Penetration and distribution of three lipophilic probes in vitro in human skin focussing on the hair follicle

ABSTRACT

Fluorescent model substances of increasing lipophilicity (Oregon Green® 488, Bodipy® FL C5 and Bodipy® 564/570 C5) were selected to enable the visualisation in the skin using Confocal Laser Scanning Microscopy. After measuring the penetration for 18 hours, the non-fixed human scalp skin was imaged from the bottom parallel to the stratum corneum and in a cross section view perpendicular to the skin surface. The images were evaluated by calculating relative accumulation values for different penetrants. The studies indicate that the penetrated amount is highest for BFL (medium lipophilicity) and lowest for B564 (high lipophilicity) whereas B564 (high lipophilicity) reveals the highest relative accumulation in parts of the hair follicle compared to OG (low lipophilicity). The addition of 30 % (v/v) ethanol to the donor phase of substance with a low lipophilicity increases the follicular delivery. From our results we conclude that delivery to the hair follicle can be improved by increasing the drugs lipophilicity and optimising the composition of the donor phase. However, no conclusion can be drawn about the actual route of transport to the hair follicle.
INTRODUCTION

Local skin targeting is of interest for the pharmaceutical and the cosmetic industry. In case of skin diseases and in case of cosmetic products, delivery to sweat glands or to the pilosebaceous unit is essential for the effect of the drug. Local delivery can be improved by two approaches. First, the choice of formulation such as particulate carriers and variation in medium additives such as ethanol can optimise local targeting. Secondly, the physicochemical parameters of the drug itself, e.g. size, charge and lipophilicity, can affect the degree of delivery as well. In cases where formulation variation is not feasible, more attention has to be paid to the optimisation of the penetrant itself.

In the early years Scheuplein et al. [1,2] and Keister et al. [3] described the importance of the follicular pathway in the initial penetration process and its dependency on the membrane diffusion constant. Numerous studies followed presenting experimental data mainly based on permeation studies, which have been reviewed by Lauer et al. [4]. Up to now, various authors reported delivery to the hair follicle by variation of the formulation. Weiner et al. [5] summarised delivery studies of liposomal formulations, which showed enhanced delivery to the pilosebaceous unit for peptides, antibodies and DNA. In addition iontophoresis is also known to occur mainly via the follicular route where the stratum corneum barrier is non-continuous [6]. Next to liposomal or current related targeting, particles of 7 µm [7] or of 5 µm [8,9] were reported to be localised in the pilosebaceous unit after topical application. However titaniumoxide particles (size not stated) remain in the follicle orifice [10]. Lieb et al. [11] observed that large molecular weight dextrans and oligonucleotides were encountered in the hair follicle after topical application. Additionally they proposed that transport occurs between the inner and the outer root sheath namely the companion layer. The influence of ethanol on the delivery to the hair follicle has not been studied intensively. Tata et al. [12] and Touitou et al. [13] reported that the effect of minoxidil application is increased when using ethanol or ethanol in combination with vesicular systems with no information presented on the detailed localisation within the hair follicle. It has also been stated that the appendageal route contributes to the penetration when the vehicle contains propylene glycol, dimethylsulfoxide or ethanol [14].

In contrast to the influence of formulation, limited groups have studied the influence of drug lipophilicity on the effect of follicular deposition mainly focussing on permeation studies. Hueber et al. [15,16] reported a significant contribution of the pilosebaceous unit to the penetration of lipophilic drugs into the skin. The delivery to the pilosebaceous unit of hydrophilic low molecular weight model substances from various vehicles was studied by Lieb et al. [11]. They observed that liposomes were more effective than solutions containing...
ethanol, propylene glycol or no additive. Reviews on the effect of vehicle, drug and available skin models on follicular delivery are given by Lauer et al. [4,17], Illel [18] and Agarwal et al. [19].

In the studies reported in literature, it is striking that frequently animal skin with post-experimental fixation has been used. During fixation either cryo-freezing or embedding have been used. Both methods bear the danger of delocalisation of the substance during fixation and modification of cell structures. In previous studies a method was introduced in which confocal laser scanning microscopy (CLSM) was used to visualise label localisation in 200 µm thick skin without embedding [20]. Very recently this method was extended to full thickness skin and combined with a new relative quantification model [21]. Advantages of this method are high resolution, no fixation or embedding thereby avoiding delocalisation of the dye, specific and sensitive detection due to use of fluorophores, visualisation of deeper skin layers due to optical sectioning and calculating changes of accumulation in skin layers. The disadvantages such as the necessity of dyes and the limitation to in vitro use do not interfere with this study. Additionally, it has to be pointed out that this method does not give any information about the absolute amount of permeant in the skin however information is obtained about changes in distribution and thus on delivery to various skin compartments. Therefore in the present study the influence of permeant lipophilicity on the penetration and distribution in non-fixed human scalp skin is examined using the new relative quantification method. Especially the influence of permeant lipophilicity on the delivery of model substances to the hair follicle is investigated.

MATERIALS AND METHODS

Model penetrants

Fluorescent labels used as model compounds (Oregon Green® 488 (OG), Bodipy® FL C5 (BFL) and Bodipy® 564/570 C5 (B564)) were purchased from Molecular Probes, The Netherlands.

Determination of the octanol/buffer partition coefficient

The water phase consisted of either a 50 mM citric acid buffer (CAB) pH 5.0 or phosphate buffered saline (PBS) pH 7.4 (139 mM NaCl, 2.5 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 25 mg/L Streptomycin and 25000 U/L Penicillin). The solutions are filtered through a Nylon 66 membrane (0.2 µm *47 mm) from Supelco (Bellefonte, PA, USA) before use. These two buffers were chosen, since they represented the donor (CAB) and the acceptor phase (PBS) of the diffusion
Penetration and distribution of three fluorophores

During the experiment, the fluorophore was dissolved in the octanol phase followed by the addition of the aqueous phase and vigorous mixing (2 minutes). The two-phase-system was additionally shaken for one hour at 600 rpm, 25 °C. Ensuring a complete equilibrium of the dye between the two phases, the samples were left for 48 h at room temperature while protected from light. Subsequently, the samples were centrifuged at 3000 rpm for 30 min to obtain complete separation of the octanol and the buffer. The concentration of the fluorophore in each phase was determined using a Perkin-Elmer MPF-4 fluorescence spectrophotometer. In order to calculate a partition coefficient independent of the concentration, it was necessary to measure three different label concentrations and extrapolate to infinite low concentrations. The logP_{oct/buf} values for the different concentrations of each fluorophore were determined 10 times.

Diffusion experiment

_Fresh human scalp skin_ was obtained 4 to 5 hours after face-lift surgery and stored on a filter paper soaked with PBS until used the same day. Hairs were cut with scissors prior to dermatoming to a thickness of 1100 +/- 15 µm followed by cleaning of the skin surface with a PBS- and 70 % (v/v) ethanol-wipe. At least 5 skin pieces of at least 3 different donors were used. The tested donor vehicles consisted of 0.25 mg/ml OG (80% saturated), BFL (50% saturated) or B564 (100% saturated with crystals present) in CAB containing 30 % (v/v) ethanol. Diffusion experiments were performed using flow-through diffusion cells (PermeGear). Exposing a 38.5 mm² area of the fresh skin occlusively to 250 µl of the donor phase, hourly fractions of the PBS containing acceptor phase were collected over a period of 18 hours. This 18-hour diffusion experiment was followed by label visualisation using CLSM. Additionally, 0.1 mg/ml OG in CAB (100 % saturation) and 0.25 mg/ml OG or BFL in CAB containing 30 % (v/v) ethanol were exposed to the skin over 72 hours with fractions collected every hour up to 18 hours followed by 3-hour interval. The concentration of the different labels in each fraction was determined using an HPLC system comprised of an Isochrom LC pump, a Chromguard precolumn SS 10*3 mm, a LiChrosorb RP18 column (3*100 mm) with a particle size of 7 µm, a Gilson 234 autoinjector and a Jasco Version 6.01 821 FP fluorescence detector. The mobile phase consisted of 50 % acetonitril in purified water (flow rate = 1.0 ml/min). Oregon Green® 488 and Bodipy® FL C₅ were detected at 488/514 nm (excitation/emission), whereas the wavelengths for Bodipy® 564/570 C₅ were set at 564/574 nm, respectively. The limit of quantification was 93 ng/ml (OG), 130 ng/ml (BFL) and 466 ng/ml (B564) respectively.
Confocal Laser Scanning Microscopy

The visualisation of the fluorophores in the non-fixed scalp skin was carried out using a Bio-Rad MRC 600 unit followed by an evaluation of their distribution by relative quantification as previously described [21]. Images are taken from the bottom (parallel to the skin surface) and the cross section (perpendicular to the skin surface). The parallel images reveal information about label distribution in the hair follicles and the dermis, while the cross sectional view provides information about label distribution in dermis, epidermis and stratum corneum. The cross sectional view of the skin is obtained by using a modified cutting device according to Meuwissen et al. [20], which is used as a sample holder at the same time. From the confocal images the relative accumulation factor \(f_{acc}\) is calculated. A detailed explanation of the method is described elsewhere [21]. Briefly, the intensity is measured in the various skin parts after which the intensities between the follicular and the cross sectional image are normalised. Subsequently, relative intensities of various compartments in an assumed skin block are calculated. After normalising steps the relative fluorescence \(F_{rel}\) in percent for the non-follicular (stratum corneum, epidermis, dermis) and the follicular (outer root sheath, inner root sheath, cuticle, hair shaft) volume is obtained within this assumed skin block. By comparing this \(F_{rel}\) in one skin region with the relative intensity assuming a homogeneous distribution in the model skin block, a relative accumulation value \(f_{acc}\) for each skin compartment is obtained. These regional values serve to estimate the change in accumulation when label lipophilicity or donor solutions are modified.

RESULTS

Partition Coefficient

The partition coefficients of the labels increased in the order OG, BFL and B564 (Table 1) indicating that B564 is the most lipophilic and OG the most hydrophilic label for both, pH 5 and pH 7.4. However, at pH 7.4 the degree of lipophilicity for all labels was reduced compared to pH 5. A change in pH from 5 to 7.4 increased the ionisation of the carboxylic groups of the substances. At pH 7.4, OG has a negative \(\log P_{oct/buf}\) value, which indicates a higher affinity for the aqueous phase than for octanol.
**Table 1.** Partition coefficient of three model penetrants expressed as \( \log P_{oct/buf} \) in a citric acid buffer solution at pH 5.0 (donor phase) and in a phosphate buffered saline solution at pH 7.4 (acceptor phase) is depicted in this table.

<table>
<thead>
<tr>
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<th>Citric acid buffer pH 5.0</th>
<th>Phosphate buffered saline pH 7.4</th>
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<tbody>
<tr>
<td>Oregon Green® 488</td>
<td>1.6</td>
<td>-2.5</td>
</tr>
<tr>
<td>Bodipy® FL C₅</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Bodipy® 564/574 C₅</td>
<td>4.3</td>
<td>3.0</td>
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**Diffusion**

The diffusion profiles of the three model substances during 18 hours have been plotted in Figure 1A. No steady state flux was reached. B564 revealed a 250 times lower cumulative flux after 18 hours than BFL, which was significant \((p<0.01)\). All other cumulative amounts did not differ significantly from each other. Since only a very small difference in flux between the OG and BFL was observed we decided to examine the diffusion profile of these labels also for a period of 72 hours. The cumulative amount is plotted in Figure 1B. A significant difference in cumulative amount of the two labels was detected after 24 hours of diffusion. This difference increases with an increasing diffusion period. The steady state flux and lag-time are provided in Table 2. For BFL a decreasing flux rate is observed after 57 hours (Figure 1B). This is most likely due to depletion in the donor phase since after 54 hours 47 % of the applied BFL has been detected in the acceptor fluid. A decrease in flux is not observed for OG.

**Table 2.** Diffusion parameters of 0.25 mg/ml label applied on human scalp skin in a citric acid buffer vehicle containing 30 % (v/v) of ethanol.

<table>
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<tr>
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<th>Oregon Green® 488</th>
<th>Bodipy® FL C₅</th>
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<tr>
<td>Steady State flux ([\mu\text{mol}/\text{cm}^2\times\text{h}])</td>
<td>1.26</td>
<td>6.79</td>
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<td>Lag time ([\text{h}])</td>
<td>27</td>
<td>22</td>
</tr>
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Figure 1A. Diffusion (18 hours) of 0.25 mg/ml of three model permeants (OG-Oregon Green® 488, BFL-Bodipy® FL C5 and B564-Bodipy® 564/570 C5) through fresh full thickness human scalp skin applied occlusively in citric acid buffer pH 5.0 containing 30 % (v/v) ethanol. The applied dose was 440 nmol/cm² (OG), 507 nmol/cm² (BFL) and 9 nmol/cm² (B564). Depicted is the mean including standard error with n=6 for BFL and n=8 for OG and B564.

Figure 1B. 72 hour diffusion of Oregon Green® 488 (OG, 0.25 mg/ml) and Bodipy® FL C5 (BFL, 0.25 mg/ml) from a 30 % (v/v) ethanol containing citric acid buffer vehicle pH 5.0. The applied dose was 440 nmol/cm² (OG) and 507 nmol/cm² (BFL). Depicted is the mean including standard error with n=6 for BFL and OG.
In order to study the effect of ethanol on the skin barrier we decided to examine the permeation also in the absence of ethanol. The least lipophilic label OG was chosen since the effect of ethanol on the barrier function was expected to increase the flux of a hydrophilic dye more efficient than that of the more lipophilic dye BFL. The diffusion profile was measured during 72 hours. After 18 hours of diffusion (time point of CLSM images), no significant difference between the cumulative amount of OG in the absence and presence of ethanol is observed. The difference in flux was significant after 24 hours. 72 hours of diffusion resulted in a cumulative amount of OG in the presence of ethanol which is 25.7-fold higher (56.5 μmol/cm²) than in the absence of ethanol (2.2 μmol/h*cm²) (Figure 1C).

**Figure 1C.** 72 hour diffusion experiment of nearly saturated Oregon Green® 488 (OG) solution (0.25 mg/ml, 440 nmol/cm²) from a citric acid buffer vehicle containing 30 % (v/v) ethanol and a saturated OG solution (0.25 mg/ml, 176 nmol/cm²) without additives (CAB) are shown. Depicted is the mean including standard error with n=6 for OG in 30 % (v/v) ethanol and n=3 for OG in CAB only.

**Distribution**

Autofluorescence could only be detected in a follicle at 100 % laser intensity. Since images of the experiments were taken at laser intensities <30 %, no interference from autofluorescence occurred. In general, the permeation pathway across the stratum corneum resides in the intercellular lipid domains (not shown). This is in contrast to the viable epidermis, in which the fluorophore is not
restricted to the cell membranes only, but also accumulates in the cytosol of the various cells. A dependency on the lipophilicity of the applied substance was observed in the accumulation of the label in stratum corneum and viable epidermis. While the lipophilic Bodipy® labels exhibit bright staining of the epidermis and a much lower staining of the stratum corneum (Figure 2C, E), the

**Figure 2.** Distribution of 0.25 mg/ml label from citric acid buffer pH 5.0 containing 30 % (v/v) ethanol in human scalp skin after 18 hours diffusion. A, C, E: Manual cross section of non-fixed tissue with stratum corneum (sc), epidermis (epid) and dermis (derm). Bottom view of the dermis parallel to the skin surface (B, F) and cross section view perpendicular to skin surface (D) showing a hair follicle with outer root sheath (ors), inner root sheath (irs), cuticular area (cut), hair shaft (sha) and dermis (derm). A, B: Oregon Green® 488; C, D: Bodipy® FL C5; E, F: Bodipy® 564/570 C5.
more hydrophilic OG shows a dominating staining in the stratum corneum compared to that in the viable epidermis (Figure 2A). The dermis exhibited a less intense staining than in the viable epidermis for all fluorophores. The hair follicle, which reaches down into the dermis and subcutaneous fat reveals a characteristic and similar staining of the follicular parts for all three labels. While the outer root sheath and the cuticular area depict good labelling, staining of the inner root sheath was much weaker and hardly any label was observed in the hair shaft itself (Figure 2B, D, F). The degree of staining varied between the labels. When OG was applied to the skin the follicular region was more difficult to detect due to the low staining, not the lower quantum efficiency, in the deeper skin regions. Since after application of the Bodipy® labels, the deeper skin layers were more intense, detection of the hair follicles was easier.

Accumulation

Label lipophilicity

In the assumed skin block, less than 10 % of the fluorescence is present in the follicular volume in case of OG and BFL (Table 3) with no significant difference between the less lipophilic labels. In case of the more lipophilic dye B564, this percentage increases significantly and reaches a value of 12.4 %. Although the accumulation factors (f_{acc}) (Figure 3A) of several skin parts exhibit accumulation values above 1, only the values of OG in the stratum corneum, B564 in the epidermis, outer root sheath, inner root sheath and the cuticle revealed a significant difference. This indicates an accumulation of the labels in these skin regions. High label lipophilicity does not significantly influence the accumulation factor in the epidermis and dermis, but results in a significant decrease (p<0.01) of the accumulation factor in the stratum corneum.

Vehicle effect

Deeper skin layers are easier visualised when ethanol is present in the donor phase. This observation is confirmed by the F_{rel} percentages. An addition of 30 % (v/v) ethanol to the CAB vehicle has the tendency to increase the F_{rel} percentage for the hair follicle from 5.6 to 9.1. When comparing the accumulation factors (Figure 3B) of the same vehicles these values reveal that the stratum corneum contains a very high fluorescent intensity (f_{acc} value of 10.1) in the absence of ethanol. Also in the presence of ethanol the accumulation in stratum corneum is rather high (f_{acc} value of 5.2). No difference in accumulation in the presence or absence of ethanol can be observed in the dermis and epidermis, while the f_{acc} values of the outer root sheath, the inner root sheath and the cuticle reveal the trend of an increase in f_{acc} in the presence of ethanol.
Table 3. Relative fluorescence ($F_{\text{rel}}$) in the non-follicular (stratum corneum, epidermis, dermis) and the follicular area (outer root sheath, inner root sheath, cuticle, hair shaft) after 18 hours of diffusion of OG, BFL and B564 in either citric acid buffer (CAB) or CAB containing 30 % (v/v) of ethanol (CAB/EtOH). The $F_{\text{rel}}$ is a means to estimate how much of the calculated fluorescence is present in the follicular area in an assumed skin block in comparison to the non-follicular area.

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<tr>
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<th>$F_{\text{rel}}$ (non-follicular) [%]</th>
<th>$F_{\text{rel}}$ (Follicular) [%]</th>
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<tr>
<td></td>
<td>Average    Standard error</td>
<td>Average Standard error</td>
</tr>
<tr>
<td>OG in CAB</td>
<td>(n=3)      94.4 1.5</td>
<td>5.6 1.5</td>
</tr>
<tr>
<td>OG in CAB/EtOH</td>
<td>(n=4)      90.5 1.0</td>
<td>9.5 1.0</td>
</tr>
<tr>
<td>BFL in CAB/EtOH</td>
<td>(n=3)      92.0 0.4</td>
<td>8.0 0.4</td>
</tr>
<tr>
<td>B564 in CAB/EtOH</td>
<td>(n=4)      87.6 0.9</td>
<td>12.4 0.9</td>
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</table>

DISCUSSION

Diffusion

During the first 10 hours the flux is low whereas after 10 hours it increases noticeable. No tendency is observed that either the more hydrophilic or the more lipophilic substance is permeating the human scalp skin faster. The most lipophilic substance (B564) exhibits extremely low fluxes, which might be due to the high log $P_{\text{oct/buf}}$ value. Its lipophilic character will minimise the partitioning from the dermis into the acceptor phase thereby minimising the diffusion of B564. However at a pH of 5.0 (pH of the donor solution and the stratum corneum) the as hydrophilic considered OG has an affinity for the lipophilic phase. In the diffusion process, when the pH changes to values of about 7.4 in deeper skin strata, the affinity of OG for the aqueous environment dominates (hydrophilic) while the Bodipy® labels are still lipophilic (Table 1).

The diffusion profile exhibits a long lag-time. This might be due to the lipid lamellae in the stratum corneum that may act as a depot for the more lipophilic labels. Alternatively the full thickness human skin used in the present studies increases the permeation path length and affects the lag-time.
In pilot studies (unpublished results) skin degradation was observed after 72 h, however not after 18 hours. Since a significant difference in flux between OG and BFL is already seen after 24 hours, degradation is not expected to be the main cause for the difference in flux. This difference in flux might be due to i) a different degree of saturation of the two labels, ii) a difference in the diffusion coefficient of each label in the skin or iii) a difference in permeation pathway. Since BFL exhibits a higher flux but at the same time has a lower thermodynamic activity (saturation of ca. 50 %) than OG (saturation of ca. 80 %), either a difference in partition coefficient, diffusion coefficient and/or penetration pathway is responsible for the difference in flux.

Finally our diffusion results reveal that addition of ethanol to the donor phase results in a much higher flux of OG. This strongly indicates that ethanol is a very good penetration enhancer for OG. Whether ethanol enhances the solubility of OG in the skin or changes the lipid organisation in the stratum corneum is not known. Ghanem et al. [22] postulated, that smaller ethanol volume fractions (<25 %) in the donor phase resulted in a permeation enhancement due to partitioning of ethanol into the stratum corneum, while larger volume fractions (>50 %) result in a dehydration and pore formation in the skin. Berner and Liu [23] summarise the effect of ethanol as a penetration enhancer. They differentiate the enhancement effect of lipophilic and hydrophilic penetrants and mention that the primary action of ethanol for lipophilic compounds is to dissolve in the lipid regions thereby increasing the solubility of the lipophilic penetrants therein.

**Accumulation**

The distribution of the various labels was determined by calculating the degree of accumulation (relative accumulation factor = $f_{acc}$) in the various skin areas. It has to be realised that the $f_{acc}$ value gives no indication for the total amount of fluorophore in the skin. If rather high $f_{acc}$ values have been determined, it is still possible, that only a low total amount of label has reached the plane of imaging and vice versa. However a good measure is obtained for changes in label distribution and thus for a change in deposition in the various skin compartments.

Although the label was applied on top of the stratum corneum only low relative accumulation was observed for the more lipophilic labels in this skin barrier. This can be explained by predominant penetration of the label across the lipid domains of the stratum corneum. Since, the lipid lamellae constitute only a small region of the stratum corneum compared to the corneocytes, this will “dilute” the label concentration in the stratum corneum. The adjacent viable epidermis is much brighter stained than the stratum corneum. Since in this layer the cornified envelope has not been developed yet, the label can distribute throughout the entire epidermis, which results in a brighter appearance. The
deeper layer of the skin (dermis) shows only low labelling (Figure 2A, C, E) and no accumulation of dye (Figure 3A).

In the hair follicle the outer root sheath exhibits the highest relative accumulation whereas the adjacent inner root sheath reveals lower relative accumulation values. Compared to the outer root sheath, which is the least keratinised layer of the follicle, the inner root sheath starts its keratinisation process already shortly after cell formation in the lower part of the follicle. Therefore keratinisation is thought to protect a cell from label penetration. The cuticle of the hair shaft is keratinised in early stages as well and therefore protects the soft hair cortex from environmental influences [24]. This might lead to the accumulation of the dye in the cuticular area. Previously Kelch et al. [25] showed that a fluorophore accumulates in the soft endocuticle and the cell membrane complex.

**Influence of permeant lipophilicity**

In the stratum corneum accumulation of BFL and B564 is less than of OG. Since OG is expected to be less soluble in the lipids of the stratum corneum than the Bodipy® dyes, the high intensity of OG is most probably caused by an accumulation of this label on the surface. Generally, the relative accumulation in the follicular unit increases with high lipophilicity as seen from the $F_{rel}$ and $f_{acc}$ values. This can on the one hand be attributed to an increased penetration through the epidermis to the outer root sheath, since the outer root sheath is directly connected to and has similar features as the epidermis [26]. However, a second route of transport might be the gap between the skin and the hair shaft. The gap is filled with the lipophilic sebum and allows movement of the keratinised hair in the skin. Since in the deeper regions of the epidermal invagination the stratum corneum along the hair shaft becomes thinner, the permeability of the skin is expected to increase in these regions. A combination of the mentioned routes of penetration is most likely to occur since penetration via all routes includes the overcoming of a lipophilic barrier. Reviewed by Lauer et al. [4] and Agarwal et al. [18], literature reports that the follicular duct contains neutral and non-polar lipids, and that the hair follicle favours the permeation of polar compounds. Up to now no proof for either of the routes can be given from our experiments.
Penetration and distribution of three fluorophores

Figure 3. Relative accumulation ($f_{acc}$) including standard error (n=3) of Oregon Green® 488 (OG), Bodipy® FL C5 (BFL) and Bodipy® 564/570 C5 (B564) in the stratum corneum (SC), epidermis (Epid), dermis (Derm), outer root sheath (ORS), inner root sheath (IRS), cuticular area (Cut) and the hair shaft (Sha). A depicts the influence of lipophilicity on the relative accumulation when the fluorophores are applied in a citric acid buffer solution containing 30 % (v/v) ethanol. B shows the $f_{acc}$ values when a hydrophilic dye is applied in citric acid buffer with (EtOH) or without ethanol (CAB). $f_{acc}$ values of ≤1 imply that no accumulation is present. Stars indicate a significant difference (p<0.05) in the specific skin part of the $f_{acc}$ value from 1.
Vehicle effect

Our results indicate that the presence of 30 % (v/v) ethanol targets Oregon Green® 488 to the hair follicle as indicated by the $f_{acc}$ and $F_{rel}$ values. There are two possible influences of ethanol on the penetration route. By increasing the relative amount of dye in the epidermis, more dye is available for the diffusion via the direct connection to the outer root sheath and thereby to the hair follicle. Simultaneously the ethanol can interact with the lipids excreted into the follicular duct by the sebaceous gland making the duct more accessible for penetration. One advantage of the penetration via the follicular duct is the decreased barrier function of the stratum corneum.

CONCLUSIONS

From the diffusion studies we conclude, that ethanol (30 % (v/v) in CAB) increases the penetration rate of Oregon Green® 488 across the skin. However no conclusion can be drawn whether this influence is due to changes in permeation pathway or stratum corneum structure. At the same time, although not significant in this study ethanol seems to promote the transport of Oregon Green® 488 into the hair follicle. Increasing permeant lipophilicity to a certain degree results in an increase in penetration rate across human scalp skin as determined by diffusion studies. Simultaneously, the relative distribution in the skin is also affected by the lipophilicity of the permeating dye. A high lipophilicity of the label promotes the deposition of the label in the hair follicle. This has clearly been demonstrated by the increased $F_{rel}$ and $f_{acc}$ values. Therefore, delivery to the hair follicle can be improved by the use of a lipophilic substance. No conclusions can be drawn concerning the actual route of transport across the skin and to the hair follicle since permeation is a dynamic process. Therefore time-resolved experiments have to be performed in future.

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