J. Pharm. Pharmacol. 2000, 52: 487–494
Received November 17, 1999
Accepted January 11, 2000

Investigation of the Absorption of Hypericin into the Skin of Hairless Mice

APPOLINARY R. KAMUHABWA, KAREL R. GEBOES* AND PETER A. DE WITTE

*Laboratorium voor Farmaceutische Biologie en Fytofarmacologie, Faculteit Farmaceutische Wetenschappen and *Laboratorium voor Histochemie en Cytochemie, Faculteit Geneeskunde, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

Abstract

The skin absorption of hypericin was evaluated in hairless mice to develop an optimised hypericin topical formulation that could be used in the clinical study of psoriasis.

Hypericin (0·01–1·0%) in Beeler basis, polyethylene glycol ointment, carbopol gel, cetomacrogol cream, petrolatum or emulsifying ointment, with and without skin-absorption enhancers (isopropylidene glycerol and diethylene glycol monoethyl ether), was tested in-vivo on hairless mice skin. Using a skin-stripping technique and the intrinsic fluorescence of hypericin under standardised UV_{365} irradiation, it was demonstrated that the absorption of hypericin very much depended on the vehicle used. The concentrations of hypericin in the skin were then estimated by HPLC analysis. For this purpose, two vehicles were employed, with which hypericin penetrated the skin of hairless mice well (emulsifying ointment with isopropylidene glycerol) or very poorly (polyethylene glycol ointment). In the case of emulsifying ointment with isopropylidene glycerol (0·05% hypericin), a substantial concentration of hypericin ($8{\cdot}6 \pm 3{\cdot}2\,\mu g\,g^{-1}$) (mean ± s.d., n = 5) was found in the skin. With polyethylene glycol ointment, however, only a limited hypericin skin concentration ($0{\cdot}38 \pm 0{\cdot}34\,\mu g\,g^{-1}$, n = 5) was achieved.

These results show that emulsifying ointment with polyethylene glycol holds promise as an effective topical vehicle for the treatment of skin diseases, such as psoriasis, with hypericin.

Hypericin (Figure 1), a hydroxylated phenanthroperylenequinone derivative, is a constituent of a number of plants of the genus *Hypericum*. Hypericin is a potent photosensitizer and its photosensitising properties exhibit interesting biological activity both in-vitro and in-vivo. The compound is under consideration as a potential tool in photodynamic therapy (Diwu & Lown 1994). Hypericin also has a high fluorescence yield, so the presence of hypericin in body tissues can be easily visualised. In vehicles or solvents, the intensity of fluorescence indicates the degree of solubility of the compound (Yamazaki et al 1993; Lavie et al 1995).

Some data suggest that photoactivated hypericin might be a potential antipsoriatic agent. For instance, it has been shown that the photoactivated compound exhibits a powerful in-vitro inhibition of the enzymatic activity of casein kinase 2 (CK-2; a Ser/Thr kinase) (Agostinis et al 1995) and epidermal growth factor (EGF; a tyrosine kinase) receptor (de Witte et al 1993; Agostinis et al 1996). It has been suggested that both of these enzymes are involved in the aetiology of psoriasis (Nanney et al 1986; Mitev et al 1992). Moreover, epidermal hyperproliferation is a prominent feature of psoriasis, and photoactivated hypericin has been reported to exhibit antiproliferative effects on different human cell lines (Ritcher & Davies 1995; Ugwu et al 1998).

Thus far, only a few photosensitizers such as 5-aminolevulinic acid (Szeimies et al 1994) have been employed topically for the photodynamic therapy of skin diseases, probably because of the limited skin penetration of the compounds. The possibility of using hypericin, a natural photosensitizer, topically for dermatological purposes has not been explored. Consequently, as a first step

Correspondence: P. A. de Witte, Laboratorium voor Farmaceutische Biologie en Fytofarmacologie, Faculteit Farmaceutische Wetenschappen, Van Evenstraat 4, B-3000 Leuven, Belgium. E-Mail: peter.dewitte@farm.kuleuven.ac.be

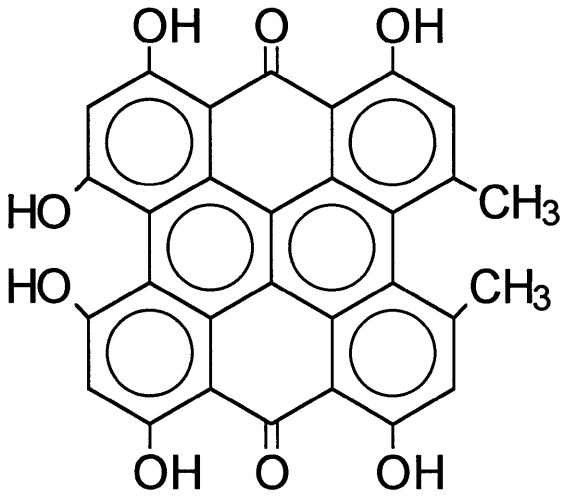

Figure 1. The chemical structure of hypericin.

this study was undertaken to evaluate and optimise the in-vivo absorption of hypericin in hairless mice skin by using topical formulations with different compositions. By utilising the intrinsic fluorescence of hypericin, a skin-stripping technique was employed as a straightforward way of screening hypericin topical vehicles. Some results obtained with the skin-stripping technique were quantitatively confirmed using HPLC. The results show that hypericin incorporated in a suitable vehicle is absorbed by the skin of hairless mice.

Materials and Methods

Hypericin

Hypericin was synthesised as described by Falk et al (1993). Briefly, 2·5 g of emodin (isolated from cortex frangulae) was dissolved in 125 mL acetic acid and refluxed. $SnCl_2.2H_2O$ (30·0 g) dissolved in 75 mL conc. HCl was added. After 2 h, the flask was cooled at room temperature and the emodin anthrone that was formed was filtered off and dried in vacuum (yield 2·0 g; 80%). Emodin anthrone (2·0 g) was heated in a mixture of pyridine/piperidine (10 : 1, v/v), 4·0 g pyridine 1-oxide, and 0·1 g $FeSO_4.7H_2O$ at 110°C under nitrogen and in darkness. Crude protohypericin was obtained after precipitation (by adjusting the solution to pH 4 with HCl) and centrifugation. The crude mixture was then further purified by silica chromatography (Merck, 0·063–0·200 mm) with ethylacetate/water (100 : 2·5, v/v), followed by ethylacetate/acetone/water (80 : 20 : 2·5, v/v) as eluent.

Protohypericin was dissolved in acetone and converted to hypericin by light irradiation, as monitored by thin-layer chromatography (Alugram Sil G/UV_{254} plates (Macherey-Nagel, Duren, Germany)) with the solvent toluene/ethylacetate/formic acid (5 : 4 : 1, v/v). The latter was further purified by Sephadex LH20 (Pharmacia, Uppsala, Sweden) column chromatography with dichloromethane/methanol/acetone (11 : 6 : 3, v/v) as eluent. The yield was 28% and its purity was $>99\%$ as assessed by HPLC.

Hypericin was characterised with ^{1}H NMR (Gemini 200 MHz, Varian), LSI mass spectrometry (Kratos Concept IH), and UV/Vis spectrophotometry (Hewlett-Packard, CA). The data obtained were comparable with literature data (Falk & Schoppel 1992; Falk et al 1993).

Topical vehicles

The vehicles used were petrolatum, polyethylene glycol ointment (polyethylene glycol 400, 60% and polyethylene glycol 4000, 40%), emulsifying ointment (30 g emulgent cetyl-stearyl alcohol, 50 g petrolatum and 20 g liquid paraffin), Beeler basis (15 g cetyl alcohol, 1 g cera alba, 10 g propylene glycol, 2 g sodium lauryl sulphate and 72 g water), carbopol gel (2 g carboxypolymethylene, 15 g propylene glycol, 0·05 g disodium ethylenediamine tetra-acetate, 2·5 g triethanolamine and 80·45 g water) and cetomacrogol cream (7·2 g lanette O, 1·8 g cetomacrogol 1000, 15 g white petrolatum, 6 g liquid paraffin and 70 g water). These vehicles were supplemented (or not) with 10% of the skin absorption enhancers diethylene glycol monoethyl ether or isopropylidene glycerol. Compositions of the vehicles were taken from the Belgian National Formulary (NF VI) or Pharmacopoeia (Ph. Belg. V).

Preparation of hypericin formulations

When skin-absorption enhancers were not used, the appropriate amount of hypericin was first dissolved in acetone. Then acetone solutions of hypericin (200–500 μL) were pipetted onto a flat glass plate. After acetone evaporation, given weights of vehicles were added and thoroughly mixed to give the desired hypericin concentrations (0·01–1·00%). When skin-absorption enhancers were used, hypericin was first dissolved in the enhancer followed by addition of the vehicle.

Application of hypericin formulations onto the hairless mice skin

Male and female hairless mice, aged 8–12 weeks and weighing 24–26 g (NMRI-HR-HR, Charles River, Germany), were used. Control and test

vehicles containing varying amounts of hypericin with or without skin-absorption enhancers were uniformly and gently applied with a spatula ($20\,mg\,cm^{-2}$) onto the dorsal side of the mice anaesthetised with sodium pentobarbital (i.p., $90\,mg\,kg^{-1}$). Four different vehicles were applied to each mouse. After predetermined application times (1–6 h), the excess ointment was washed off, and the mice were killed. Treated skin samples of uniform surface were excised and mounted on a flat glass plate for stripping.

Skin stripping

Skin samples were stripped by repetitively applying and removing a piece of transparent adhesive polyethylene tape. For each strip, a fresh piece of tape was used. In order to standardise the skin-stripping technique, a 1-kg weight was placed on the tape in contact with the skin for 30 s before the peeling. Immediately after every 5, 10, 15, 20, or 25 strippings, the skin samples were visualised under standardised UV_{365} irradiation and pictures taken to document the presence and extent of fluorescence of hypericin in the skin. The skin could be stripped up to 20–25 times, after which it glistened and could no longer be efficiently stripped.

In evaluating tissue removal with skin stripping, the skin was treated with the vehicles described including different concentrations of hypericin. After 4 h, the treated skin was excised and stripped as described. Skin samples were immediately fixed with formalin and routinely processed after paraffin embedding. Eight semi-serial sections of each sample were stained with haematoxylin and eosin (H&E) and examined by light microscopy.

Determination of hypericin concentration in hairless mice skin

Hypericin in emulsifying ointment with isopropylidene glycerol (0·05% hypericin) or polyethylene glycol ointment (0·5% hypericin) was applied onto the skin as described. After 4 h, the unabsorbed ointment was washed off, the mice were killed, and the treated skin samples excised. Freshly excised skin samples were heated in water for 1 h at 70°C to dislodge the fatty layer from the rest of the skin. This treatment did not extract hypericin from the epidermis or dermis since hypericin is virtually insoluble in water. Consequently, hypericin was not found in the water used to heat the tissues. The fatty layer was then carefully peeled off from the skin with the help of tweezers. The remaining skin tissue (epidermis and dermis, as confirmed by histological microscopy) was weighed and homogenised (Ultra-Turrax) in 2 mL of water. The samples were then further homogenised with 8 mL acetone and centrifuged ($11\,000 \times g$, 10 min). The supernatants were removed and the samples were re-extracted using the same procedure. The pooled supernatant fractions were dried under reduced pressure, and the residue dissolved in dimethylsulphoxide. Finally, the dimethylsulphoxide samples were centrifuged ($7000 \times g$, 5 min) to remove debris, and the supernatants analysed by HPLC.

To determine the yield of the extraction procedure described above, the following experiment was carried out. Skin samples ($1\,cm^2$) were excised from the dorsal side of the hairless mice and were heated in water for 1 h at 70°C to remove the fatty layer. The rest was then homogenised in 2 mL water and incubated with known amounts of hypericin at 37°C for 1 h. Acetone (8 mL) was then added, after which the samples were processed as described above.

Hypericin analysis

An HPLC (L-6200 intelligent pump; Merck Hitachi, Tokyo, Japan) with a Lichrosphere 100 RP-18 (5 μm, 4×100 mm) column (Merck) and fluorescence detection (F-1050, Merck Hitachi, Tokyo, Japan) (excitation wavelength: 470 nm, emission wavelength: 600 nm) was used to analyse the samples. As eluent, an isocratic mixture (20 : 80) of 30 mM citrate buffer (pH 4) and methanol/acetone (2 : 1, v/v), with the flow rate set at $1\,mL\,min^{-1}$, was used. The retention time of hypericin was 6 min. The method was linear over the range 0·05–50 ng of hypericin (r^2: 0·9998). The reproducibility of the method was determined by replicate analysis ($n = 5$) of 3·1 ng hypericin. The relative standard deviation obtained was 2·4%.

Statistical analysis

Results are presented as means ± s.d., $n = 5$. Standard statistical evaluation of the data was performed with Student's *t*-test. $P < 0{\cdot}05$ was considered significant.

Results

Tissue removal associated with skin stripping

Microscopic analysis of the stripped skin tissues revealed gradual removal of parts of the stratum corneum with successive stripping. No effect was

Table 1. The extent of hypericin absorption into the skin of hairless mice, after 4 h of application of different hypericin concentrations in different vehicles with or without skin-absorption enhancers.

Vehicle	Absorption enhancer	Hypericin concn (%)	Fluorescence intensity
Petrolatum	None	0·01–1·0	−
Polyethylene glycol ointment	None	0·01–1·0	−
	Isopropylidene glycerol	0·001–1·0	−
	Diethylene glycol monoethyl ether	0·01–1·0	−
Carbopol gel	None	0·01–1·0	−
	Isopropylidene glycerol	0·01–1·0	−
	Diethylene glycol monoethyl ether	0·01–1·0	−
Beeler basis	None	0·01	+
	None	0·05–1·0	+ +
	Isopropylidene glycerol	0·01	+ +
	Isopropylidene glycerol	0·05–1·0	+ + +
	Diethylene glycol monoethyl ether	0·01	+ +
	Diethylene glycol monoethyl ether	0·05–1·0	+ + +
Emulsifying ointment	None	0·05–1·0	+
	Isopropylidene glycerol	0·05–1·0	+ + +
	Diethylene glycol monoethyl ether	0·05–1·0	+ +
Cetomacrogol cream	None	0·05–1·0	+*
	Isopropylidene glycerol	0·05–1·0	+ +*
	Diethylene glycol monoethyl ether	0·05–1·0	+ +*

−, no fluorescence of hypericin detected in the skin; +, + + and + + +, intensity of the fluorescence of hypericin in the skin (+ < + + < + + +). *Lateral spreading of hypericin in the skin.

observed on the stripping efficiency after application of the different vehicles used. Microphotographs of the stripped mouse skin indicated almost complete removal of the stratum corneum over large areas of skin after 20 strippings (results not shown).

Absorption of hypericin into the skin

The extent of hypericin absorption into the skin was assessed by visualisation of its fluorescence under standardised UV_{365} irradiation. The results of different hypericin concentrations incorporated in different vehicles following 4 h of application on the hairless mice skin are listed in Table 1. Figure 2 shows some typical fluorescence present in the hairless mice skin, as visualised under standardised UV_{365} irradiation after 4 h of topical application with some vehicles containing hypericin with and without skin-absorption enhancers, before and after 5, 15, and 20 strippings of the treated skin samples.

Hypericin was found to penetrate the skin when incorporated in Beeler basis, emulsifying ointment and cetomacrogol cream. However, the absorption into the skin from cetomacrogol cream was different from that of Beeler basis or emulsifying ointment. Hypericin with cetomacrogol cream appeared to be absorbed in a lateral spread, as indicated by scattering of the hypericin fluorescence over a much larger surface area in the skin. Thus, hypericin fluorescence intensity in the skin from cetomacrogol cream could not be compared very well with that from Beeler basis or emulsifying ointment, with which the hypericin was concentrated on the applied spot.

There was virtually no hypericin absorption into the skin when the compound was incorporated in polyethylene glycol ointment, petrolatum or carbopol gel. With petrolatum and carbopol gel, there was no fluorescence of hypericin detected in the skin after the first stripping, which indicates that there was no hypericin absorption into the skin from these vehicles. Hypericin delivered from polyethylene glycol ointment could no longer be detected in the skin after the first five strippings, so the compound was only absorbed into the very superficial areas of the epidermis. Comparison of the different concentrations of hypericin in the vehicle showed no significant differences in terms of absorption, except for the lowest hypericin concentration (0·01%), which produced less absorption of hypericin than the other concentrations (Table 1).

Addition of the skin-absorption enhancers (isopropylidene glycerol and diethylene glycol monoethyl ether) markedly increased hypericin absorption from Beeler basis and emulsifying ointment at all hypericin concentrations (Table 1). However, in the emulsifying ointment, isopropylidene glycerol appeared to be much more effective than diethylene glycol monoethyl ether, while in Beeler basis the two enhancers were equally effective. With skin-absorption enhancers, there were also no significant differences in the amount of hypericin absorbed in the skin for the different hypericin concentrations used, except for

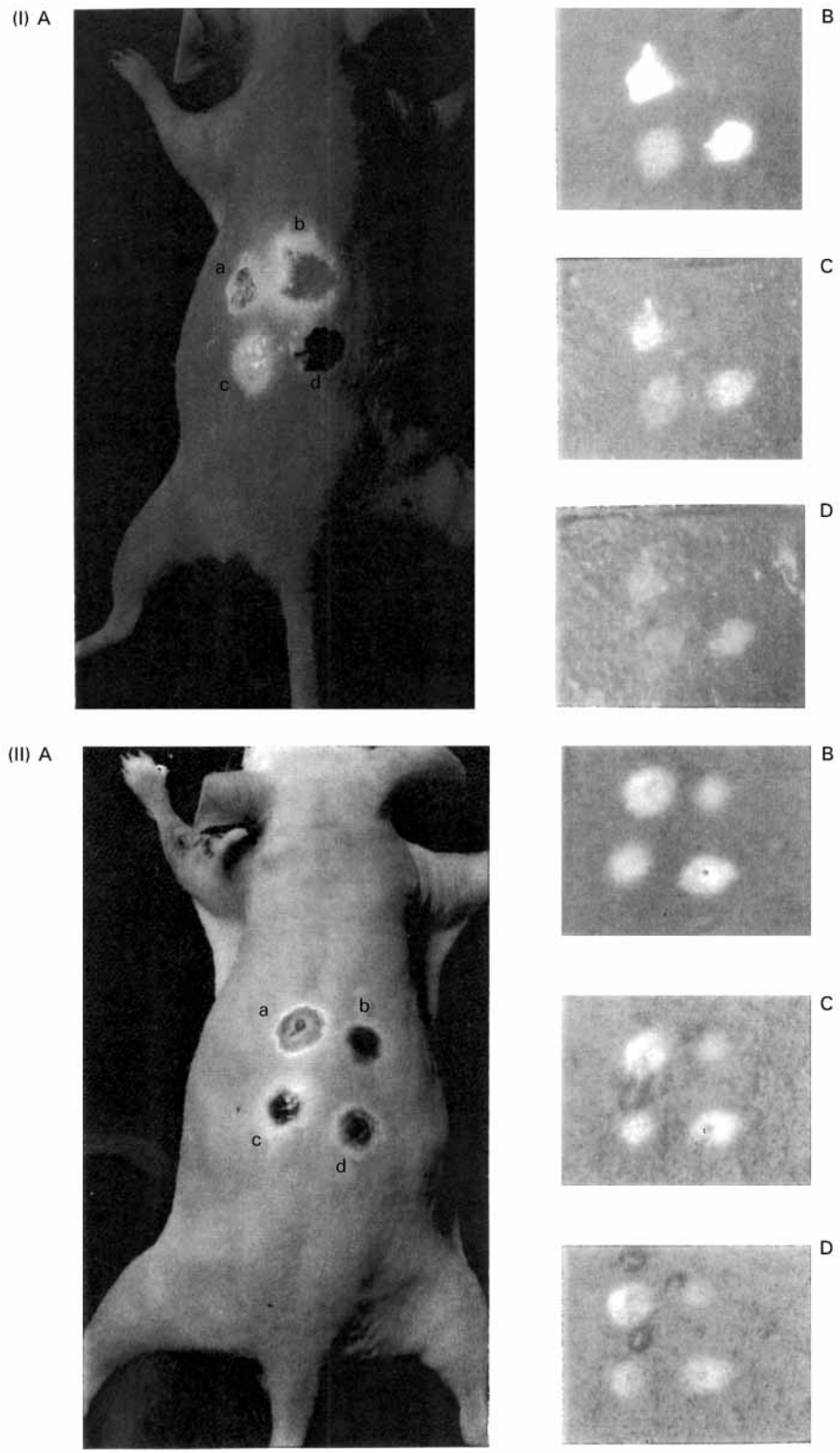

Figure 2. Photographs showing the fluorescence of hypericin in hairless mice skin (4-h application). The following vehicles were used: (I) (A) hypericin (0·05%) incorporated in (a) Beeler basis, (b) polyethylene glycol ointment, (c) emulsifying ointment and (d) hypericin (0·5%) in Beeler basis, before washing off the unabsorbed ointments and after (B) 5, (C) 15 and (D) 20 strippings of the skin, and (II) hypericin (0·05%) in Beeler basis with (a) diethylene glycol monoethyl ether or (d) isopropylidene glycerol and in emulsifying ointment with (b) diethylene glycol monoethyl ether or (c) isopropylidene glycerol, (A) before washing off the unabsorbed ointments and after (B) 5, (C) 15, and (D) 20 strippings of the skin.

the lowest concentration (0·01%), which showed less absorption of the compound. There was no hypericin absorption with carbopol gel, petrolatum or polyethylene glycol ointment, even after the addition of the skin-absorption enhancers.

For those vehicles which allowed better absorption of hypericin into the skin (Beeler basis, emulsifying ointment, cetomacrogol cream), the amount of hypericin appeared to increase with increasing application time. After 1 h of application, hypericin could be detected only in the first 5–10 strippings, while the compound could still be detected even after 25 strippings after being applied to the skin for 4 and 6 h (results not shown).

Hypericin concentration in hairless mice skin

Extraction yield. The extraction yield experiment was carried out to determine the efficiency of the extraction procedure employed in extracting hypericin from the skin. The extraction yield was determined with three doses of hypericin (20 ng, 100 ng, and 300 ng) added to a homogenate of the epidermis and dermis present in 1 cm^2 skin. The choice of these three doses was based on the results of the amounts of hypericin (20 and 200 ng) that were extracted from the skin following the topical application of the compound in polyethylene glycol ointment or emulsifying ointment with isopropylidene glycerol, respectively. About 65–86% of the original amount of hypericin incubated with skin homogenate could be recovered.

Amount of hypericin extracted from skin. Hypericin penetrated into the skin much better with some vehicles than with others. To corroborate quantitatively these visual observations, the concentrations of hypericin in the skin were estimated by HPLC analysis. For this purpose, two vehicles were used with which hypericin penetrated well (emulsifying ointment with isopropylidene glycerol) or very poorly (polyethylene glycol ointment) into the skin of hairless mice. As a control, an experiment was conducted to determine the amount of unabsorbed hypericin that could not be washed away before the samples were analysed. In this case, the ointments were applied to the skin for only 1 min.

The results of the control experiment and the 4-h application periods are presented in Table 2. With polyethylene glycol ointment, the average hypericin concentration present was limited after the 4-h application time ($0{\cdot}38 \pm 0{\cdot}34\ \mu g\ g^{-1}$ skin (epidermis and dermis), $n = 5$) and was not significantly different from that of the control experiment ($P > 0{\cdot}05$). Conversely, with emulsifying ointment plus isopropylidene glycerol, the concentration of hypericin in the skin for the control experiment was very small compared with that measured for the 4-h application time ($P < 0{\cdot}01$). After a 4-h application, the average hypericin concentration was $8{\cdot}61 \pm 3{\cdot}18\ \mu g\ g^{-1}$, $n = 5$. The average hypericin concentration was approximately 23 times higher in the skin when emulsifying ointment with isopropylidene glycerol was used than when the hypericin was delivered in polyethylene glycol ointment ($P < 0{\cdot}01$).

Discussion

After being topically applied in a vehicle, hypericin present in the skin could be visualised well under strong UV_{365} irradiation because of its bright fluorescence. This allowed the evaluation of the progressive fading of fluorescence that accompanied the stripping of the skin, which gradually removed layers of the stratum corneum. Consequently, the effectiveness of different vehicles in delivering hypericin into the skin could be investigated. The decrease in fluorescence of the tissue was due to removal of cell layers containing hypericin and not due to photodegradation of hypericin under UV_{365} irradiation, since prolonged irradiation of tissue containing hypericin without stripping did not reduce the fluorescence.

Table 2. Average concentration of hypericin present in hairless mouse skin following topical application either in polyethylene glycol ointment or in emulsifying ointment with isopropylidene glycerol.

Vehicles	Application time	Hypericin concn in skin ($\mu g\ g^{-1}$)
Polyethylene glycol ointment	1 min (control)	$0{\cdot}21 \pm 0{\cdot}14$
	4 h	$0{\cdot}38 \pm 0{\cdot}34$
Emulsifying ointment plus isopropylidene glycerol	1 min (control)	$0{\cdot}40 \pm 0{\cdot}028$
	4 h	$8{\cdot}61 \pm 3{\cdot}18$**

Values are expressed as means ± s.d., $n = 5$. **$P < 0{\cdot}01$ compared with application of hypericin in polyethylene glycol ointment for 4 h.

The fluorescence yield of hypericin in the vehicles also appeared to be higher in some vehicles than in others. For instance, hypericin showed high fluorescence in polyethylene glycol ointment and carbopol gel while moderate to low fluorescence was observed in the other vehicles. Fluorescence of hypericin in the vehicle or solvent indicates the extent of its solubility, and an increase in fluorescence indicates an increase of solubility (Yamazaki et al 1993; Lavie et al 1995). It is well known that hypericin readily dissolves in polar organic solvents (e.g. polyethylene glycol) to develop red fluorescent solutions, but forms non-fluorescent high-molecular-weight aggregates in aqueous media (Lavie et al 1995). In addition, hypericin does not dissolve, and consequently is not fluorescent, in vegetable oils and non-polar organic solvents (data not shown). The higher fluorescence of hypericin in carbopol gel is likely to be due to the dissolution of hypericin caused by the presence of propylene glycol. Moreover, hypericin is known to form ion pairs with organic bases such as triethanolamine, which is present in carbopol gel. The various ion pairs of hypericin have varying solubility rates in water depending on the cation (Falk & Schmitzberger 1992) and it is likely that, in combination with propylene glycol, triethanolamine enhanced the solubility of hypericin in the aqueous medium.

It is interesting to note that there was an inverse correlation between observed fluorescence and the skin absorption of hypericin: the compound was poorly absorbed in the skin from the vehicles in which it was highly fluorescent but readily absorbed from those vehicles in which it showed moderate to low fluorescence. It is not obvious from this study why hypericin tends to penetrate the skin from those vehicles in which it is only slightly soluble but not from those vehicles in which it is well solubilized. It can, however, be speculated that in the former vehicles there is high affinity between the drug and the vehicle that might prevent the release of the compound from the vehicle to the skin. Alternatively, it can also be assumed that in the vehicles in which hypericin is insoluble, the compound is also not able to leave the vehicle and penetrate the skin due to the bigger sizes (e.g. aggregates).

The results of this study further demonstrate the influence of the application time on the absorption of hypericin into the skin. Hypericin diffuses slowly through the stratum corneum layers. Thus, after the hypericin diffused from the vehicle, its absorption into the layers of the skin is determined by the stratum corneum. The lipophilicity as well as the hydrogen-bonding potential of hypericin, which might facilitate its interaction with the stratum corneum constituents, may explain why hypericin forms a depot in the stratum corneum, as documented for other lipophilic drugs (Michaels et al 1975).

This study also shows that hypericin skin absorption is enhanced by the addition of skin-absorption enhancers (isopropylidene glycerol and diethylene glycol monoethyl ether) to the vehicles. The two enhancers, at 10% concentration, have been found by other investigators to be non-toxic to mice and other animal skins (Lashmar et al 1989) and effective in promoting percutaneous absorption of different drugs (Harrison et al 1996). In this study it is interesting to note that this enhancement was only observed with those vehicles from which the compound could already efficiently absorb into the skin. This might imply that the action of these enhancers was on the skin rather than on the vehicles. However, there was a small increase in hypericin fluorescence in the vehicles after the addition of the skin-absorption enhancers, which indicates an effect on the solubility of hypericin by the enhancers themselves.

The quantitative results obtained using an extraction procedure and HPLC further demonstrate the validity of the skin-stripping technique. Substantially higher concentrations of hypericin were extracted from the skin when it was applied in emulsifying ointment with isopropylidene glycerol than when it was applied in polyethylene glycol ointment. This large ($\sim$23-fold) difference is consistent with the visual results obtained with the skin-stripping technique, indicating that absorption of hypericin into the skin indeed depends on the vehicle that is used to deliver the compound to the skin.

The extraction procedure employed to extract hypericin from the skin indicates that 65–86% of the applied dose could be extracted. These data are in agreement with the recovery of hypericin reported from human urine (63–75%) but somewhat lower than the recovery of hypericin from plasma of mice, monkeys and humans ($96{\cdot}6 \pm 5{\cdot}8\%$) (Liebes et al 1991). The relatively lower recovery of hypericin in this study could be explained by the fact that hypericin interacts with body fluid components such as human serum albumin and lipoproteins (Lavie et al 1995). In addition, mice that received hypericin in their diets remain sensitive to light for more than a week after transfer to hypericin-free food, which indicates that the pigment has a high affinity for tissue matter (Giese 1980). In this regard, it is likely that hypericin also strongly interacts with skin constituents, leading to a lower recovery of the compound from the skin homogenate.

We conclude that the absorption of hypericin into the skin very much depends on the composition of the incorporating vehicle. We have proved that the solubility of hypericin in the vehicle, as assessed by the fluorescence of the compound, could be used as a good indication for its delivery to the skin. Hypericin absorption into the inner layers of the skin is controlled by the stratum corneum. Emulsifying ointment with isopropylidene glycerol promises to be an effective topical vehicle in the treatment of skin diseases (such as psoriasis) with hypericin.

References

Agostinis, P., Vandenbogaerde, A., Donella-Diana, A., Pinna, L. A., Lee, K. T., Goris, J., Merlevede, W., Vandenheede, J. R., de Witte, P. (1995) Photosensitised inhibition of growth factor-receptor protein kinases by hypericin. Biochem. Pharmacol. 49: 1615–1622

Agostinis, P., Donella-Diana, A., Cuveele, J., Vandenbogaerde, A., Sarno, S., Merlevede, W., de Witte, P. (1996) A comparative analysis of the photosensitised inhibition growth factor regulated protein kinases by hypericin-derivatives. Biochem. Biophys. Res. Commun. 220: 613–617

de Witte, P., Agostinis, P., Van Lint, J., Merlevede, W., Vandenheede, J. R. (1993) Inhibition of epidermal growth factor receptor tyrosine kinase activity by hypericin. Biochem. Pharmacol. 16: 1929–1936

Diwu, Z., Lown, W. (1994) Phototherapeutic potential of alternative photosensitizers to porphyrins. Pharmacol. Ther. 63: 1–35

Falk, H., Schmitzberger, W. (1992) On the nature of "soluble" hypericin in *Hypericum* species. Monatshefte für Chemie. 123: 731–739

Falk, H., Schoppel, G. (1992) On the synthesis of hypericin by oxidative trimethylemodin anthrone and emodin anthrone dimerization: isohypericin. Monatshefte für Chemie. 123: 931–938

Falk, H., Meyer, J., Oberreiter, M. (1993) A convenience semisynthetic route to hypericin. Monatshefte für Chemie. 124: 339–341

Giese, A. C. (1980) Hypericin. Photochem. Photobiol. Rev. 5: 229–255

Harrison, J. E., Watkinson, A. C., Green, D. M., Hadgraft, J., Brain, K. (1996) The relative effect of Azone® and Transcutol® on permeant diffusivity and solubility in human stratum corneum. Pharm. Res. 13: 542–546

Lashmar, U. T., Hadraft, J., Thomas, N. (1989) Topical application of penetration enhancers to the skin of nude mice: a histopathological study. J. Pharmacol. 41: 118–121

Lavie, G., Mazur, Y., Lavie, D., Meruelo, D. (1995) The chemical and biological properties of hypericin – a compound with a broad spectrum of biological activities. Med. Res. Rev. 15: 111–119

Liebes, L., Mazur, Y., Freeman, D., Lavie, D., Lavie, G., Kudler, N., Mendoza, S., Levin, B., Hochster, H., Meruelo, D. (1991) A method of quantification of hypericin, an antiviral agent, in biological fluids by high-performance liquid chromatography. Anal. Biochem. 195: 77–85

Michaels, A. S., Chandrasekran, S. K., Shaw, J. E. (1975) Drug permeation through human skin: theory and in-vitro experimental measurement. AIChE. J. 21: 985–996

Mitev, V. I., Miteva, L., Dourmishev, A. (1992) Casein kinase II activity in psoriatic epidermis. Arch. Dermatol. Res. 284: 111–113

Nanney, L. B., Stoscheck, C. M., Magid, M., King, L. E. (1986) Altered [^{125}I]epidermal growth binding and receptor distribution in psoriasis. J. Invest. Dermatol. 86: 260–265

Ritcher, A., Davies, D. E. (1995) Effects of anthralin and hypericin on growth factor signalling and cell proliferation in-vitro. Biochem. Pharmacol. 50: 2039–2045

Szeimies, R. M., Sassy, T., Landthaler, M. (1994) Penetration of topical applied delta-aminolevulinic acid for photodynamic therapy of basal cell carcinoma. Photochem. Photobiol. B. 6: 143–148

Ugwu, F., Vandenbogaerde, A. L., Merlevede, W. J., de Witte, P. A. (1998) A comparative study on phototoxicity of hypericin on A431 cells using three different assays. Anticancer Res. 18: 1181–1184

Yamazaki, T., Ohta, N., Yamazaki, I., Song, P.-S. (1993) Excited-state properties of hypericin: electronic spectra and fluorescence decay kinetics. J. Phys. Chem. 97: 7870–7875