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EVALUATION OF IN VITRO SKIN PERMEATION OF UV FILTERS

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Abstract

The skin permeation and the skin primary irritation of two UV filters from caprylic capryl triglyceride (oil), oil in water (O/W) and water in oil (W/O) emulsions, were evaluated. We selected octyl methoxycinnamate (OMC) broadly used in cosmetics and polymeric sunscreen agent (PSA, average MW: 2,000) synthesized by the coupling reaction of 2-ethylhexyl 4-hydroxycinnamate with poly vinylbenzyl chloride, as model UV filters.

For in vitro skin permeation experiments, Franz diffusion cells (effective diffusion area:1.766cm) and the excised skin of female hairless mouse aged 8 weeks were used. Oil or emulsion containing UV filters was applied in the donor compartment. The skin primary irritation was evaluated with female guinea pigs (8-10 weeks, 350-400 g).

In oil and emulsions, the skin permeability and the skin primary irritation of PSA were lower than those of OMC. The skin permeability of UV filters was lower when they were in oil-in-water emulsion (O/W) than water-in-oil emulsion (W/O).

We suggest that O/W system would be more useful when compared with W/O system, and PSA could be a good candidate for a future sunscreen agent for reducing the skin irritation.

Introduction

Ultra-violet chemical filters are widely used in sunscreen preparation to protect human skin from UV radiation. Since UV filters have been published as irritant in cosmetics(1), it is important to develop materials safe to skin. In view of skin toxicology, the safety factor to skin depends on the chemical structure of UV filter and skin permeation.(2) The skin permeability of UV filters depends on chemical structure, the type of emulsion or vehicle properties.(3) Low skin permeation profiles and high photoprotective efficacy play a very important role in any safe cosmetic sunscreens. Polymeric sunscreen agent (PSA, average MW:2,000) has been developed by the coupling 2-ethylhexyl 4-hydroxy cinnamate with poly vinyl benzyl chloride for reducing skin irritation.(4)

In the present study, the skin permeability and the skin primary irritation of PSA were evaluated and compared with octyl methoxycinnamate (OMC) which is broadly used in cosmetics. We investigated the effect of skin permeability on the type of emulsion (water-in-oil emulsion W/O, oil-in-water emulsion O/W) and discussed the relationship between the skin permeation and the skin primary irritation.

Experimental

Materials & Preparations

OMC (Parsol MCX) was from Givaudan Co. (USA). PSA was from LG chemical Ltd. (Korea). Hydrocortisone, n-Octanol, Potassium phosphate, Sodium chloride, Sodium phosphate were obtained from Sigma Co. (USA). HPLC grade Methylene chloride, Aceton, Tetrahyrofuran(THF) were all purchased from J.T.Baker Chemical Co. (USA). Polyoxyethylene oleyl ether (Volpo 20) was from Croda Inc. (USA). Caprylic capryl triglyceride was obtained from Inolex Chemical Co. (USA). All other ingredients were reagent or cosmetic grade.

Caprylic capryl triglyceride solution containing 30 % of OMC or 30 % of PSA, O/W and W/O emulsions containing 5 % of OMC or 5 % of PSA were prepared.

Skin Permeation Studies : Penetration Procedure for Sunscreen Preparation

Vertically assembled Franz type diffusion cell (Microette transdermal diffusion system, Hanson Research Corporation, Chatsworth, CA, USA) were used for in vitro skin permeation experiments. The system consisted of Franz type diffusion cells with an effective diffusion area of 1.776 cm^2 and receptor volume of 7.0 ml, autosampler and cell drive system with rpm controller. The fundamental experiments were performed according to the method given in our previous report.(5) Briefly, the excised skin of female hairless mouse was obtained from 8-9 weeks old, 27-33 g animals. The above skin was mounted on diffusion cell, and the receiver compartment was filled up with 7 ml of 50 mM phosphate buffered saline pH 7.4 (PBS) with 2 % Volpo 20 (a nonionic surfactant, HLB=16) maintaining at 32 C by water which circulates within a jacket around the lower chamber. Volpo 20 was used to insure that solubility in receptor solution would not limit penetration through skin. Diffusion characteristics of skin are unaffected by Volpo 20 exposure.(6) Samples containing UV filters were applied in the donor compartment. Caprylic capryl triglyceride solution was uniformly distributed with a micropipette on the skin surface (20µl). Each emulsion was uniformly rubbed on the skin surface (20 mg). The receptor fluid was mixed by a magnetic stirrer throughout the experiment. The receptor fluid was collected from the receiver compartment at predetermined time (9 hour after sample application) and replaced by fresh fluid.

At the end of the experiment (18 hour after sample application), receptor fluid was collected, and donor compartment washed with 500µl of aceton. The washing was repeated three times. Washes were assessed by HPLC. After completion of the preset time (18 hour), skin samples were taken out of diffusion cells. The epidermis was separated from dermis by gentle teasing using the pinch and then collected in vials. Epidermis was treated by 1 ml of methylene chloride and placed in an ultrasound bath for 2 hour. After filtration on Millex filter FG (pore size: 0.2µm, millipore), solutions were assessed by HPLC. Dermis was treated by 2 ml of 1 N NaOH and kept in a incubator at 40 C for 48 h. Samples were neutralized with hydrochloric acid (1 N) and extracted by 4 ml of methylene chloride and injections in HPLC were made. Five hundred microliters of receptor fluid withdrawn from the receiver compartment at predetermined times was treated 1 ml of methylene chloride and shaken by a vortex mixer. Following centrifugation (13,000 rpm), the supernatant was subjected to HPLC.

Penetration Procedure for a Reference Compound: Hydrocortisone

The experimental procedure was conducted as stated for sunscreen preparation. A PBS was compared with the receptor fluid containing 2 % Volpo 20.

Solution of 1 % hydrocortisone was applied on the skin surface (20µl). Collections were made 4, 8, 12, 16 and 24 hours after product application. At the end of experiment (24 hours), skin surface was washed three times with 1 ml of ethanol. Washes were analyzed by HPLC. At the end of the experiment, skin was taken off and epidermis was separated from the dermis. Epidermis and dermis were treated by the above method. After filtration on Millex filter FG (pore size: 0.2µm, millipore), solutions were assessed by HPLC. Receptor fluid was filtered (pore size: 0.2µm, MFS-13, Micro filtering systems, USA) and injected onto HPLC.

Determination of UV Filters Solubility in the Receptor Solution

Excess of both UV filters were added to receptor solution and mechanically shaken at 32 C for 24 hours. After centrifugation (13,000 rpm for 15 min) and filtration using a Millex filter FG (pore size:0.2µm, millipore), 1ml of supernatant was treated with 1 ml of methylene chloride. The saturated concentration of both UV filters was determined by HPLC.

High Performance Liquid Chromatography

The HPLC consisted of solvent delivery pump (Waters, 600 pump, Waters Co., MA, USA), GPC column (Waters Styragel HR3, 7.8 x 300 mm, Millipore, USA), UV detector (waters, 486 UV detector) and data process system (Waters millennium). The mobile phase was 100 % THF and flow rate was 1 ml/min. Wavelength of 310 nm was selected and temperature of the column was kept at 40 C. The retention time was 7.5 min for polymeric sunscreen agent and 10.5 min for OMC.

Determination of Skin Primary Irritation

Female guinea pigs (8-10 weeks, 350-400 g) were shaved along the dorsal surface of back, 24 h in advance of the procedure. Two test sites in shaven area delineated with picric acid. One test site remained intact. Approximately 0.1 ml of caprylic capryl triglyceride solution with 20 % UV filters was placed onto a gauze pad and applied directly to the animals back. The gauze pad was covered with an occlusive bandage and tightly secured to the animal. The patches were removed following 48 h of exposure and each test site was wiped with dry disposable paper towels. The sites were evaluated for dermal irritation approximately 1 h, 48 h and 72 h after patch removal using the Draize method of scoring.

Results and Discussion

Two preliminary studies were conducted in order to validate the operating conditions necessary for the skin permeation protocol:

Determination of UV Filters Solubility in the Receptor Solution

The low solubility of OMC and PSA in the receptor solution limits the diffusion of them to receiver compartment across skin. For increasing the solubility of these UV filters, 2 % of Volpo 20 solution was used as a receptor solution.

The solubility of OMC and PSA in the receptor solution was 2017 μg/ml and 90 μg/ml. The solubility corresponds to more than 100 % and 63 % applied doses of OMC and PSA.

Validation of the Receptor Solution Containing 2 % of Volpo 20

The receptor solution was a non-physiologic medium in which the UV filters are more soluble than in PBS. We demonstrated experimetally that this receptor solution has no effect on the skin barrier function by the evaluation of skin permeation of control compound: hydrocortisone (Figure 1). There was no significant difference between skin permeations of hydrocortisone using the 2% of Volpo 20 and PBS.

Skin Permeation Study of UV Filters

Table 1 and Figure 2 represent the permeated amounts of OMC and PSA in oil (Caprylic capryl triglyceride) and emulsions (O/W and W/O) 18 hours after application to the excised hairless mouse skin.

In both oil and emulsions, skin permeability of PSA was lower than that of OMC. In caprylic capryl triglyceride, the permeated amount of OMC was 4 times greater than that of PSA; In O/W emulsion, 7 times and W/O emulsion, 5 times.

It was considered that this result was due to decrease of diffusion coefficient and partition coefficient of PSA by coupling octyl hydroxycinnamate with poly vinyl benzyl chloride. It has already been reported that when the physicochemical properties of drugs are similar, the skin permeation of drugs decrease as molecular weights become large.(8) OMC has a high octanol/water partition coefficient (log P = 5.96) and PSA does not dissolve in n-octanol, which could serve as a reference standard because of the solvent similar to skin polarity. This indicates that partition of PSA to skin may be lower than that of OMS.

The skin permeability of UV filters (OMC and PSA) from various preparations showed the following order ; W/O emulsion > O/W emulsion > Caprylic capryl triglyceride.

In general, it has been considered that efficient fraction of drug for percutaneous penetration is that which exists in the outer phase free from entrapment in emulsions and micelles.(9) We assumed that the skin permeability of UV filters was higher in W/O than O/W emulsion, because the free UV filters were more in the outer phase.

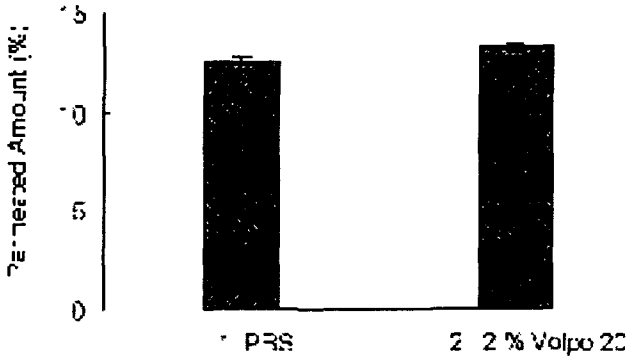

Figure 1 Permeated amount (%) of hydrocortisone across the excised hairless mouse skin using PBS and 2% Volpo 20 as receptor solutions

Table I. Total permeated amount (%) of UV filters from various preparations across the excised hairless mouse skin.

Preparation	Permeated Amount (%)	
	OMC	PBA
Oil solution	2.58	0.67
O/W	7.03	0.97
W/O	11.65	2.14

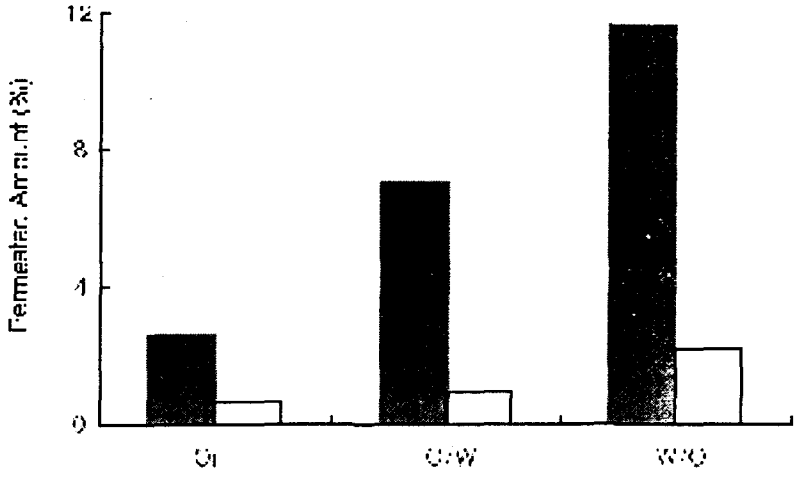

Figure 2 Total permeated amount (%) of UV filters 18h after application from various preparations. Shaded bars represent results of OMC, open bars represent results of PBA

Table 2. Distributed amount (%) of UV filters 18h after application to hairless mouse skin

Source	Distributed Amount (%)					
	OMC			PSA		
	Oil	O/W	W/O	Oil	O/W	W/O
Surface	92.05	90.01	85.22	94.57	97.78	94.51
Epidermis	[illegible]	1.33	3.86	0.38	0.8	1.86
Dermis	1.43	3.8[illegible]	6.29	0.22	0.38	0.56
Receptor	0.57	1.53	1.5	0.02	0.00	0.00
Total	95.62	97.01	96.64	95.16	98.76	96.85

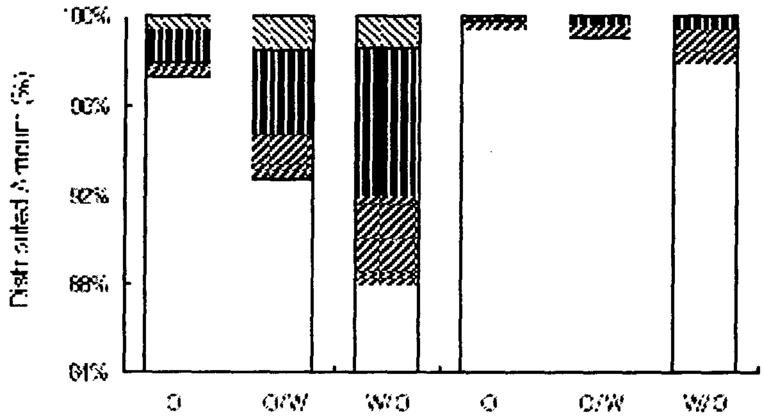

Figure 3. Distributed amount (%) of UV filters according to preparations
□ Surface ▨ Epidermis ■ Dermis ▧ Receptor

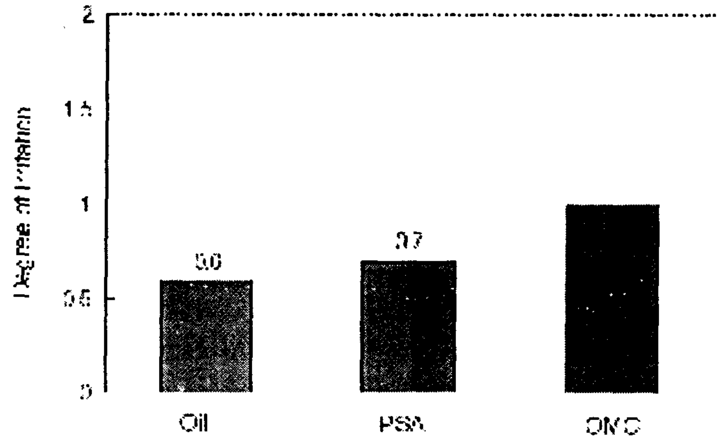

Figure 4. Skin primary irritation of UV filters
Oil: Caprylic capryl triglyceride, PSA: 20% PSA in oil, OMC: 20% OMC in oil.

Table 2 and Figure 3 demonstrate the distribution amount of UV filters in skin according to preparation. Amounts of UV filters determined in washes, epidermis, dermis and receptor 18 hours after sample application for capryric capryl triglyceride, O/W and W/O. In all cases, the amounts of OMC were much higher than those of PSA. Especially, the distributed amounts of OMC were higher in dermis than epidermis in all preparations. However, those of PSA were higher in epidermis than dermis. These results showed that fluxes of OMC were greater than those of PSA and the epidermis including stratum corneum was to be the large barrier of skin permeation of PSA.

Figure 4 shows the skin primary irritation of UV filters in caprylic capryl triglyceride. The skin primary irritation of PSA was lower than OMC. This result suggests that the skin primary irritation of UV filters increases with an increase of skin permeability, which was in good agreement with our previous report.(10)

Conclusion

In conclusion, the skin permeability and the skin primary irritation of PSA were lower than those of OMC. The skin permeability of UV filters was lower when they were in O/W than W/O, and the skin primary irritation showed the same tendency as it. We suggest that PSA could be a good candidate for sunscreen agent for reducing the skin irritation.

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