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Permeation Characteristics of 8-Methoxypsoralen Through Human Skin; Relevance to Clinical Treatment*

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Abstract

The permeation characteristics through human skin of 8-methoxypsoralen (8-MOP) and its physical attributes were investigated.

The log octanol/water partition coefficient and saturated aqueous solubility of 8-MOP at 32°C were 1·98 and 55·8 $\mu g\,mL^{-1}$ respectively. 8-MOP showed Fickian diffusion, with its flux being linearly related to the concentration of drug in the donor solution. The permeability coefficient of 8-MOP through human skin from different concentrations of aqueous solutions and a 2·6 $\mu g\,mL^{-1}$ bath lotion (as used in clinics) were statistically identical with mean values of $1{\cdot}76 \pm 0{\cdot}12 \times 10^{-2}$ and $1{\cdot}70 \pm 0{\cdot}32 \times 10^{-2}$ cm h^{-1} respectively ($P \geq 0{\cdot}05$). An ethanol/water (1 : 1 w/v) receptor solution did not improve the clearance of 8-MOP from the dermis when compared with an aqueous vehicle. Complete removal of the stratum corneum by tape stripping from full-thickness membranes produced a threefold increase in the flux of 8-MOP thus suggesting that the main barrier to 8-MOP permeation resides in the stratum corneum although the aqueous epidermal and dermal tissue provide a significant resistance to transdermal drug permeation. The equilibrium uptake of 8-MOP into psoriatic plaques and the 8-MOP aqueous/plaque partition coefficient were found to be more than twofold greater than for normal stratum corneum. The absorption of 8-MOP from the total applied topical dose (396 mg) was assessed as ~0·25% and only 2·5% of an oral dose, a significant reduction in the possible toxic hazard. The peak concentration of 8-MOP permeating through the skin was observed at about 35 min after limited exposure for 15 min.

Our results suggest that following a 15 min bath in the drug solution, there may be a need for an interval of about 20 min before patients are irradiated to ensure the optimization of photosensitizer with UVA irradiation (PUVA) therapy. Alternatively, UV irradiation could be applied at a lower flux over a longer time.

Photochemotherapy, involving the combined application of a photosensitizer with UVA irradiation (PUVA), has for long been utilized in the treatment of dermatological disorders such as psoriasis vulgaris, atopic eczema, vitiligo and leucoderma (El-Mofty 1948; Walter & Voorhees 1973; Kenney 1974). Psoralen derivatives such as 5-methoxypsoralen (5-MOP), trimethylpsoralen (TMP) and 8-methoxypsoralen (8-MOP) have been used in the treatment of psoriasis (Weber 1974; Fisher et al 1980). Originally, in the photochemotherapy of psoriasis with psoralens, ointment, cream or lotion was applied topically but all these dosage forms produced a patchy and persistent hyperpigmentation response and an uneven phototoxic response (Petrozzi et al 1977). This mode of treatment was therefore replaced with oral PUVA (Steiner et al 1978; Wagner et al 1979). Systemic psoralen therapy, however, has several shortcomings, such as the possibility of headache, nausea, a higher degree of systemic toxicity with possible damage to internal organs including the eyes and poor or impaired gastrointestinal absorption (Wagner et al 1979; Pham & Koo 1993). Topical PUVA therapy using dosage forms such as an emulsion, alcoholic gel (Neild & Scott 1982), ointment, cream and paint (Petrozzi et al 1977) has been described in the literature. These dosage forms, however, produced plasma levels of the same order as oral therapy thus providing no safety from the risks of systemic toxicity. Pham & Koo (1993) have recently reported minimal systemic absorption from 8-MOP paint in patients with palmoplantar psoriasis following localized application. This was estimated from plasma concentrations and actual amounts of the drug in tissues were not measured.

An alternative approach to topical PUVA with 8-MOP has been the delivery from bath water. In this method a gelatin capsule containing 8-MOP crystals or a commercially available lotion formulation is dissolved in large volumes of body-temperature bath water to provide a final concentration of 2·6–3·7 $\mu g\,mL^{-1}$ (Lowe et al 1986; Thomas et al 1991). The patient is then immersed in this solution for a time ranging from 5–15 min. The patient is then irradiated with a dose of UV light in the range of 330–400 nm followed by a shower to remove excess 8-MOP. Thomas et al (1991) have reported the clinical effectiveness of this treatment as well as undetectable plasma levels of 8-MOP following bath-water delivery. This mode of therapy thus has potential for long term treatment with its attendant need to minimize systemic toxicity.

Of the studies that have been reported, most have dwelt on the determination of plasma levels of 8-MOP following

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oral or bath-water delivery. Fewer have addressed the issue of 8-MOP penetration kinetics in the target tissue (Kammerau et al 1976; Schalla et al 1976; Gazith et al 1978). The studies reported now were therefore designed to investigate the fundamental penetration kinetics of 8-MOP into and its permeation through human skin from aqueous solutions and a commercially available lotion formulation (PUVASORALEN-8). The effects of different receptor solutions on 8-MOP clearance, and partitioning and uptake of 8-MOP into stratum corneum and psoriatic plaques were also studied. Other investigation involved the determination of relevant physical attributes of 8-MOP such as its saturated aqueous solubility and its octanol/water partition coefficient.

Materials and Methods

Materials

8-Methoxypsoralen (Fig. 1) was obtained from Aldrich Chemical Co., Gillingham, UK; 8-[Methoxyl-^{3}H]methoxypsoralen spec. act. 360 mCi mg^{-1} was obtained from Amersham International plc, Little Chalfont, UK. 8-MOP bath lotion, PUVASORALEN, 1·2% w/v containing Dehydol DS3 (30·0% w/v), Cetiol HE (34·0% w/v), Emulgin RO40 (20·0% w/v) and Texapon WW99 to 100% was obtained as a gift from Crawford Pharmaceuticals, Milton Keynes, UK. Caucasian abdominal skin was obtained post mortem and stored at −20°C before use (Harrison et al 1984).

Full-thickness membranes

Full-thickness human skin membranes were prepared by a method similar to that of Coldman et al (1969). Excess subcutaneous fat was trimmed off skin samples which were then clamped between stainless steel plates with the stratum corneum surface covered with a polythene sheet. The samples were refrozen at −20°C. After 2 h, the preparation was removed from the freezer and the top plate and polythene sheet were removed. The stratum corneum surface was gently thawed with a hot water bag until just movable to touch. The skin was cut using a Duplex electrodermatome to a thickness of about 430 μm consisting of the stratum corneum, epidermis and some of the dermis. The membrane was floated on 0·002% w/v aqueous sodium azide to hydrate for 72 h before use. Full-thickness membranes were prepared from 9 donors (6 male, 3 female) with a mean age of 60·3 ± 17·9 years.

Stripped full-thickness membranes

Samples of full-thickness membranes prepared as described above were stripped with adhesive tape (Clipper tape) to remove the stratum corneum. Usually 25–30 strippings were needed to remove the entire stratum corneum from the full-thickness membrane. Fully hydrated stratum corneum is about 30 μm thick such that when completely removed, stripped full-thickness membranes provide a thickness of approximately 400 μm.

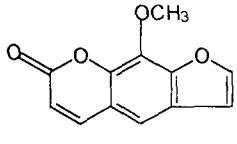

FIG. 1. Chemical structure of 8-methoxypsoralen.

Epidermal membranes

Epidermal membranes were prepared by the heat separation method (Kligman & Christophers 1963). Excess subcutaneous fat was removed from the skin which was then immersed in water at 60°C for 45 s. The epidermis was gently teased off the underlying dermis taking care not to unduly stretch the membranes. The epidermal membranes were hydrated in 0·002% w/v aqueous sodium azide for 72 h to ensure essentially full hydration of the stratum corneum before use. Epidermal membranes were prepared from a total of 19 donors (10 male, 9 female) with a mean age of 74·2 ± 7·5 years. Epidermal membranes provide a thickness of ~80 μm comprising 30 μm of fully hydrated stratum corneum and 50 μm of viable epidermis (Marks 1981).

Stratum corneum membranes

Stratum corneum membranes were prepared from epidermal membranes. The epidermal membranes were floated overnight on an aqueous solution of trypsin (0·0001% w/v) and sodium hydrogen carbonate (0·5% w/v) at 20 ± 1°C. The digested epidermal cells were removed by swabbing. The sheets of stratum corneum were rinsed in water and left to dry. The membranes were then rinsed in cold acetone for 10 s to remove contaminants such as sebaceous lipids and subcutaneous fat which adhere to the surface of the membranes during preparation. The clean stratum corneum membranes were stored in an evacuated desiccator until required. Sheets of stratum corneum membranes were prepared from 11 donors (2 male, 9 female), with a mean age of 70·6 ± 8·6 years.

Psoriatic plaques

These were obtained from the Dermatology Department of the General Infirmary at Leeds, UK and stored at −20°C before use. Samples from five donors (3 male, 2 female) with a mean age of 51·4 ± 17·1 years were used.

Determination of saturated aqueous solubility at 32°C

A solution, 12 μg mL^{-1} of 8-MOP in ethanol, was scanned from 190 to 400 nm in a Philips variable band-width UV/VIS spectrophotometer. The absorbencies of serial dilutions of the ethanolic solution were determined at λ max 219 nm. A calibration curve was plotted of the absorbance against concentration. A suspension of excess 8-MOP crystals in water was sonicated for 1 h with the temperature not exceeding 60°C and maintained at 32 ± 0·5°C for 72 h with constant stirring to equilibrate. The solution was filtered through a cellulose nitrate membrane filter (pore size, 0·1 μm; Whatman Ltd, UK) maintained at 32 ± 0·5°C and immediately diluted with water. The absorbance of the dilute solution was then determined at 219 nm and its concentration calculated using the calibration equation. Five replicates of the determination were performed.

Determination of the octanol/water partition coefficient

The octanol/water partition coefficient, $P_{oct/water}$, of 8-MOP was determined by the shake-flask method as described in

the British Pharmaceutical Codex using radiolabelled 8-MOP. The two phases were pre-equilibrated by saturating each with 5% v/v of the other, with shaking, and left to stand overnight. Two hundred microlitres (0·04 mCi) of an ethanolic solution of ^{3}H-labelled 8-MOP was evaporated under vacuum at 25°C and re-dissolved in 20 mL octanol phase. Twenty millilitres aqueous phase was added and the two liquids were mixed by vigorous hand shaking for about 5 min to ensure complete distribution of the drug between the two phases.

After standing for 48 h at 20 ± 1°C, the two phases were separated and centrifuged at 4500 rev min^{-1} for 10 min to ensure complete separation of any entrapped opposite phase. One millilitre of each phase was dissolved in 5 mL OptiPhase 'HiSafe' 3 scintillation fluid (LKB Scintillation Products Ltd, UK). The concentrations of 8-MOP in each phase were determined by scintillation counting on a Packard Tri-Carb Liquid Scintillation Analyser, 1600 Model TR in counts min^{-1}. The determination was performed in triplicate.

Preparation of radiolabelled drug solutions for diffusion and uptake experiments

Two millilitres of ethanolic tritium-labelled 8-[methoxyl-^{3}H]methoxypsoralen (0·4 mCi mL^{-1}) was evaporated under nitrogen. Thirty millilitres of saturated aqueous solution of cold 8-MOP was added to the tritium-labelled fraction. The solution was equilibrated at 32 ± 0·5°C with constant stirring for 48 h. The aqueous radiolabelled 8-MOP solution was serially diluted with water as required.

Ethanol was also evaporated from 1 mL radiolabelled 8-MOP and 20 mL 8-MOP bath lotion diluted to a concentration of 2·6 μg mL^{-1} was added to the radiolabelled drug. The solution was equilibrated at 32 ± 0·5°C with constant stirring for 48 h.

In-vitro diffusion experiments

In-vitro diffusion studies used an automated diffusion apparatus as described by Akhter et al (1984), consisting of metal flow-through cells, each with a diameter of 4 mm providing an effective diffusional area of 0·126 cm^2.

Full-thickness or epidermal membranes were floated for 72 h on 0·002% w/v aqueous sodium azide to ensure full hydration of the stratum corneum. The membranes were clamped between the two chambers of the metal cells and the cells mounted on a heated carrier arm which maintained the membrane temperature at approximately 32°C. The flow-through receptor rate was set at 2 mL h^{-1} and degassed receptor fluid was pumped continuously beneath the membrane with the aid of a peristaltic pump thus maintaining sink conditions. Either 0·002% w/v aqueous sodium azide, which has bactericidal properties, or ethanol/water (1 : 1 v/v) was used as receptor fluid.

Similar experiments as described above were performed with stripped full-thickness membranes (i.e. membranes from which the stratum corneum had been completely removed).

Aliquots (150 μL) of the donor drug solutions were applied to the membranes from the donor compartments which were covered with glass cover slips to prevent evaporation. For experiments in which steady-state data were required, the drug solution was left in contact with the membrane for the duration of the experiment, usually 8–10 h.

Finite dose experiments were also performed using epidermal membranes. Aliquots (150 μL) of the permeant, radiolabelled 8-MOP bath lotion (2·6 μg mL^{-1}) were applied and left in contact with the membranes for a total of 15 min, after which excess surface drug solution was removed.

Samples of receptor solution were collected at regular intervals, set on the multi-channel auto-reset timer, into vials containing 5 mL OptiPhase 'HiSafe' 3 scintillation fluid. Since the flow rate was constant, the volume of receptor collected in each vial was dependent on the time interval, such that, for samples collected at intervals of 10 min, the receptor volumes were 0·33 mL and those collected at 2 h intervals were 4 mL. Depending on the experiment, the samples of lower volumes were adjusted to 2 mL or 4 mL with appropriate receptor fluid to maintain a constant radiocounting channel ratio. The concentrations of 8-MOP in the receptor samples were determined using a Packard TriCarb 1600TR liquid scintillation analyser.

8-MOP uptake and skin/aqueous partitioning studies

Discs of dry stratum corneum membranes (1·3 cm^2 area) were cut, weighed and floated on a 0·002% w/v aqueous sodium azide solution for 72 h to hydrate. The hydrated stratum corneum discs were taken up flat on tissue paper, blotted dry, re-weighed and placed in near saturated solutions of radiolabelled 8-MOP (40 μg mL^{-1}; 0·29 mCi mL^{-1}) or bath lotion at the same concentration for times ranging from 10 s to 48 h. The stratum corneum discs were then removed, blotted dry to remove excess drug solution from the surface, weighed and dissolved in 1 mL Soluene-350 tissue solubilizer (Packard). Glacial acetic acid (100 μL) was added to each vial to neutralize the Soluene-350. Scintillation fluid (5 mL) was added to each vial which was then vortexed and left for 24 h for chemiluminescence to subside. The concentrations of 8-MOP in the membranes and donor drug solutions after partitioning were determined by liquid scintillation counting.

Because the clinically relevant concentration of 8-MOP is 2·6 μg mL^{-1}, equilibrium uptake and the stratum corneum/water partition coefficient $P_{sc/aq}$ were also determined using an aqueous solution and the bath lotion at 2·6 μg mL^{-1}. In the clinic, however, the patients are typically treated with drug for a maximum of 15 min before irradiation and so 8-MOP uptake into stratum corneum and partitioning were measured following 15 min contact between hydrated discs and aqueous drug solution and bath lotion, both at a concentration of 2·6 μg mL^{-1}. The procedure outlined above was repeated using hydrated psoriatic plaques to obtain equilibrium values following 24-h contact with the bathing drug solutions.

Data analysis

Following liquid scintillation counting, the octanol/water partition coefficient, $P_{oct/water}$, was calculated from the equation:

$$P_{oct/water} = \frac{C_{oct}}{C_{water}} \quad (1)$$

where C_{oct} is the concentration of 8-MOP in octanol in counts min^{-1} and C_{water} is the concentration of 8-MOP in water in counts min^{-1}.

The skin/water partition coefficient, $P_{skin/aq}$ for either stratum corneum or psoriatic plaques was calculated as the ratio of the concentration of drug in the stratum corneum or plaques measured as counts min^{-1} per dry weight of membrane to the concentration of drug in the bathing solution:

$$P_{skin/aq} = \frac{\text{amount of drug in membrane/dry weight}}{\text{concentration of drug in bathing solution}} \quad (2)$$

Under in-vitro experimental conditions of permeation, the membrane separates two compartments, a donor and a receptor with a concentration gradient existing between the two. Sink conditions prevail and after sufficient time has elapsed, the concentration of the diffusing molecule remains constant at all points in the separating membrane. The cumulative amount of drug permeating a unit area of the skin is calculated from the raw scintillation count data. A typical permeation profile for a drug diffusing through the skin in a simple zero-order situation is obtained by plotting the cumulative amount of the drug crossing unit area of the membrane against time. The steady state flux, J, which is defined as the rate of change of drug mass per unit area is determined from the slope of the linear portion of the plot. The permeability coefficient, K_p, can be calculated from the pseudo steady-state flux from the equation:

$$K_p = \frac{J}{C} \quad (3)$$

where C is the concentration of the applied donor solution. Extrapolation of the linear portion of the steady state plot to the time axis yields the lag time, L.

All statistical analyses used Student's t-test.

Results and Discussion

The aqueous solubility of 8-MOP at 32°C was 55·8 ± 0·932 $\mu g\,mL^{-1}$ (s.e.m., n = 5). The $P_{oct/water}$ was 95·03 ± 2·31 (n = 3); log $P_{oct/water}$ for 8-MOP is thus 1·98. In terms of skin transport, the partition coefficient of a drug and its aqueous solubility (Valvani & Yalkowsky 1980) influence

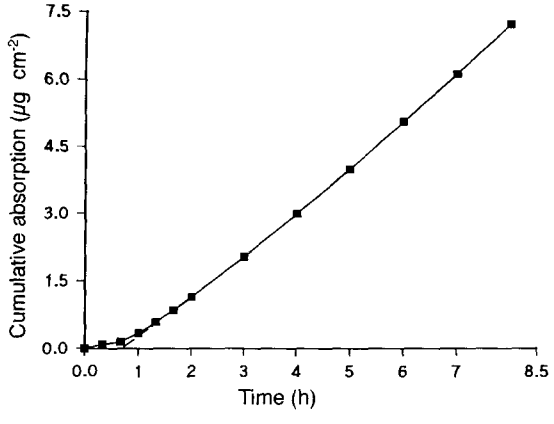

FIG. 2. A sample profile showing the cumulative absorption of 8-methoxypsoralen through epidermal membrane from a saturated aqueous solution.

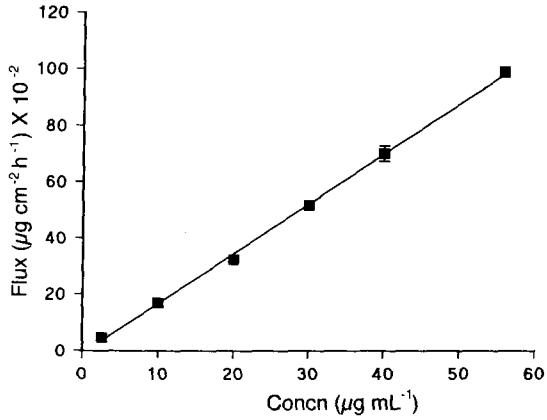

FIG. 3. Fickian behaviour of 8-methoxypsoralen.

the route it travels following topical application as well as its rate of absorption.

To investigate Fickian diffusion behaviour, the permeation of 8-MOP through epidermal membranes from aqueous solutions was measured. Fig. 2 shows a typical permeation profile from saturated aqueous solution. Fluxes increased with 8-MOP concentration; 8-MOP thus showed Fickian behaviour with a linear relationship (r = 0·9995) between flux and concentration (Fig. 3). The lag time varied from 30–100 min.

Table 1 summarizes permeability coefficients (K_ps) from aqueous solutions and the bath-lotion formulation diluted to the clinical concentration, 2·6 $\mu g\,mL^{-1}$. Permeability coefficients were similar, the mean being 1·72 ± 0·030 × 10^{-2} cm h^{-1} (n = 72) for the aqueous solutions and 1·70 ± 0·32 × 10^{-2} cm h^{-1} (n = 12) for the bath lotion. There was no significant difference between the fluxes from the bath lotion and aqueous solution at the same concentration. This suggests that the surface-active agents, at their respective concentrations; Texapon WW99 (32 $\mu g\,mL^{-1}$), Emulgin RO40 (43 $\mu g\,mL^{-1}$), Dehydol DS3 (65 $\mu g\,mL^{-1}$) and Cetiol HE (73·7 $\mu g\,mL^{-1}$) in the bath lotion at the clinically used level of 2·6 $\mu g\,mL^{-1}$ are not penetration enhancers.

There was limited information available on the permeation kinetics of 8-MOP. It was considered, however, that 8-MOP being a lipophilic drug (log P, 1·98), and relatively poorly water soluble (aqueous solubility, 55·8 ± 0·932 $\mu g\,mL^{-1}$, n = 5), may partition well into the stratum corneum but may not be removed efficiently by an aqueous receptor fluid from the more aqueous environment of the viable epidermis and dermis.

Table 1. A summary of 8-methoxypsoralen permeability coefficients (K_p) from aqueous solutions of different concentrations and bath lotion.

Solution	Concn ($\mu g\,mL^{-1}$)	$K_p \times 10^{-2}$ (cm h^{-1})
aqueous	55·8	1·77 ± 0·321
aqueous	40·0	1·75 ± 0·388
aqueous	30·0	1·72 ± 0·254
aqueous	20·0	1·61 ± 0·361
aqueous	10·0	1·68 ± 0·323
aqueous	2·6	1·79 ± 0·198
bath lotion	2·6	1·70 ± 0·320

Table 2. A summary of the permeation data for 8-methoxypsoralen from a saturated solution through different layers of human skin.

Membrane	Receptor solution	$K_p \times 10^{-2}$ (cm h^{-1})	Tissue resistance (h cm^{-1})	Number of replicates
Skin samples a–l				
Stratum corneum + epidermis	aqueous sodium azide (0·002% w/v)	1·77 ± 0·321	56·5 ± 0·852	60
	ethanol/water (1 : 1 v/v)	1·69 ± 0·230	59·2 ± 2·77	9
Skin samples m–u				
Full thickness stratum corneum + epidermis + dermis	aqueous sodium azide (0·002% w/v)	1·73 ± 0·206	57·8 ± 1·13	12
	ethanol/water (1 : 1 v/v)	2·11 ± 0·520	47·4 ± 3·64	12
Skin samples m–u				
Stripped full thickness epidermis + dermis	aqueous sodium azide (0·002% w/v)	5·49 ± 0·380	18·2 ± 0·434	9
	ethanol/water (1 : 1 v/v)	6·11 ± 0·335	16·4 ± 0·308	9

Values are mean ± s.e.m.

Using a less polar receptor could lead to better clearance as evident by a higher flux. The effect of two receptor solutions, aqueous sodium azide, 0·002% w/v and ethanol/water (1 : 1 v/v) on the permeation of aqueous 8-MOP through epidermal membranes was therefore assessed. Ethanol/water receptor solutions have been widely used to mimic in-vivo physiological perfusates. The results show no significant differences in the flux from a saturated aqueous solution through epidermal membrane using ethanol/water as receptor ($94·3 \pm 12·8 \times 10^{-2}$ $\mu g\,cm^{-2}\,h^{-1}$, n = 9) and 0·002% w/v aqueous sodium azide ($96·0 \pm 3·40 \times 10^{-2}$ $\mu g\,cm^{-2}\,h^{-1}$, n = 60) ($P \geq 0·05$). Thus, clearance would not pose a problem for 8-MOP delivery through the epidermal membrane.

The contributions of the various skin layers to the resistance to permeation was considered next (Table 2). The effects of the two receptor solutions on permeation through full-thickness skin from a saturated aqueous donor were investigated. The fluxes ± s.e.m. of 8-MOP using aqueous sodium azide and ethanol/water (1 : 1 v/v) were $96·5 \pm 11·5$ and $118 \pm 29·0 \times 10^{-2}$ $\mu g\,cm^{-2}\,h^{-1}$, respectively (n = 12), and were not significantly different ($P \geq 0·05$). These results thus suggest that clearance from nucleate epidermis and dermis in the in-vitro situation is not a problem.

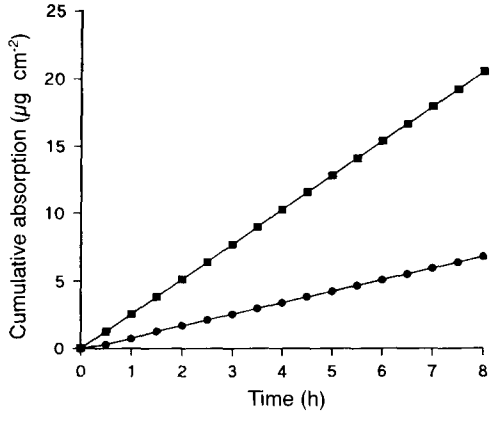

FIG. 4. Representative plots of cumulative absorption of 8-methoxypsoralen through stripped (■) and unstripped (●) full-thickness human skin using aqueous receptor fluid.

To investigate if the nucleate epidermis and dermis contribute significantly to the skin barrier, the permeation of through intact full-thickness and stripped full-thickness skin was compared with permeation through epidermal membranes (Fig. 4). Slopes differed and following stripping the lag time was undetectable.

If we regard whole skin as a laminate with each layer behaving as an isotropic medium, the total resistance is R_T. The resistance offered by the various layers is the reciprocal of the relevant permeability coefficient and thus (Barry 1983):

$$R_T = \frac{1}{K_p} = \frac{h_{SC}}{D_{SC}K_{SC}} + \frac{h_E}{D_E K_E} + \frac{h_D}{D_D K_D} \qquad (4)$$

where subscripts SC, E and D refer to the stratum corneum, viable epidermis and dermis respectively; h, D and K refer to the thickness of the membrane, the diffusion coefficient and partition coefficient in the different layers. Since the viable epidermis and dermis are both essentially aqueous phases and present a similar environment to the diffusing molecule, the resistance of the intact skin becomes:

$$R_T = \frac{h_{SC}}{D_{SC}K_{SC}} + \frac{h}{DK} \qquad (5)$$

where h equals $h_E + h_D$, $D = D_E = D_D$ and $K = K_E = K_D$. Since it is impractical to strip epidermal membranes, assessment of the enhancement ratio for 8-MOP achieved by complete removal of the stratum corneum used stripped full-thickness membrane (about 400 μm). Of the 80 μm provided by an intact epidermal membrane, 30 μm represents the fully hydrated stratum corneum; the viable epidermis is thus 50 μm, representing about one eighth of the thickness provided by stripped full-thickness membrane. The resistance of the intact epidermal membrane can thus be represented as:

$$R = \frac{h_{SC}}{D_{SC}K_{SC}} + \frac{h}{8DK} \qquad (6)$$

There is no significant difference between the resistances of epidermal and full thickness membranes ($P \geq 0·05$). The resistance of intact full-thickness skin is however significantly higher than that of stripped full-thickness skin ($P \geq 0·05$, see Table 2). This indicates that the main barrier to permeation is the intact stratum corneum. Fluxes through

epidermal membranes ($95{\cdot}6 \pm 3{\cdot}40 \times 10^{-2}\,\mu g\,cm^{-2}\,h^{-1}$) and unstripped full-thickness membranes ($96{\cdot}5 \pm 11{\cdot}5 \times 10^{-2}\,\mu g\,cm^{-2}\,h^{-1}$) using an aqueous receptor, are not significantly different ($P \geq 0{\cdot}05$). This most likely resulted from the variability of human skin as different skin samples were used (Table 2). However, there is a threefold difference between the fluxes, $96{\cdot}5 \pm 11{\cdot}5 \times 10^{-2}$ and $306 \pm 21{\cdot}2 \times 10^{-2}\,\mu g\,cm^{-2}\,h^{-1}$ through unstripped and stripped full-thickness membranes respectively using aqueous receptors.

Williams & Barry (1991) observed a five-fold increase in the flux of β-oestradiol through stripped full-thickness membrane as compared with intact full-thickness membrane. If we apply the same concept to 8-MOP, we can consider this increase in flux of 8-MOP from stripped full-thickness membrane as the enhancement ratio for 8-MOP provided by tape stripping.

If we assume that the viable epidermis and dermis present similar resistances to a lipophilic molecule, the permeability coefficient will be about 8 times greater through 50 μm of viable epidermis than 400 μm of viable epidermis and dermis. The maximum enhancement ratio for 8-MOP across epidermal membranes with the stratum corneum severely damaged or removed will be 24. Applying the enhancement ratio concept to β-oestradiol and 5-fluorouracil, Williams & Barry (1991) found the maximum enhancement ratios to be 42 and 8400, respectively. They considered that for hydrophilic 5-fluorouracil, the unmodified permeability coefficient through intact epidermal membranes is low and thus has greater potential for increase on stratum corneum removal whereas lipophilic β-oestradiol already has a comparatively higher permeability coefficient and thus has lower scope for increase following damage to the stratum corneum. This concept clearly applies to lipophilic 8-MOP.

Diffusivity is estimated assuming that the stratum corneum is isotropic and that in determining the stratum corneum/aqueous partition coefficient, the whole stratum corneum volume was used. In the absence of geometric considerations (neglecting tortuosity considerations), the apparent diffusion coefficient, D, can be calculated from lag-times (eqn 7) or fluxes (eqn 8).

$$D_{app} = \frac{h^2}{6L} \tag{7}$$

$$D_{app} = \frac{J_{ss}h}{K_m C} \tag{8}$$

where L is lag time, J_{ss} is steady-state flux, K_m is the membrane/vehicle partition coefficient and C is concentration. The apparent diffusivity of 8-MOP assuming a stratum corneum thickness of 30 μm is $3 \times 10^{-6}\,cm^2\,h^{-1}$ calculated from mean lag time of 30 min ($n = 72$) and $2{\cdot}12 \times 10^{-6}\,cm^2\,h^{-1}$ when calculated from steady-state flux. Thus for 8-MOP, the two approaches provide agreement as would be expected for a lipophilic molecule.

Most dermatological products are applied in finite doses such that data obtained from steady-state in-vitro experiments may not accurately mirror the in-vivo situation. With 8-MOP, the patient may be immersed in a bath water containing $2{\cdot}6 \times 10^{-2}\,\mu g\,mL^{-1}$ for 15 min. We therefore designed a finite dose in-vivo mimic experiment in which 150-μL aliquots of solution of 8-MOP bath lotion at $2{\cdot}6 \times 10^{-2}\,\mu g\,mL^{-1}$ were applied to epidermal membranes.

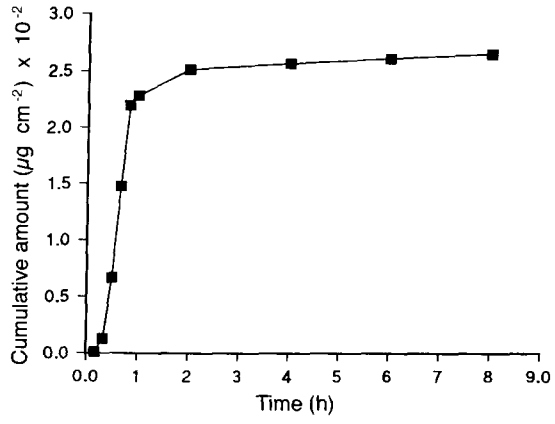

FIG. 5. Cumulative amounts of 8-methoxypsoralen obtained following a finite dose application through epidermal membrane measured as a function of time.

Following 15-min contact time, the solution was removed and membranes were exposed to ambient conditions while receptor samples were collected (Fig. 5).

The maximum cumulative amount of 8-MOP permeating across the epidermis was $\sim 0{\cdot}02\,\mu g\,cm^{-2}$ compared with $0{\cdot}15\,\mu g\,cm^{-2}$ obtained by Schalla et al (1976) 30–300 min post-application using a 1% ointment.

Fig. 6 presents instantaneous fluxes plotted at midpoint times and values calculated similarly from steady-state experiments. The finite-dose fluxes increased to a maximum of $\sim 2{\cdot}5 \times 10^{-2}\,\mu g\,h^{-1}$ at ~35 min post-application. Subsequently, fluxes dropped rapidly as drug depletes. The maximum flux was similar to that from infinite dose application for the same time. This most likely results from the natural variability between skin samples.

Since patient exposure to 8-MOP is limited to 15 min, it is useful to measure the drug in the stratum corneum at this time. Hydrated discs of stratum corneum were therefore incubated in triplicate with near-saturated aqueous solutions of 8-MOP ($40\,\mu g\,mL^{-1}$) (Fig. 7). The mean uptake at equilibrium and $P_{sc/aq}$ were $1{\cdot}01 \pm 0{\cdot}248\,mg\,g^{-1}$ and $25{\cdot}3 \pm 6{\cdot}20$ respectively. For comparison, uptake from the same concentration of the lotion formulation was also measured. The trend for both treatments was similar. The

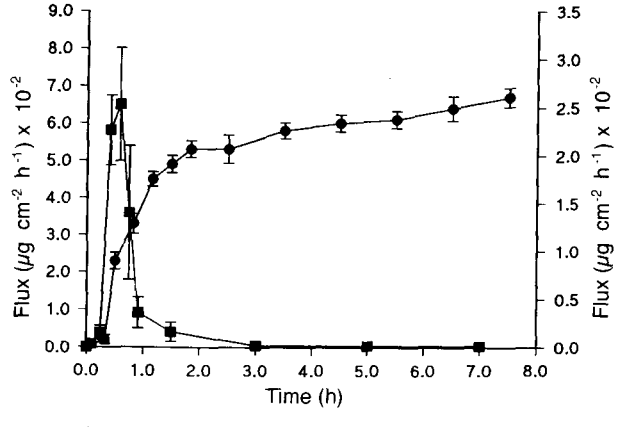

FIG. 6. Plots of 8-methoxypsoralen flux with time following infinite dosing (●) and finite drug dose application (■).

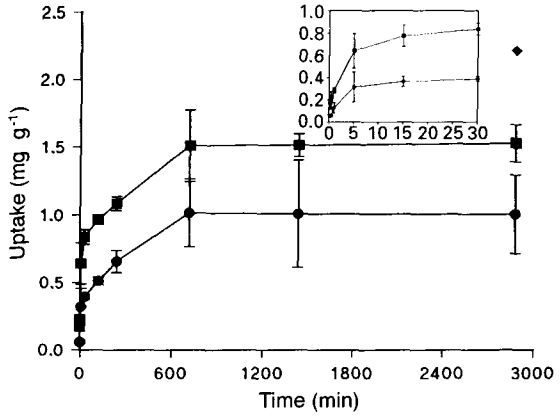

FIG. 7. Uptake with time of 8-methoxypsoralen into stratum corneum from a near saturated aqueous solution (40 μg mL^{-1}) (●), lotion formulation at the same concentration (■) and equilibrium uptake into psoriatic plaque (◆). Insert is the same graph showing uptake at short contact times up to 30 min.

mean uptake of at equilibrium and $P_{sc/aq}$ from the lotion formulation were $1{\cdot}52 \pm 0{\cdot}231$ mg g^{-1} and $38{\cdot}0 \pm 5{\cdot}78$.

8-MOP bath lotion is, however, used clinically at a much lower concentration, 2·6 μg mL^{-1}. The uptake at equilibrium and $P_{sc/aq}$ at this strength were therefore also determined and were $6{\cdot}53 \pm 0{\cdot}639 \times 10^{-2}$ mg g^{-1} and $25{\cdot}1 \pm 2{\cdot}45$, respectively (Table 3).

The partition coefficient is thus not significantly different ($P \geq 0{\cdot}05$) than from a near-saturated aqueous solution but the amount of drug taken up from the bath lotion at $2{\cdot}6 \pm \mu$g mL^{-1} is significantly lower because of the differences in the concentrations. The uptake from aqueous drug solution and bath lotion of concentration 2·6 μg mL^{-1} following 15-min contact were $1{\cdot}56 \pm 0{\cdot}367 \times 10^{-2}$ (n = 3) and $1{\cdot}71 \pm 0{\cdot}493 \times 10^{-2}$ mg g^{-1} (n = 3), respectively.

In a typical psoriatic patient, parts of the skin are diseased while others remain uninvolved. Since an aim of this study was to provide clinically relevant kinetic data, the equilibrium uptake into psoriatic plaques and partition coefficient ($P_{pp/aq}$) were also determined. The mean uptake and $P_{pp/aq}$ from an aqueous solution (2·6 μg mL^{-1}) were $14{\cdot}3 \pm 1{\cdot}65 \times 10^{-2}$ mg g^{-1} and $55{\cdot}0 \pm 6{\cdot}35$, respectively. From the bath lotion at the same concentration, the mean uptake into psoriatic plaques and $P_{pp/aq}$ were $14{\cdot}4 \pm 1{\cdot}20 \times 10^{-2}$ mg g^{-1} and $55{\cdot}4 \pm 4{\cdot}62$, respectively. Results from aqueous solution and bath lotion are not significantly different ($P \geq 0{\cdot}05$). Comparing these data with those obtained for stratum corneum shows a more than two-fold significant increase in the partition coefficient and uptake of 8-MOP into psoriatic plaques ($P \geq 0{\cdot}05$).

In psoriasis, stratum corneum is damaged and epidermal intercellular spaces are larger than in healthy skin. The barrier properties are thus compromised. A possible way of explaining the higher amount of drug taken up by psoriatic plaques is to consider the physical nature of the plaques. The plaques are spongy, unlike normal stratum corneum. The water content of the plaques may be higher and thus the amount of 8-MOP dissolved within the intercellular spaces may be higher.

The maximum flux and therefore the maximum concentration of 8-MOP at the basal layer of the epidermis, assumed to be the site of drug action, following a 15-min finite dosing with a 2·6 μg mL^{-1} drug solution was achieved at about 35 min from time of application (Fig. 6). The clinical treatment regimen is such that patients towel off excess surface drug after 15 min contact and are immediately irradiated. A conservative estimation, if it takes patients an average of 1–2 min to dry their body, puts the time of irradiation at 16–17 min from the time of first drug exposure. Our study suggests that the drug is not at its optimal concentration in the target tissue at 16–17 min. Neither 8-MOP nor UV light alone are beneficial in psoriasis treatment (Weber 1974; Morison 1991). Any drug reaching the epidermis before or after irradiation is not primed for binding with DNA and only contributes to the body's toxic load. Since the goal of PUVA therapy is to maximize the use of photosensitizer and UV light, we suggest that following bathing in drug solution, there should be a delay of about 20 min before patients are irradiated with UV light. Alternatively, instead of the usual dose of UV light applied for about 2 min post-bathing, a lower irradiation flux could be given over a longer time such as 20 min selected to cover as much of the peak time as possible.

The total body load of a drug can be estimated as the sum of the amount entering the stratum corneum and that removed into the systemic circulation. From in-vitro experiments this latter amount is the cumulative amount in the receptor solution. At short times, the amount entering the stratum corneum can be estimated from steady state as (Cleek & Bunge 1993):

$$M = 2AP_{sc/aq}C\sqrt{\frac{Dt}{\pi}} \qquad (9)$$

where M is the amount absorbed, D is the diffusivity (eqn 8),

Table 3. A summary of results of 8-methoxypsoralen partition coefficient and uptake (± s.e.m.) into stratum corneum and psoriatic plaque.

Solution	Tissue	Determination	Uptake × 10^{-2} (mg g^{-1})	Partition coefficient
Aqueous (40 μg mL^{-1})	Stratum corneum	Equilibrium	101 ± 24·8	25·3 ± 6·20
Bath lotion (40 μg mL^{-1})	Stratum corneum	Equilibrium	152 ± 23·1	38·0 ± 5·78
Bath lotion (2·6 μg mL^{-1})	Stratum corneum	Equilibrium	6·53 ± 0·639	25·1 ± 2·45
Aqueous (2·6 μg mL^{-1})	Psoriatic plaque	Equilibrium	14·3 ± 1·65	55·0 ± 6·35
Bath lotion (2·6 μg mL^{-1})	Psoriatic plaque	Equilibrium	14·4 ± 1·20	55·4 ± 4·62
Aqueous (2·6 μg mL^{-1})	Stratum corneum	15 min	1·56 ± 0·367	—
Bath lotion (2·6 μg mL^{-1})	Stratum corneum	15 min	1·71 ± 0·493	—
Bath lotion (2·6 μg mL^{-1})	Psoriatic plaque*	15 min	5·39	—

*Estimated value.

t is time, A is surface area, $P_{sc/aq}$ is the partition coefficient between stratum corneum and vehicle and C is the concentration, assumed to remain constant.

The estimated amount of 8-MOP entering the stratum corneum from a 2·6-$\mu g\,mL^{-1}$ solution at 15 min was $0{\cdot}672 \times 10^{-2}\,\mu g\,cm^{-2}$. In a diffusion study and clinically, only one side of the membrane is exposed to the drug solution and molecules move unidirectionally along the concentration gradient. In an uptake experiment, however, molecules move in two directions until equilibrium is reached such that the amount of drug in the membrane is twice that obtained in a diffusion study. Taking this into consideration, the amount of 8-MOP entering the stratum corneum from an uptake experiment (Table 3) in which membranes were incubated with a solution of the same concentration ($2{\cdot}6\,\mu g\,mL^{-1}$) for the same time (15 min) was calculated by halving the uptake value and converting the units from $mg\,g^{-1}$ to $\mu g\,cm^{-2}$. A value of $2{\cdot}57 \times 10^{-2}\,\mu g\,cm^{-2}$ was obtained which thus overestimates by 3·7 the value derived from steady-state parameters. The accurate determination of drug uptake at short contact times is difficult as any excess unremoved surface drug is more likely to overestimate the uptake at short treatment times compared with longer times such as near equilibrium. Once again, skin variability may have played a role.

The total body load following 15-min contact using bath lotion at $2{\cdot}6\,\mu g\,mL^{-1}$ can thus be estimated from steady-state diffusion as a best case scenario (minimum) and from uptake studies as the highest amount that can be delivered. Assuming the body area for an average 70 kg man to be $1{\cdot}73\,m^2$ (Haycock et al 1978) with the head $\sim 0{\cdot}13\,m^2$, and if the body excluding the head is immersed in drug solution, the area available for diffusion is about $1{\cdot}6\,m^2$. The body load of 8-MOP can thus be estimated for three situations: the patient is completely cured, the entire skin is psoriatic, or part is diseased and the remainder healthy.

Case 1. Totally healthy skin

Based on an area of $1{\cdot}6\,m^2$, the body load of 8-MOP is the sum of the amount entering the stratum corneum and that in the receptor solution in 15 min following finite dosing which is $0{\cdot}152 \times 10^{-2}\,\mu g\,cm^{-2}$. From the steady-state method, the amount entering the stratum corneum in 15 min was $0{\cdot}672 \times 10^{-2}\,\mu g\,cm^{-2}$ and from our uptake studies, $2{\cdot}57 \times 10^{-2}\,\mu g\,cm^{-2}$. The minimum and maximum body load of 8-MOP for a totally healthy skin can thus be calculated as 0·132 mg and 0·436 mg respectively (Table 4).

Case 2. Totally diseased skin

It is usually assumed that the barrier properties of skin in dermatoses are compromised. Zackeim et al (1977) found nearly a 3-fold increase in the absorption of 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine) from involved skin in *mycosis fungoides* compared with uninvolved skin. In stable psoriatic plaque, Wester et al (1983), however, found no difference in the absorption of hydrocortisone from normal and involved skin. Schaefer & Schalla (1986) reported a twofold increase in penetration of desoxymethasone through psoriatic skin compared with normal skin. Because of the limited availability of psoriatic skin, we were unable to obtain kinetic data for 8-MOP through psoriatic skin in-vitro. We could, however, measure uptake of 8-MOP at equilibrium into psoriatic plaques. Plaques are fragile so we did not attempt to measure uptake with time. They took up about twice as much 8-MOP as healthy stratum corneum. Amount of drug in stratum corneum at equilibrium was about 4 times that following 15 min contact. If the same applies to plaques, we can estimate the amount in plaques after 15 min exposure by halving the uptake value and converting units; the estimate is $\sim 5{\cdot}39 \times 10^{-2}\,\mu g\,cm^{-2}$.

Stripping mimics damage done by psoriasis in human skin (Lamaud & Schalla 1984). In-vivo, migrating molecules encounter the capillary system within 200 μm from the skin surface which is thus the effective thickness for diffusion. If the entire 30 μm of stratum corneum is removed and we assume that viable epidermis and dermis present similar resistances to a lipophilic drug such as 8-MOP, the permeability coefficient of 8-MOP would be about 3·4 times greater through 50 μm of viable epidermis than 170 μm of epidermis and dermis. We actually found a three-fold decrease in the diffusional resistance offered by stripped skin to 8-MOP.

Applying these concepts to severe psoriasis, we estimate the amount of drug passing through into the systemic circulation after 15 min from equation 3 to be $3{\cdot}58 \times 10^{-2}\,\mu g\,cm^{-2}$. We can determine the amount of 8-MOP in psoriatic plaques following 15-min exposure to a drug solution of $2{\cdot}6 \times 10^{-2}\,\mu g\,mL^{-1}$ from equation 9; this was $1{\cdot}76 \times 10^{-2}\,\mu g\,cm^{-2}$. From our uptake data, the amount in psoriatic skin after the same time would be $5{\cdot}39 \times 10^{-2}\,\mu g\,cm^{-2}$. As with healthy tissue, we can take these values to be the minimum and maximum amounts per unit area of plaque after 15-min contact. We can therefore calculate the total body load of 8-MOP for psoriatic skin; as with healthy stratum corneum, this would be $(3{\cdot}58 \times 10^{-2} + 1{\cdot}76 \times 10^{-2}\,\mu g\,cm^{-2})$ as the minimum and $(3{\cdot}58 \times 10^{-2} + 5{\cdot}39 \times 10^{-2}\,\mu g\,cm^{-2})$ as the maximum load per unit area of totally diseased skin. Assuming a body area of $1{\cdot}6\,m^2$, the minimum and maximum body load of 8-MOP would thus be $\sim$0·854 mg and 1·44 mg respectively.

Case 3. Partially diseased with healthy skin

In reality, a patient with severe psoriasis has as much as 50% of skin surface diseased (Turowski et al 1980; Neild & Scott 1982). A patient receiving bath PUVA would have involved and uninvolved skin contacting the drug solution. We can estimate the total body load of 8-MOP in a patient with 50% involved skin at the start of treatment. The minimum and maximum load would be $\sim$0·493 mg and 0·938 mg respectively. Assuming a steady recovery measured by decrease in fraction of body area remaining involved, a simple scheme for body load as a function of this area is shown in Fig. 8.

Table 4. A summary of estimates of 8-methoxypsoralen body load for healthy and diseased skin calculated on the basis of $1{\cdot}6\,m^2$ body area following exposure for 15 min.

Skin type	Minimum load (mg)	Maximum load (mg)
Totally healthy	0·132	0·436
Totally psoriatic	0·854	1·44

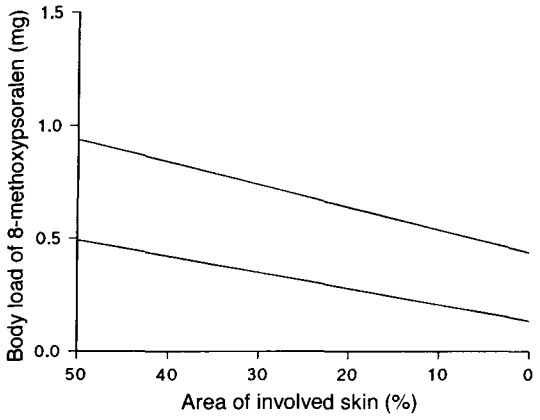

FIG. 8. A graph showing the minimum (lower profile) and maximum (upper profile) estimated body load of 8-methoxypsoralen as a function of area of psoriatic skin.

In oral PUVA, an average of 0·6 mg kg^{-1} body weight of 8-MOP is administered 1–2 h before irradiation. The plasma concentration is low and little diffuses into skin. If we estimate oral bioavailability (Schalla et al 1976) as at least 95%, in a 70-kg man receiving about 40 mg 8-MOP, much of this contributes to the systemic adverse effects associated with oral 8-MOP. Comparatively, if the patient receives bath PUVA therapy in which total applied dose is 396 mg (33 mL 1·2% lotion diluted to 140 L), maximum amount of drug absorbed if 50% of skin is diseased is 0·938 mg, i.e. ~0·24% of total applied dose and only 2·5% of an oral dose, a significant decrease in toxic hazard. Calzavara-Pinton et al (1994) studied safety and effectiveness of oral and bath water 8-MOP delivery. Their results clearly point to the advantages of bath water 8-MOP delivery, as to achieve the same therapeutic effect, there was a reduction in the cumulative UVA doses applied as well as a decrease in the number of exposures in patients who received bath-PUVA compared with those on oral therapy.

In conclusion, therefore, we recommend that to minimize the risk of systemic side effects associated with 8-MOP PUVA therapy in psoriatic patients, topical PUVA using bath lotion should be considered over oral therapy. Following a 15 min bath in drug solution it would be worthwhile investigating clinically to see if an interval of about 20 min before irradiation would ensure optimization of PUVA therapy. Alternatively, UV irradiation could be applied at a lower flux over a longer period of time.

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