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COMMUNICATION

Skin Permeation of 5-Methoxypsoralen from Topical Dosage Forms

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ABSTRACT

The topical photochemotherapy of dermatoses with psoralens (PUVA therapy) requires an adequate drug level at the target site (basal epidermis) at the time of UVA radiation. The aim of this work was to enhance 5-methoxypsoralen transport to the basal epidermis, with the goal to shorten the delay between drug application and UVA irradiation. 5-Methoxypsoralen transport through rabbit skin was studied in vitro from topical formulations (water solution, gel, and emulsion). The results obtained show that the use of the emulsion increased the flux through rabbit ear skin, even if partitioning was not favorable. Additionally, the time lag was sensibly reduced, compared with the gel and solution. Furthermore, drug accumulation in human skin in vitro was determined using the thin slicing technique. Human skin accumulation profile was significantly higher for the emulsion, compared with the gel, indicating that the delay between psoralen application and UVA irradiation can be shortened.

Key Words: Permeation; Skin; Psoralen; Topical.

INTRODUCTION

Vitiligo and psoriasis are skin diseases characterized by the formation of patches of depigmentation (vitiligo) or multilayered scales (psoriasis). For treatment of these dermatoses, 5-methoxypsoralen (5-MOP) is used in combination with UVA irradiation[1] (PUVA therapy).

According to the British Photodermatology Group, 5-MOP is typically administered per os (1.2 mg/kg), 3 hr before UVA irradiation.[2] When the number and extension of affected areas are limited,

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local application of psoralen can be advisable because it reduces the systemic side effects. To be effective, therapeutically useful drug concentrations at the target sites (i.e., basal layer of epidermis) should be provided. As a reference, we can consider as a useful 5-MOP concentration the one obtained in the basal epidermis after 3 hr from the oral administration of the typical dose.[3]

In previous reports,[3,4] 5-MOP accumulation in the different skin layers has been studied on human skin in vivo after application of a topical gel and after oral administration. In both cases, drug concentration decreased from the skin surface toward the dermis, because of a strong affinity of 5-MOP for the stratum corneum. With the gel, a minimum application time of 2 hr was required to achieve therapeutically useful concentrations of 5-MOP in the skin.

The aim of this work was to enhance 5-MOP transport to the basal epidermis, with the goal of shortening the delay between drug application and UVA irradiation. Hence, we studied the in vitro permeation of 5-MOP through rabbit ear skin from a new formulation, in comparison with the previously described gel. The formulation chosen was an emulsion, because this kind of preparation is reported to be effective in accumulating other psoralens in the skin.[5] The permeation data obtained with rabbit skin were compared with experiments on human skin, in which drug accumulation in the different layers was measured.

EXPERIMENTAL

Materials

5-Methoxypsoralen (water solubility at 37°C: 7 μg/mL) was obtained from Galeno (Prato, Italy). The reference gel, containing 0.05% 5-MOP (w/v), was prepared by dissolving 5-MOP (50 mg) in 20 mL of 2-propanol and 10 mL of propylene glycol, and mixing the obtained solution with 1.5 g of hydroxypropylcellulose (Natrosol® 250 HR, Eigenmann & Veronelli, Milan, Italy) hydrated in 70 mL of water.

The emulsion was prepared as follows: 5-MOP (0.05 g) and cholesterol (1.3 g) were dissolved in 95% (v/v) ethanol (39 g) and isopropylmyristate (14 g) under magnetic stirring. After dissolution, lecithin (Phospholipon® 80 H, Natterman Phospholipid GMBH, Köln, Germany) and water (q.s. to 100 mL) were added. The final mixture was simply stirred, giving rise to an emulsion that was stable for at least 1 year.

Methods

5-MOP Permeation Through Rabbit Skin

Rabbit ear skin (thickness: 0.036 ± 0.006 cm), obtained from a local slaughterhouse, was mounted on Franz-type glass diffusion cells (0.6 cm^2 surface area) (DISA, Milan, Italy) with the stratum corneum facing the donor chamber. The donor contained 1.0 mL of formulation, whereas the receptor chamber, thermostated at 37°C and magnetically stirred, contained 4.2 mL of β-cyclodextrin (β-CD; 1% w/v) in saline solution.

Quantification of the drug was performed by HPLC (Perkin-Elmer, Norwalk, CT) equipped with a UV detector (Perkin-Elmer) and a 3.9×150 mm C_{18} column (Nova-Pak® Waters, Milford, MA). The mobile phase was methanol:water (55:45, v/v) at a flow rate of 0.7 mL/min, and the wavelength was set at 300 nm. System suitability was assessed according to USP 24. The limit of quantification resulted in 0.05 ng/mL.

The amount of 5-MOP permeated as function of time, $Q(t)$, was fitted to the appropriate solution to Fick's second law of diffusion in a homogeneous membrane,[6] i.e.,

$$Q(t) = (KH)C_d \times \left[\frac{D}{H^2}t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-n^2\pi^2\frac{D}{H^2}t\right)\right] \quad (1)$$

where K is the partition coefficient, H is stratum corneum thickness, C_d is donor concentration, and D is apparent diffusion coefficient. The fitting was performed with n equal to 10, using Kaleidagraph™ software on a Power G3 MacIntosh computer.

All experiments were replicated six times; results are expressed as the means ± SEM.

5-MOP Accumulation in Human Skin

Human skin, obtained from abdominoplastic surgery, was mounted on Franz-type diffusion cells. Experiments were performed using the emulsion as donor and saline solution as receptor phase. After

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30 min, the skin was removed from the cell and frozen in liquid nitrogen. Skin slicing and drug quantification in the different layers were performed according to [4]. Briefly, 20 slices, 20 μm thick, were obtained by horizontal sectioning with a cryomicrotome (Miles, Elkhart, IN). Each slice was extracted with 500 μL of a mixture of methanol:water (4:1), and the supernatant was analyzed by HPLC using trimethoxypsoralen as internal standard.

All experiments were performed at least in triplicate; results are expressed as the means ± SEM.

Statistical Analysis

The significance of the differences between the values of 5-MOP recovered in the skin, as well as the permeation parameters, were assessed using the *t*-test for unpaired samples. Analysis was performed using Microsoft Excel 8.0 software running on a Power G3 MacIntosh computer.

RESULTS AND DISCUSSION

5-MOP Permeation Through Rabbit Skin

To guarantee the sink conditions in the receptor solution, β-CD was added since it increased 5-MOP solubility about five times. β-CD is reported not to alter the skin barrier.[7]

Figure 1 shows 5-MOP permeation profiles from the tested formulations through rabbit skin. The amount of 5-MOP permeated from the gel resulted significantly higher than from the saturated water solution; 5-MOP transported from the emulsion was even higher and after 6 hr was twice that measured from the gel. Assuming a nonrelevant difference in 5-MOP thermodynamic activity, this could be attributed to a change in permeability, induced by lipophilic excipients contained in the emulsion (i.e., isopropylmyristate, cholesterol, and lecithin).[8]

Penetration profiles from the different formulations were analyzed to obtain the relevant permeation parameters. Diffusivity, permeability, time lag, and partitioning—calculated using Eq. (1) (see [6,9])—are reported in Table 1.

As Table 1 shows, the excipients used in the gel and in the emulsion produced an increase of the diffusivity value of 5-MOP, compared with the water solution—most likely because of the perturbation of the stratum corneum. Assuming H as a constant, the increase in diffusivity (D/H^2) is because of the effect of formulation on drug diffusion coefficient. Compared with the solution, the gel showed a 5-fold increase of D, whereas with the emulsion, the increase was 40 times. On the contrary, the highest value of partitioning parameter KH was measured with water solution. This value decreased approximately 2 orders of magnitude in the case of gel and still more for the emulsion. As a consequence, 5-MOP permeability coefficient P (calculated as $KH \times D/H^2$) resulted 1 order of magnitude higher for the aqueous solution than using the gel or the emulsion. However, the steady-state flux (calculated as $P \times C_d$) was highest for the emulsion and extremely low for the water solution; the high solubility of 5-MOP in the emulsion overcame the unfavorable permeability coefficient.

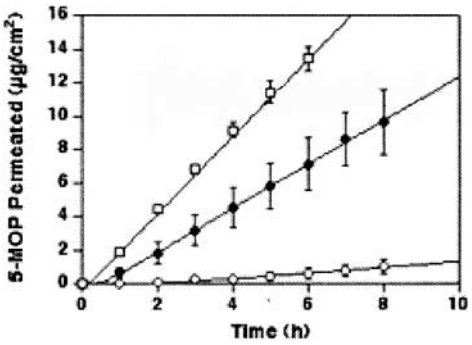

Figure 1. 5-MOP permeation profiles through rabbit ear skin from drug-saturated solution (○), gel (●), and emulsion (□). Experimental points and theoretical curves are according to Eq. (1) (mean values ± SEM, $n = 6$.)

Time lag value (calculated as $H^2/6D$) varied significantly in dependence of the formulation: from 4.5 hr for water solution, the value decreased to 1 hr for the gel and to a few minutes for the emulsion. Because use of the emulsion reduced the time lag, compared with the gel, we verified if this time lag reduction was also reflected in a quicker accumulation in human skin.

5-MOP Accumulation in Human Skin

5-Methoxypsoralen accumulation profile in human skin was measured after application of the emulsion for 30 min. The results, compared in Fig. 2 with the data already obtained with the gel,[4] show a skin concentration profile in the target region signifi-

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Table 1. Permeation parameters of 5-MOP calculated using Eq. (1) (mean ± SEM, $n = 6$).

Donor	D/H^2 [a]	$10^2 \times KH$ [b] (cm)	Time lag (hr)	$10^3 \times$ Permeability (cm hr^{-1})	Steady-state flux (μg cm^{-2} hr^{-1})
Water-saturated solution (7 μg/mL)	0.05 ± 0.03[c]	106 ± 64[d]	4.54 ± 2.40	36.6 ± 4.2[e]	0.11 ± 0.01
Gel (500 μg/mL)	0.26 ± 0.06[c]	1.1 ± 0.1[d]	0.87 ± 0.22	2.7 ± 0.5[e]	1.33 ± 0.26
Lotion (500 μg/mL)	1.97 ± 0.22	0.26 ± 0.05[d]	0.09 ± 0.01	4.6 ± 0.3[e]	2.32 ± 0.13

[a]Diffusion coefficient divided by the diffusion length squared.
[b]Partition coefficient multiplied by diffusion length.
[c]Statistically different from lotion ($p < 0.01$).
[d]Statistically different from each other ($p < 0.05$).
[e]Statistically different from each other ($p < 0.01$).

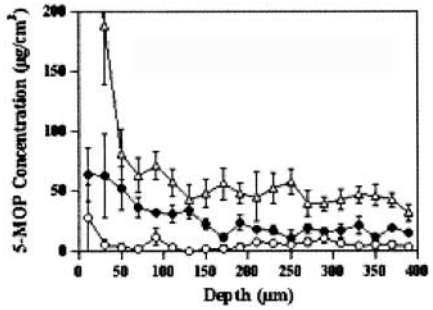

Figure 2. 5-MOP concentration profiles in human skin after 30 min of gel application (modified from [4]; ○) or emulsion (●) or after 3 hr from oral administration of the typical dose in humans (modified from ref. 3; ▲) (mean values ± SEM, $n \geq 3$).

cantly higher for the emulsion than for the gel ($p < 0.05$ from 70 to 210 μm). This demonstrates that the emulsion can quickly accumulate high quantities of 5-MOP, confirming the time lag data.

Interestingly, the amount of 5-MOP accumulated in the basal layer after 30 min of emulsion application was not significantly different from that obtained in the previous report from the gel formulation after 2 hr.[4]

Further considerations can be made about the potential therapeutic effectiveness of the emulsion. Because no literature data concerning the useful concentration at the basal epidermis are available, we considered as a reference the 5-MOP concentration obtained in the basal epidermis after 3 hr from oral administration of the typical dose. These data, already published,[3] have been reported for sake of clarity in Fig. 2. As shown, the profile obtained after oral administration is higher than that obtained after 30 min of emulsion application in vitro but, because of high variability, is not statistically different ($p > 0.05$).

CONCLUSIONS

Compared with the gel, the use of the emulsion increased the flux of 5-MOP through rabbit ear skin, even if partitioning was less favorable. In addition, the time lag was sensibly reduced, indicating a rapid uptake of 5-MOP by skin. In human skin, the emulsion increased drug concentration in all the layers considered, including the epidermal basal layer, site of action in the treatment of psoriasis. Moreover, this formulation gave rise to a drug concentration at the target site not statistically different from that obtained after oral administration. Hence, by using the emulsion, the delay between the psoralen application and the UVA irradiation can be shortened.

REFERENCES

1. McNeely, W.; Goa, K.L. 5-Methoxypsoralen. A review of its effects in psoriasis and vitiligo. Drugs **1998**, *56*, 667–690.
2. British Photodermatology Group. Guidelines for PUVA. Br. J. Dermatol. **1994**, *130*, 246–255.
3. Zucchi, A.; Raho, E.; Marconi, B.; Nicoli, S.; Santini, M.; Allegra, F.; Colombo, P.; Bettini, R.; Santi, P. Plasma and skin concentration of 5-methoxypsoralen in psoriatic patients after oral administration. J. Inv. Derm. **2001**, *117*, 379–382.
4. Colombo, G.; Zucchi, A.; Allegra, F.; Colombo, P.; Zani, F.; Santi, P. In vitro and in vivo study

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of 5-methoxypsoralen skin concentration after topical application. Skin Pharmacol. Appl. Skin Physiol. (accepted).

5. Baroli, B.; Lopez-Quintela, MA.; Delgado-Charro, M.B.; Fadda, A.M.; Blanco-Mendez, J. Microemulsions for topical delivery of 8-methoxalen. J. Controlled Release **2000**, *69*, 209–218.
6. Moser, K.; Kriwet, K.; Froehlich, C.; Kalia, Y.N.; Guy, R.H. Supersaturation: enhancement of skin penetration and permeation of a lipophilic drug. Pharm. Res. **2001**, *18*, 1006–1011.
7. Sclafani, J.; Liu, P.; Hansen, E.; Cettina, M.G.; Nightingale, J. A protocol for the assessment of receiver solution additive-induced skin permeation changes. An example with γ-cyclodextrin. Int. J. Pharm. **1995**, *124*, 213–217.
8. Naik, A.; Kalia, Y.N.; Guy, R.H. Transdermal drug delivery: overcoming the skin's barrier function. P.S.T.T. **2000**, *3*, 318–326.
9. Dias, M.; Farinha, A.; Faustino, E.; Hadgraft, J.; Pais, J.; Toscano, C. Topical delivery of caffeine from some commercial formulation. Int. J. Pharm. **1999**, *182*, 41–47.

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