Can Increasing the Viscosity of Formulations be used to Reduce the Human Skin Penetration of the Sunscreen Oxybenzone?

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The effect of adding thickening agents on the penetration of a sunscreen benzophenone-3 through epidermal and a high-density polyethylene membrane was studied using both very thick (infinite dose) and thin (in use) applications. Contradictory results were obtained. Thickening agents retard skin penetration, in a manner consistent with a diffusional resistance in the formulation, when applied as an infinite dose. In contrast, when applied as in thin (in use) doses, thickening agents promote penetration, most likely through greater stratum corneum diffusivity arising from an enhanced hydration by the thicker formulations. The two key implications from this work are (i) a recognition of the danger in the potential extrapolation of infinite dosing to in use situations, and (ii) to recognize that thicker formulations may sometimes enhance the penetration of other topical agents when applied ''in use''. ***Key words: epidermis/finite dosing/hydration/infinite dosing. J Invest Dermatol 117:147–150, 2001***

The increased awareness of protection against skin cancer has led to a rise in the use of topically applied chemical sunscreen agents. The desirable site of action of these chemicals is restricted to the skin surface or within only the upper most layers of the stratum corneum. It has been demonstrated, however, that the skin penetration and retention of sunscreen agents from commercial products can differ significantly between the formulations used (Jiang *et al*, 1998). Sunscreens formulated as emulsions, dispersions of one immiscible liquid in another in the form of tiny droplets formed and stabilized by an emulsifier, are the most common topical commercial ultraviolet protectant products. In a recent clinical study up to 2% of an applied dose of the sunscreen agent oxybenzone (benzophenone-3, BP), including metabolites, was found to be excreted in the urine of volunteers applying a commercial emulsion formulation (Hayden *et al*, 1997).

One approach used to ensure a more controlled application of products, and the production of a uniform, thick, and effective sunscreen film on the skin, is to increase the viscosity of emulsion formulations. There are relatively few studies examining the effect of vehicle viscosity on cutaneous penetration following the application of finite or small ''in use'' doses of topical drug formulations. Tsai *et al* (1999) showed that the penetration of the alkaloid berberine through rat skin *in vitro* was inversely related to applied ointment viscosity. Potter *et al* (1999) recently showed that both the uptake of radiolabeled benzo[*a*]pyrene from topically applied oils into the blood and binding of metabolites to skin DNA was significantly less when the oil viscosity was higher. In this study we sought to determine whether increasing the viscosity of an emulsion formulation could also be used to retard the cutaneous penetration of sunscreens, using BP as a prototype. We sought to determine and compare the effect of viscosity on the *in vitro* penetration of BP from four different types of emulsion formulations, at the same thermodynamic activity, through both epidermal and high-density polyethylene (HDPE) membranes, allowing us to control for any possible vehicle–skin interactions. In addition, we examined the change in percutaneous penetration and retention kinetics of BP from the emulsions following infinite and finite dose application in an attempt to define the effects of viscosity on actual ''in use'' conditions (where factors such as formulation evaporation, estimated from the rate of vehicle water loss, would be expected to have a significant contribution to release kinetics).

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GENERAL MATERIALS AND INSTRUMENTATION

Materials BP and bovine serum albumin (fraction V) were supplied by Sigma-Aldrich Chemical Co. (Sydney, NSW, Australia). Other reagents included coconut oil (The Oil Garden, Brisbane, Australia), Tego care 450 (TH Goldschmidt AG, Essen, Germany), cetomacrogol emulsifying wax BP (David Craig and Co, Brisbane, Australia), and carbomer 940 (BF Goodridge, Avon Lake, Ohio). HDPE 20 μm membrane was donated by Beaver Plastics (QLD) Pty Ltd (Coopers Plains, QLD, Australia). High performance liquid chromatography grade methanol was used for high performance liquid chromatography analysis and all other chemicals used in the study were of analytical grade.

High performance liquid chromatography instrumentation This method has been described in detail elsewhere (Jiang *et al*, 1996).

METHODS

Preparation of emulsions Two oil-in-water emulsions were prepared using a conventional emulsifier (cetomacrogol emulsifying wax) or a new

Table I. Composition of the BP o/w emulsion formulations used in the study

Ingredient	Cream 1 %	Cream 2 %	Cream 3 %	Cream 4 %
Coconut oil	30	30	30	30
BP[a]	2	2	2	2
Cetomacrogol emulsifying wax	5	0	5	5
Tego care 450	0	5	0	0
Carbomer 940	0	0	0.2	0.5
Water	qs to 100	qs to 100	qs to 100	qs to 100

[a]Concentration at 50% of its solubility in the coconut oil phase.

lipid emulsifier (Tego care 450). A summary of the final composition of the emulsions used in the study is shown in **Table I**. Briefly, the oil phase (containing BP, final concentration 2% equating to 50% of its solubility, and the emulsifier) and the water phase were heated separately to 75°C and mixed together until cooled to room temperature. Sodium hydroxide (2 M) was the neutralization agent for the carbomer to give a pH for the final product of 6. Higher viscosity emulsions were prepared by the addition of Carbomer 940 to the aqueous phase of cetomacrogol emulsions before heating and mixing, to give a final concentration in the formulation of 0.2 or 0.5%.

Viscosity The comparative viscosity of emulsions was determined at a shear rate of 0.3 r.p.m. using a Brookfield viscometer (Brookfield Engineering Labs Inc., Stoughton, MA).

Formulation evaporation rate An estimation of the relative evaporation rates of water from each of the emulsions applied as a film to the forearm skin of a human volunteer were made using a trans-epidermal water loss (TEWL) meter (Tewameter 210, Courage and Khazaka Electronic GmbH, Cologne, Germany). Baseline TEWL readings were made on untreated forearm skin and an even film of each emulsion applied to separate areas and spread to identical thickness using a glass slide. TEWL readings were then taken over the application site until no further significant changes in water loss were noticed. Plots of TEWL (g per h per m^2) *vs* time (min) were plotted and slopes of the exponential decay curves fitted to the data used as an index of relative evaporation rate.

Membrane diffusion studies Human epidermal tissue (female abdominal) from cosmetic surgery was obtained by heat separation (Kligman and Christophers, 1963). After blunt dissection of the full-thickness skin, resultant epidermis was air dried and stored at –20°C until use. Epidermis was thawed at room temperature and HDPE membranes cleaned with distilled water before mounting between the donor and receptor chambers of horizontal Franz-type diffusion cells. The area available for diffusion was approximately 1.2 cm^2 and the receptor chamber volume approximately 3.4 ml. The receptor chambers were filled with 4% bovine serum albumin in phosphate-buffered saline, pH 7.4. Diffusion cells were equilibrated at 37°C ± 0.1°C for at least 1 h prior to application of the BP emulsions. Aliquots, 1 ml of emulsions for infinite dose studies and 3–4 mg per cm^2 for the finite dose study, were introduced into the donor chambers at t = 0. The receptor fluids were stirred throughout with magnetic fleas and samples (0.1 ml) were taken from the receptor chamber periodically (up to 6 h). Six replicates were used for the epidermis and four for the HDPE membrane. At the end of the diffusion studies, remaining solution in each donor chamber was wiped out using soft tissue and the membranes cleaned with tissue and distilled water. BP remaining within the membrane (μg) was determined by methanol extraction (recovery > 99%) with quantitation by high performance liquid chromatography. The flux of BP through the membranes into the receptor fluid from each of the emulsions was determined from slopes of plots of cumulative concentration in the receptor phase *vs* time and expressed as μg per h per cm^2. For finite doses, epidermal fluxes were estimated from the initial sections of the concentration–time plots before the profiles plateaued due to depletion of dose.

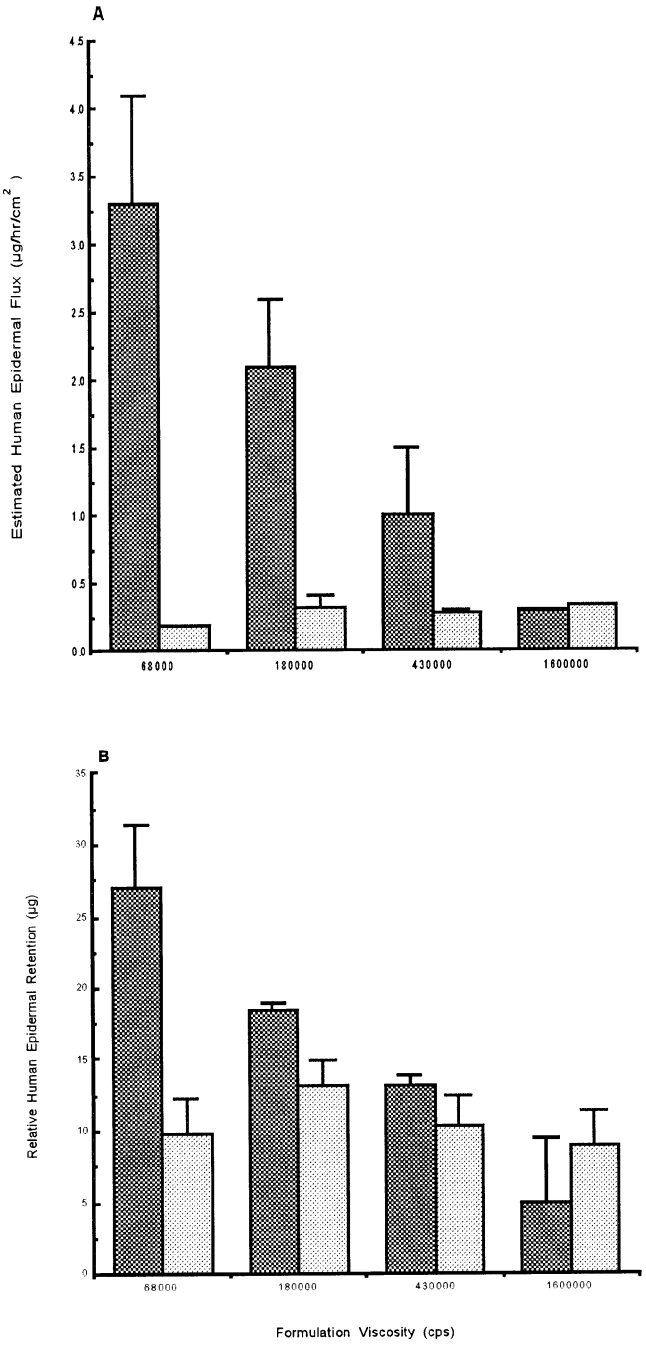

Figure 1. Epidermal flux and retention of BP. Relationships observed between (*A*) estimated human epidermal flux and (*B*) human epidermal retention and formulation viscosity (dark columns = infinite dosing; light columns = finite dosing), mean ± SD, n = 6.

RESULTS

Figure 1 shows the human epidermal flux and membrane retention for BP *vs* the viscosity of the formulations used. It is apparent that while the flux decreases with formulation viscosity for the very thick (infinite dose) formulation, the flux was increased over the control formulation with increasing viscosity for the very thin (finite or "in use" dose) (**Fig 1*A***). The epidermal membrane retention also decreases with viscosity for the infinite dose (**Fig 1*B***). In contrast, the epidermal membrane retention for the finite dose appears to be unaffected by the viscosity of the formulation used.

Table II. Physical properties and BP membrane penetration and retention characteristics for each of the emulsion formulations studied

Parameter	Cream 1 control	Cream 2 Tego care 450	Cream 3 0.2% Carbomer	Cream 4 0.5% Carbomer
Viscosity (cps)	68,000	180,000	430,000	1,600,000
Evaporation index*	0.249	0.165	0.148	0.127
Flux (μg per h per cm^2) ± SD				
HDPE—infinite	8.5 ± 2.1	8.0 ± 0.7	6.6 ± 0.7	3.7 ± 0.9
Epidermis—infinite	3.3 ± 0.8	2.1 ± 0.5	1.0 ± 0.5	0.3 ± 0.01
Epidermis—finite	0.18 ± 0.01	0.32 ± 0.09	0.28 ± 0.02	0.33 ± 0.01
Membrane retention (μg) ± SD				
HDPE—infinite	4.7 ± 0.9	3.8 ± 0.5	2.3 ± 0.3	1.7 ± 0.2
Epidermis—infinite	27.0 ± 4.4	18.4 ± 0.6	13.1 ± 0.8	5.0 ± 4.5
Epidermis—finite	9.8 ± 2.5	13.2 ± 1.7	10.3 ± 2.1	9.0 ± 2.4

[a]Exponential decay coefficient fitted to TEWL (g per h per m^2) *vs* time (min) plots.

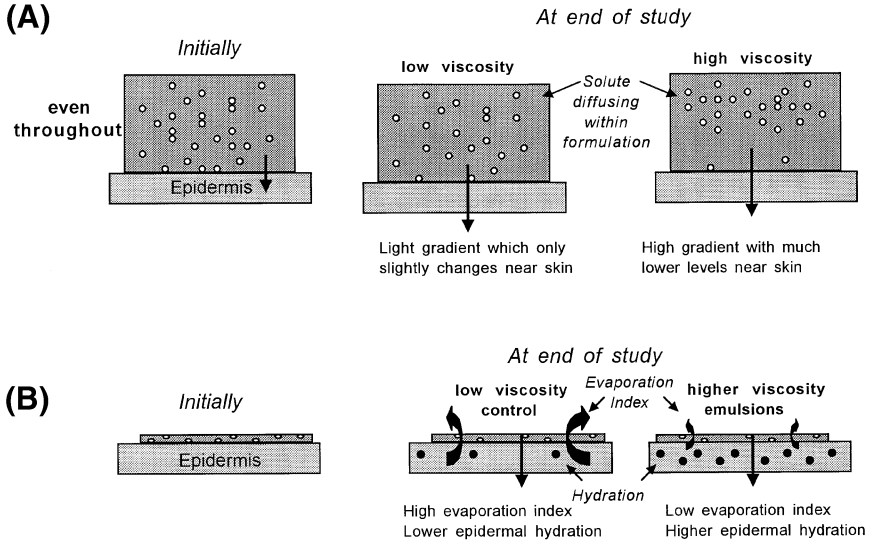

Figure 2. Contradictory effects of increasing the thickness of formulations on BP penetration through epidermal membranes using infinite and finite topical dosing. (*A*) Infinite does, thick formulation; (*B*) finite dose, thin "in use" formulation.

The human epidermal fluxes, membrane retention, and viscosities determined for each formulation used are shown in **Table II**. It is apparent that the viscosity of creams formulated with Tego care 450 emulsifier was higher than with Cetomacrogol wax and was further increased by adding carbomer 940. Also shown in **Table II** are the evaporation indices for each formulation, determined from the slopes of the exponential decay in evaporation rate for each formulation. This evaporation rate was highly correlated with the reciprocal of the formulation viscosity ($r^2 = 0.993$, $p < 0.01$). Also shown in **Table II** are the flux and membrane retention for BP when applied as an infinite dose. The penetration and retention profiles with viscosity are similar to that observed for human epidermal membranes.

DISCUSSION

This work suggests that the inclusion of thickening agents in topical formulations may have apparent contradictory effects on the skin penetration of the sunscreen agent BP when assessed using finite ("in use") and infinite (very thick application) dosing regimens. This effect was apparent for both human epidermal and plastic (HDPE) membranes. Concerns about using the more widely reported infinite dosing studies to represent "in use" membranes was raised in this journal more than two decades ago (Franz, 1978). Interestingly, our study emphasizes this concern in showing that whereas adding a thickening agent may decrease skin penetration when an infinite dose is applied, it actually facilitates penetration over control values for a finite dose.

The discrepancy in the infinite and finite dosing results are likely to arise from the differing diffusion of BP in the formulations and skin hydration arising in the two situations (**Fig 2**). In the finite ("in use") case, the residual film is unlikely to exert any significant resistance to penetration relative to the epidermal membrane barrier. Slower water evaporation, consistent with the water evaporation index (**Table II**), is likely to result in a higher water content in the residual film and an increase in skin penetration due to a higher diffusivity in a more hydrated membrane (Roberts and Walker, 1993). It is unlikely that the formulations have affected partitioning into the skin as epidermal retention for the four vehicles was similar (**Table II**). Cross and Roberts (2000) have recently shown that occlusion can cause significant increases in the diffusivity of solutes through an epidermal membrane depending on the type of vehicle occluded.

In contrast, solute diffusion through the formulation will become a significant resistance in the penetration process, and if the formulation is very thick (as in the infinite case) diffusion in the formulation becomes an even greater determinant of transport than epidermal membrane flux. Hence, as shown in **Table II**, the flux of BP through both human epidermal and plastic (HDPE) membranes decreases with increasing formulation viscosity. In this context, both macroviscosity and microviscosity contribute to the overall viscosity (Di Colo *et al*, 1980). Consistent with a formulation diffusion resistance limitation, **Table II** also shows that the membrane retention of BP in both membranes is lowest for the formulation with the highest viscosity. When the epidermal flux is

formulation diffusion controlled, the concentration at the absorption site into the skin will be lowest in the formulation showing the least BP diffusivity, i.e., the highest viscosity product and this lowest concentration is then in turn reflected by the lowest membrane retention for this formulation. It is possible that, in contrast to the finite dose formulations where the site of sunscreen action is in the upper layers of the stratum corneum, within the thicker layers of infinite dose formulations could be the site of action for these products.

The clinical implication from this study is that caution should be exercised in assuming that thicker formulations applied to the skin may retard the penetration of topically applied substances such as sunscreens. This work has shown that, whereas indeed thicker formulations impede the skin penetration of BP under infinite dosing conditions, thicker formulations may lead to faster skin penetration using thin, ''in use'' formulations. Further, this study highlights the dangers of trying to extrapolate infinite dose skin penetration data to finite, ''in use'' situations.

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