotherapy and a combination of calcipotriol and betamethasone valerate after 2 weeks'

treatment with calcipotriol in the topical therapy of psoriasis vulgaris: a multicentre, double-blind, randomized study. Br. J. Dermatol. **1998**, *138* (2), 254–258.
8. Lebwohl, M. Topical application of calcipotriene and corticosteroids: combination regimens. J. Am. Acad. Dermatol. **1997**, *37* (3), S55–S58.
9. Didriksen, E.; Høy, G. *Pharmaceutical Composition*; January 27, 2000, International patent application no. WO 00/64450.
10. Patel, B.; Siskin, S.; Krazmien, R.; Lebwohl, M. Compatibility of calcipotriene with other topical medications. J. Am. Acad. Dermatol. **1998**, *38* (6), 1010–1011.
11. Loftsson, T.; Petersen, D.S. Cyclodextrin solubilization of water-insoluble drugs: Calcipotriol and EB-1089. Pharmazie **1997**, *52* (10), 783–785.
12. Traulsen, J.; Hughes-Formella, B.J. The atrophogenic potential and dermal tolerance of calcipotriol/betamethasone dipropionate ointment compared with betamethasone dipropionate ointment. Dermatology **2003**, *207* (2), 166–172.
13. Franz, T.J. Percutaneous absorption. on the relevance of in vitro data. J. Invest. Dermatol. **1975**, *64* (3), 190–195.
14. Skelly, J.P.; Shah, V.P.; Maibach, H.; Guy, R.H.; Wester, R.C.; Flynn, G.L.; Yacobi, A. FDA and AAPS report on the workshop on principles and practices of in vitro percutaneous penetration studies: relevance to bioavailability and bioequivalence. Pharm. Res. **1987**, *4* (3), 265–267.
15. Dick, I.P.; Scott, R.C. Pig ear skin as an in-vitro model for human skin permeability. J. Pharm. Pharmacol. **1992**, *44* (8), 640–645.
16. Douglas, W.S.; Poulin, Y.; Decroix, J.; Ortonne, J.P.; Mrowietz, U.; Gulliver, W.; Krogstad, A.L.; Larsen, F.G.; Iglesias, L.; Buckley, C.; Bibby, A.J. A new calcipotriol/betamethasone formulation with rapid onset of action was superior to monotherapy with betamethasone dipropionate or calcipotriol in psoriasis vulgaris. Acta Derm.-Venereol. **2002**, *82* (2), 131–135.
17. Guenther, L.; van de Kerkhof, P.C.; Snellman, E.; Kragballe, K.; Chu, A.C.; Tegner, E.; Garcia-Diez, A.; Springborg, J. Efficacy and safety of a new combination of calcipotriol and betamethasone dipropionate (once or twice daily) compared to calcipotriol (twice daily) in the treatment of psoriasis vulgaris: a randomized, double-blind, vehicle-controlled clinical trial. Br. J. Dermatol. **2002**, *147* (2), 316–323.
18. Papp, K.A.; Guenther, L.; Boyden, B.; Larsen, F.G.; Harvima, R.J.; Guilhou, J.J.; Kaufmann, R.; Rogers, S.; van de Kerkhof, P.C.; Hanssen, L.I.; Tegner, E.; Burg, G.; Talbot, D.; Chu, A.C. Early onset of action and efficacy of a combination of calcipotriene and betamethasone dipropionate in the treatment of psoriasis. J. Am. Acad. Dermatol. **2003**, *48* (1), 48–54.
19. Kaufmann, R.; Bibby, A.J.; Bissonnette, R.; Cambazard, F.; Chu, A.C.; Decroix, J.; Douglas, W.S.; Lowson, D.; Mascaro, J.M.; Murphy, G.M.; Stymne, B. A new calcipotriol/betamethasone dipropionate formulation (daivobet) is an effective once-daily treatment for psoriasis vulgaris. Dermatology **2002**, *205* (4), 389–393.