Skin Penetration and Sun Protection Factor of Ultra-Violet Filters from Two Vehicles

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Purpose. In order to improve our knowledge on the efficacy and safety of sunscreen products, we measured the skin penetration profiles of ultra-violet (UV) filters in vitro and in vivo, and the corresponding sun protection factors (SPF) from two vehicles (an O/W emulsion-gel and petroleum jelly).

Methods. The UV filters tested were oxybenzone (5%, A), 2-ethylhexyl 4-methoxycinnamate (7.5%, B), and 2-ethylhexylsalicylate (3%, C). Two mg/cm^2 were applied for 2 min to 6 h. In vitro penetration measurements were performed with static diffusion cells. In vivo, horny layer concentrations were measured after stripping and the SPF evaluated as recommended by the COLIPA-guidelines.

Results. Significant differences between vehicles were noticed in vitro as well as in vivo. In vitro, the emulsion-gel generated higher epidermal concentrations than petroleum jelly. Values at 6 h, expressed as percent of the applied dose for A, B, and C were 4, 9, and 7% for the emulsion-gel and 2, 1, and 2% for petroleum jelly. An opposite trend was noticed, mainly for A, in the deeper skin layers with concentrations of 2% in the dermis and 5% in the receptor fluid for petroleum jelly and 0.6% and 1% for the emulsion-gel respectively. In vivo, for each UV filter, maximal stratum corneum levels (15 strips) were obtained at 0.5 h with percentages of the applied doses of 50% for the emulsion-gel and 15% for petroleum jelly. SPFs, measured 0.5 h after application amounted to 14 for the emulsion-gel and 5 for petroleum jelly, and decreased in both cases by a factor 2.2 after removal of non penetrated product.

Conclusions. These preliminary results demonstrated that UV filters penetration and retention as well as expected SPF could be optimized by a suitable vehicle.

KEY WORDS: sunscreen; UV filter; human skin; percutaneous absorption; stratum corneum.

INTRODUCTION

It has been known for decades that sunscreens are capable of protecting man from harmful effects of solar radiation such as actinic aging or cutaneous cancer (1). Basic and applied research concerning sun protection has become a major concern. Today, several ultra-violet (UV) absorbing chemicals are available for sunscreen preparations. Although most aspects of these compounds have been fully investigated, there is little published data describing either their penetration into the skin or their permeation through the skin (2) and possible effects on the sun protection factor (3) (SPF). Our aim was to look at the skin penetration profiles of UV filters in vitro in order to investigate a possible influence of the formulation, and to measure the concentrations in the horny layer and corresponding SPFs in vivo. A concentration of UV filters within the outer layers of the stratum corneum would be highly desirable to increase rubbing and sweat resistance and decrease toxicological risk. At the same time this concept could mean an improvement of the water-resistance even if no lipophilic vehicle, known to decrease cosmetical acceptance, is used. In the present study, we compared the skin penetration of UV filters after their application in an O/W emulsion-gel or in petroleum jelly.

MATERIALS AND METHODS

Sunscreen Products

Daylong 16 (Spirig AG, CH-Egerkingen), an emulsion-gel containing 70% of water, ethanol, phospholipids, carbopol, sorbitol, silicone, amphisol, cetyl alcohol, tocopherol, triethanolamine, preservatives, and a preparation with white petroleum jelly as vehicle were used throughout. UV filters in both formulations were: oxybenzone (A, 5%), 2-ethylhexyl 4-methoxycinnamate (B, 7.5%), and 2-ethylhexylsalicylate (C, 3%). Their physicochemicals properties are indicated in Table I. Measurements of their solubility in the receptor fluid were done in triplicate at room temperature by adding an excess of compound, stirring for 8 h and filtrating through a 0.2 μm filter.

Chemicals

UV filters were obtained from BASF, D-Ludwigshafen (A) and from H&R, D-Holzminden (B, C), purity was >98%. Crystallized bovine albumine was purchased from Fluka (CH-Buchs) and had a purity >98% and a pH (1.5% water solution) of 6.85.

In Vitro Skin Penetration

Skin samples were obtained from two caucasian women (source 1 and 2) undergoing abdominoplasty. Before use, the skin was dermatomed to a thickness of 600 μm (air dermatome, Zimmer, Ohio, USA-Dover). Skin penetration was measured using static diffusion Franz cells (4) (Crown Glass, Somerville, NJ) with a 1.76 cm^2 surface area of exposed skin. The preparations were applied at a dose of 2.26 ± 0.21 mg/cm^2 and 2.52 ± 0.4 mg/cm^2 for the emulsion-gel and the petroleum jelly respectively. The receptor fluid was physiological saline with albumine (1.5% w/v) maintained at 36.5 ± 1°C. Four application times were investigated (2 min, 0.5 h, 2 h, 6 h). Afterwards, the receptor fluid was removed, the skin surface was quickly washed twice with 2 ml of 60% methanol in water containing 0.5% tween 80 by cotton swabs. Two 8 mm biopsy punchs (Stiefel, CH-Winterthur) were performed and epidermis and dermis were separated on a hot plate (60°C for 2 min). For each skin compartment, both biopsies were powdered together in a steel ball grinder mill (Retsch, D-Haan) working under liquid nitrogen and extracted with 2 ml of methanol.

In Vivo Stratum Corneum Penetration

After informed consent, four healthy volunteers, aged 22–31 years, participated in the study. Areas (10 × 10 cm) were randomly allocated on the back, and each product was applied at a dose of 2 mg/cm^2. Half an hour, 2 and 6 h later, non penetrated product was removed with a paper towel, and

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Table I. Physicochemical Properties of the UV Filters

UV filters	MW[a]	MP[b](C°)	log P[c]	SRF[d](μg/ml)	AM[e](nm)
oxybenzone	228	63	3.58	76.6 ± 0.8	288–325
2-ethylhexyl 4-methoxycinnamate	290	oil	5.96	11.9 ± 1.3	311
2-ethylhexylsalicylate	250	oil	6.02	10.3 ± 2.1	307

[a] Molecular weight.
[b] Melting point.
[c] Octanol/water partition coefficient calculated with CLOG™ program (Biobyte Corp., CA, Claremont-USA).
[d] Solubility in the receptor fluid (mean ± sd, n = 3).
[e] Absorption maxima.

15 strips (10 × 20 mm) were performed with cellux tape (Sellotape, CH-Rorschach). Strips 1–5, 6–10, and 11–15 were pooled separately, powdered as described above and extracted with 2 × 2 ml of methanol.

Extraction Procedures

Skin: Samples without treatment were proceeded on as described above. Before performing the pulverization 50 μl methanol containing (μg) A: 25/2.5, B: 37.5/3.75, C: 15/1.5 were added to the epidermis/dermis samples respectively. Receptor fluid at a concentration of A = 1.25, B = 1.87, C = 0.75 (μg/ml) was also prepared.

Strips: Five tapes (10 × 20 mm) received each on the sticky side 20 μl of methanol containing 200 μg/ml of each UV filter. After evaporation, the tapes were pooled, pulverized, and extracted as described for skin biopsies.

SPF Measurements

These measurements were performed on the same volunteers 0.5 h after the application of the products under similar conditions as for the stratum corneum investigations, and following the recommendations of the COLIPA-guidelines (5). The light source was a multiport solar ultraviolet simulator, model 601 (Solar Light Co, PA, USA-Philadelphia). SPFs were measured again after removal of non penetrated product with a paper towel.

HPLC Assays

The chromatographic conditions were as follows: the detection wavelength was set at 288 nm and the eluent was methanol: water (83:17) containing glacial acetic acid at a concentration of 0.01% v/v. UV filter concentrations were determined from standard curves of pure compounds A, B, and C in methanolic solution. Typical chromatogram is shown in Figure 1. Values below the detection limit were arbitrarily recorded as zero.

Calculations and Statistics

The penetration results were expressed as percentages of the applied dose. SPF was calculated by the ratio minimal erythemal dose (MED) for protected skin/MED for unprotected skin (mean ± sd). Statistical analysis was realized with an

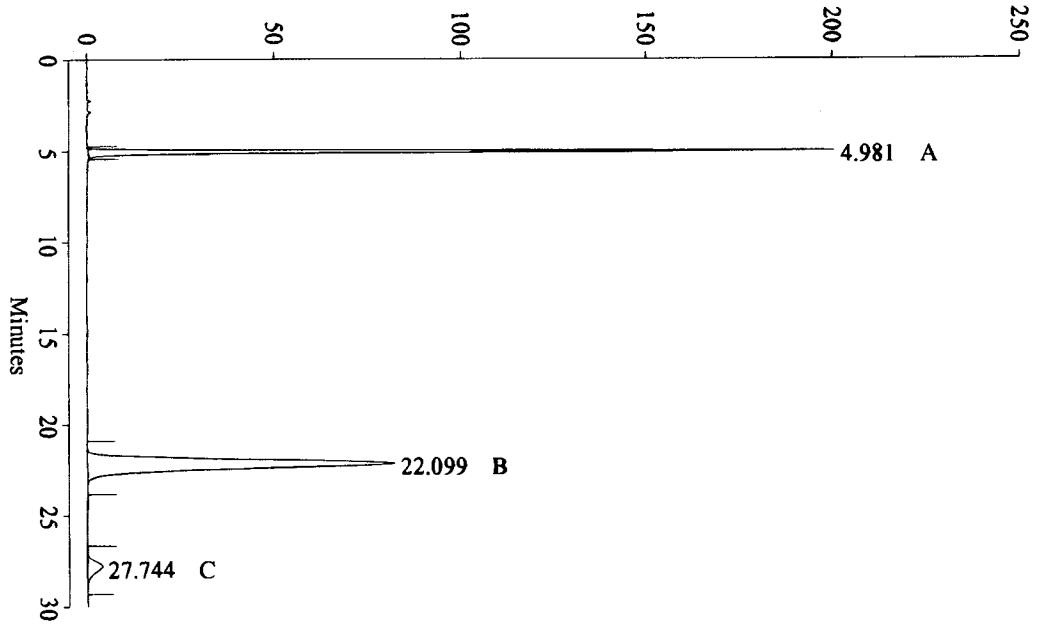

Fig. 1. HPLC chromatogram of A, B, C in pure standard. A = oxybenzone, B = 2-ethylhexyl 4-methoxycinnamate, C = 2-ethylhexylsalicylate.

Table II. Extraction Recovery[a] (%)

UV filters	Epidermis	Dermis	Receptor fluid	Strip
oxybenzone	99.8 ± 3.2	90.2 ± 7.0	79.4 ± 1.4	96.3 ± 23.1
2-ethylhexyl 4-methoxycinnamate	103.9 ± 5.0	95.2 ± 6.2	86.1 ± 4.9	88.3 ± 15.5
2-ethylhexylsalicylate	99.6 ± 6.4	99.1 ± 28.0	92.7 ± 9.3	95.5 ± 16.6

[a] Indicated data are means ± sd, n = 3–4.

analysis of variance (ANOVA). The significance of the differences between vehicles was tested by the non parametric Mann-Whitney U test.

RESULTS

Extraction Procedure

The results are given in Table II. Extraction recovery was 79 to 103%. UV filters were most difficultly extracted from the receptor fluid compartment. High standard deviations were noticed for compound C in the dermis and for all filters in the strips.

Skin Penetration Using Franz Cells

All UV filters were readily soluble in the receptor fluid as indicated in Table I. Penetration data are shown in Table III. The ANOVA did not reveal any significant differences regarding penetration data of the UV filters but application time as well as vehicle effect were found to be significant.

The application time of 2 min was chosen to check the recovery of the washing procedure. After this short application time the three UV filters could be detected in the epidermis but values were below 1% and 2% for the emulsion-gel and the petroleum jelly respectively. The quantities recovered from the dermis were minor and showed the same trend as for the epidermis (higher values for the petroleum jelly). We were not able to measure any of the 3 filters in the receptor fluid after this application time.

For all other application times, significant differences between vehicles were noticed, mainly in the epidermis after 0.5 h and 6 h, where penetration values of the emulsion-gel were much more important than those for petroleum jelly. On the contrary, this was not the case in the dermis, where the concentrations of A were higher after petroleum jelly applications (2 h and 6 h application times). Dermal concentrations of the UV filter C were often below the detection limit. Only A was detectable in the receptor fluid. In this compartment too, higher values (application times of 2 and 6 h) were found for petroleum jelly. Total recoveries were greater for the emulsion-gel than for petroleum jelly. They were highest for the application time 2 min for both vehicles.

Stratum Corneum Content

The data pointed out an obvious vehicle effect but neither UV filter concentrations nor application times were found significantly different. Results (0.5 h) are indicated in Figure 2. The amounts contained in the stratum corneum were 40–50% for the emulsion-gel and 10–15% for the petroleum jelly. Maximal levels were reached at 0.5 h, both other time points showed values slightly inferior (data not shown) The differences between both vehicles were higher in the superficial parts of

Table III. Penetration Values of UV Filters Expressed as Percentage of the Applied Dose[a]

		Wash		Epidermis		Dermis		Receptor fluid		Total recovery	
		E[b]	P	E	P	E	P	E	P	E	P
2 min[c]	A[d]	102 ± 15	79 ± 10	0.17 ± 0.1	0.39 ± 0.2	0.08 ± 0.06	0.13 ± 0.1	0 ± 0	0 ± 0	102 ± 16	80 ± 10
	B	103 ± 16[e]	75 ± 8	0.68 ± 0.4[e]	1.96 ± 0.6	0.04 ± 0.06	0.37 ± 0.2	0 ± 0	0 ± 0	104 ± 16[e]	77 ± 5
	C	112 ± 20[e]	51 ± 15	0.94 ± 0.4	1.81 ± 0.7	0 ± 0	0.46 ± 0.8	0 ± 0	0 ± 0	113 ± 20[e]	54 ± 17
0.5 h	A	79 ± 7	74 ± 3	0.50 ± 0.2[e]	0.19 ± 0.05	0.09 ± 0.03	0.06 ± 0.04	0 ± 0	0 ± 0	79 ± 8	74 ± 3
	B	81 ± 7[e]	54 ± 2	1.86 ± 0.8[e]	0.48 ± 0.3	0.09 ± 0.06	0 ± 0	0 ± 0	0 ± 0	83 ± 8[e]	55 ± 2
	C	81 ± 9[e]	39 ± 3	2.13 ± 0.7[e]	0.60 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	83 ± 11[e]	40 ± 4
2 h	A	72 ± 7[e]	59 ± 2	0.69 ± 0.2[e]	1.43 ± 0.1	0.2 ± 0.05[e]	1.06 ± 0.1	0.14 ± 0.1	0.27 ± 0.1	73 ± 9	62 ± 2
	B	77 ± 6[e]	62 ± 3	1.71 ± 0.6	1.88 ± 0.6	0.03 ± 0.04	0.30 ± 0.2	0 ± 0	0 ± 0	79 ± 8[e]	64 ± 2
	C	78 ± 4[e]	45 ± 5	1.54 ± 0.3	1.97 ± 0.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	80 ± 4[e]	47 ± 6
6 h	A	62 ± 6[e]	52 ± 1	3.78 ± 1.6[e]	1.83 ± 0.3	0.65 ± 0.2[e]	1.86 ± 0.2	1.05 ± 0.2[e]	4.87 ± 0.5	68 ± 5[e]	60 ± 1
	B	63 ± 5	60 ± 6	8.62 ± 2.2[e]	1.28 ± 0.1	0.78 ± 0.3	0.43 ± 0.3	0 ± 0	0 ± 0	73 ± 5	62 ± 7
	C	61 ± 8	52 ± 1	7.29 ± 1.8[e]	1.96 ± 0.2	0.51 ± 0.7	0 ± 0	0 ± 0	0 ± 0	68 ± 8[e]	54 ± 1

[a] Indicated data are means ± sd, n = 3.
[b] E = emulsion-gel and P = petroleum jelly.
[c] Application time of 2 min was investigated with skin source 1, the others with skin source 2 (see materials and methods).
[d] A = oxybenzone, B = 2-ethylhexyl 4-methoxycinnamate, and C = 2-ethylhexylsalicylate.
[e] Indicates $p < 0.05$ between vehicles.

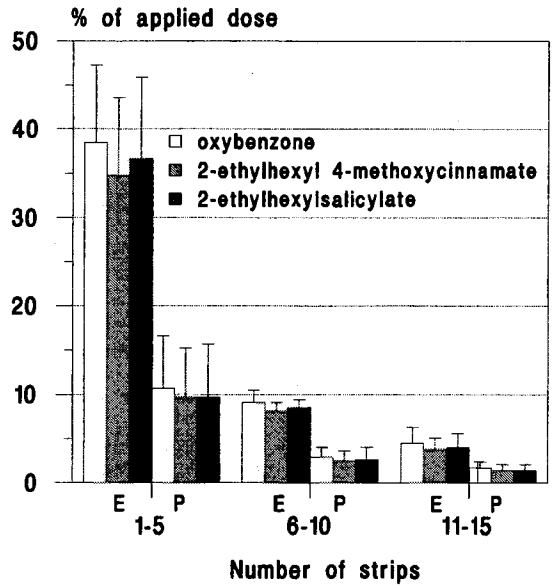

Fig. 2. Amount of UV filters in the stratum corneum after an application time of 0.5 h (mean ± sd, n = 4). E = emulsion-gel and P = petroleum jelly. Differences between vehicles were significant ($p < 0.05$).

the stratum corneum (strips 1–5) compared to the deeper part (strips 11–15), demonstrating that the enhancer effect of the emulsion-gel formulation was more important in the upper layers of the stratum corneum.

SPF Measurements

SPF measurements performed 0.5 h after application showed values of 14.2 ± 3.6 and 5.4 ± 1.3 for emulsion-gel and petroleum jelly respectively (Figure 3). For both formulations, SPF values were decreased by a factor of 2.2 after removal of non penetrated product. In every cases, the protection afforded by the emulsion-gel was significantly higher than the one of petroleum jelly.

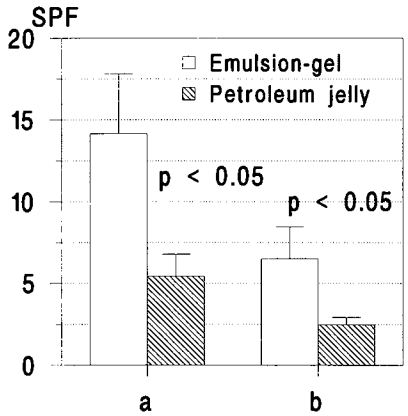

Fig. 3. Sun protection factor (SPF) measurements (mean ± sd, n = 4), before (a) and after removal of non penetrated product (b) for emulsion-gel and petroleum jelly formulations. Differences between vehicles were significant.

DISCUSSION

These results show that the skin penetration of UV filters and the corresponding SPFs can be significantly modulated by product formulation. In vitro, the emulsion-gel formulation generated higher epidermal concentrations of UV filters, with the exception of the 2 h application time. This was particularly pronounced for oxybenzone. An opposite trend was noticed in the dermis and in the receptor fluid, mainly for oxybenzone again, as higher concentrations were measured after petroleum jelly application. These results suggest that the emulsion-gel led to a greater penetration and a better retention over time in the epidermis than petroleum jelly. This last vehicle appeared to favour rather a diffusion through the skin than a retention phenomenon. The three investigated molecules are lipophilic and thus, the nature of the receptor fluid could have accentuated this phenomenon. The solubility measurements (Table I) demonstrated that the receptor fluid was not rate-limiting in the diffusion process. Differences in physicochemical properties of the compounds may explain the absence of the more lipophilic UV filters B and C in the receptor fluid, pointing to the dermis as a permeation limiting factor. This does however not represent the in vivo situation where microcirculation favours resorption, especially for lipophilic drugs. The high oxybenzone concentration in the receptor fluid agrees with its recently published in vivo percutaneous absorption (when formulating at 10% in petroleum jelly) in the rat (6). It is known that permeation differences between rat skin and human skin exist (7), but these results would confirm that oxybenzone readily penetrates into the skin. The percutaneous absorption of another UV filter, paraaminobenzoic acid (amphiphilic), was already investigated by measuring in human total radioactivity in urine for 5 days, and was shown to be 28% of the applied dose (8). Our total recoveries were sometimes low especially for petroleum jelly and compounds B and C. Protein binding, metabolisation and lost of the applied product on the head of the cell during the washing of the skin surface with the methanol/water mixture could explain incomplete recoveries. Such a mixture is likely to remove part of the compounds from superficial stratum corneum layers but, because of its short contact time (less than one min), we do not believe it to significantly perturb the compounds distribution within the deeper skin layers. Moreover, washing mixtures containing up to 95% of alcohol are usually employed to assess drug concentrations in the stratum corneum (9) and the cutaneous layers (10).

In vivo results from the stratum corneum clearly confirm the vehicle effect on penetration of the UV filters. The penetration enhancement might be explained by the presence of phospholipids in the emulsion-gel, forming liposomes (11), and/or by product spreadibility (3) as well as by changes occuring in the formulation after application, such as evaporation, acting directly on the thermodynamic activity of the UV filters. We did not find any differences between the 3 filters measured in the stratum corneum, but the variability of the extraction procedure (Table II) could have prevented us from detecting such differences. If this was not the case, we could hypothesize that the stratum corneum reservoir filling was not influenced by the physicochemical properties of these lipophilic molecules.

In contrast to the petroleum jelly, higher SPF values were generated with the emulsion-gel formulation. Its spreadibility and evaporation probably concentrated the UV filters in a thin

layer on the skin surface, thus improving their efficiency to absorbe UV light. The value of 14.2 for the emulsion-gel was slightly below the claimed SPF [16], but only 4 subjects were investigated in these experiments that did not aim at measuring an already known SPF. The same reservations apply, of course, for the SPF of the petroleum jelly formulation, but we consider a meaningful comparison still possible. After removal of non penetrated product, an SPF of 6.5 was measured for the emulsion-gel, in agreement with the percentage of UV filters found in the stratum corneum 0.5 h after application (50% of the applied dose). This demonstrates the ability of the filters to act within the stratum corneum. The SPF measured after removal of the petroleum jelly formulation was reduced by the same factor as for the emulsion-gel (2.2), while the amount of UV filters contained in the stratum corneum was only 10–15% of the applied dose. The non-linear curve (12) (dose of UV filter vs. SPF) as well as the different distribution and concentration of the compounds within the stratum corneum might explain this apparent discrepancy.

In conclusion we could demonstrate in vitro and in vivo that the penetration of UV filters into the skin can be optimized by using a suitable vehicle. Using an emulsion-gel formulation, high epidermal concentrations were obtained without reaching notable accumulation in deeper skin layers in vitro. Stratum corneum contents and SPF values agree with these results, stressing the UV filters ability to act within the stratum corneum. This property should be helpful to optimize sun protection.

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