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European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 239–247
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Modulation of Dihydroavenanthramide D release and skin penetration by 1,2-alkanediols

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Received 7 August 2007; accepted in revised form 9 April 2008
Available online 14 April 2008

Abstract

If a semisolid vehicle does not allow for the sufficient penetration of the incorporated drug, the addition of enhancers, e.g. glycols, is an option. Propylene glycol is most frequently applied in dermal products. Other 1,2-alkanediols like pentylene glycol were found to exhibit moisturizing effects and good anti-microbial activity. In the present study, the influence of propylene glycol and mainly butylene glycol (BuG) and pentylene glycol (PeG) on release and skin penetration of Dihydroavenanthramide D (DHAvD) was investigated. DHAvD release increased twice up to fourfold within 30 min if 2% of a mixture of BuG and PeG was added to a lipophilic as well as to a hydrophilic cream. Incorporation of single 1,2-alkanediols into the hydrophilic cream resulted in a linear slope of the released DHAvD amount with increasing chain length of the glycol. Trends found in the release model were also reflected in penetration studies on full thickness human breast skin using Franz diffusion cells. Here, the hydrophilic cream containing the BuG/PeG mixture was compared to the glycol-free reference. Already within 30 min the amount that penetrated into the viable skin layers doubled using the glycol-containing vehicle. After 300 min 12% of the applied dose was detected in the viable epidermis and dermis following application of the pure cream compared to 41% from the improved formulation. Dermal availability was further enhanced by administration of a polymer-stabilized hydrodispersion gel which also contained the glycol mixture. Due to their favorable biopharmaceutical and technological properties, longer chain 1,2-alkanediols represent a valuable class of ingredients for dermal products.

Keywords: Pentylene glycol; Butylene glycol; Skin penetration; Release; Enhancer; Dihydroavenanthramide D

1. Introduction

The choice of the appropriate vehicle for dermal administration of drugs depends on several factors, e.g. physicochemical properties of the drug itself, localization of pharmacological targets within the skin, the known penetration enhancing effect of a vehicle or single constituents as well as the state of the skin.

Traditionally, drugs are dispersed in semisolid formulations. Depending on the needs of the treated skin and the aim of the therapy, a variety of vehicles like creams and hydrogels are available. Two-phase formulations stabilized by surface-modified polymers instead of classic surfactants (e.g. hydrodispersion gels) are also of interest [1,2].

If the pure vehicle does not allow for sufficient penetration properties, the addition of enhancers can be an option. Alcohols and glycols are often used for this purpose. The most frequently applied glycol in dermal products is the propylene glycol, which is known to act as a co-solvent and penetration enhancer [3,4]. However, other vicinal diols are also available for dermal use. Because of their increased amphiphilicity they might offer a further solubi-

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doi:10.1016/j.ejpb.2008.04.005

lizing capacity for poor water-soluble drugs and, hence, could improve the penetration behavior. Also other technologically useful properties were attributed for 1,2-alkanediols. E.g., pentylene glycol was found to be an excellent moisturizer with additional anti-microbial activity [5].

This study aimed at the modulation of release and dermal availability of a model drug by propylene glycol (PrG), butylene glycol (BuG) and pentylene glycol (PeG). According to Bendas et al., a simple *in vitro* model is suited for investigating the glycol effect [6]. Therefore, this model was employed for pre-examinations on the general impact of glycols on drug release and a possible restriction to a certain type of cream (o/w and w/o). Furthermore, it should be evaluated whether the above-mentioned glycols could influence the release to a different extent. Penetration experiments were conducted to revise the respective predictions. The time dependent uptake of the model drug into full thickness human skin resulting from the administration of a glycol-free and glycol-containing formulation was compared. Besides a conventional o/w cream, a polymer-stabilized hydrodispersion gel was tested.

Dihydroavenanthramide D (DHAvD, M_w 285.3, Fig. 1) represented the model drug. Its pK_a values are given with $pK_{a1} = 3.51 \pm 0.36$ and $pK_{a2} = -1.15 \pm 0.50$, and $\log P$ is 3.34 ± 0.27 [7]. DHAvD is a stable synthetic analogue to naturally occurring avenanthramides – phenolic anthranilic acid amides present in oat and structurally very close to tranilast (*N*-(3′,4′-dimethoxycinnamonyl) anthranilic acid). The latter is an anti-allergic, anti-inflammatory and analgesic drug, which besides its oral administration in asthma and arthritis [8] has been shown to be effective in the topical treatment of skin diseases like keloids [9] and in corneal haze [10]. DHAvD itself was found to reduce histamine related skin disorders like itching, redness and wheal [11,12] and is used as an active ingredient in cosmetic products. Further anthranilic acid derivatives with increased lipophilicity, which were studied for dermal administration, are anti-inflammatory and analgesic drugs like flufenamic acid [13]. All the mentioned drugs have in common that they first need to overcome the stratum corneum as the outermost permeability barrier to reach their target structures within the viable parts of the skin or the blood circulation, respectively. Therefore, DHAvD is an appropriate model drug to investigate the effects of vehicle and potential enhancer molecules on release and skin penetration.

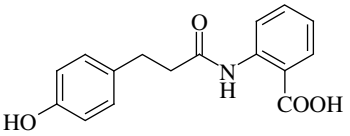

Fig. 1. Chemical structure of DHAvD.

2. Materials and methods

2.1. Materials

Chemicals and reagents were obtained from the following commercial sources: Dihydroavenanthramide D (2-[[3-(4-hydroxyphenyl)-1-oxopropyl]amino]-benzoic acid; DHAvD), 1,2-pentylene glycol (Hydrolite-5, PeG), 1,2-butylene glycol (BuG), SymCalmin (solution of 5% DHAvD in a 1:1(m/m) mixture of PeG and BuG) and hydrodispersion gel (HDG): courtesy of Symrise GmbH & Co. KG, Holzminden, Germany; propylene glycol (PrG), collodion 4% DAC: Caelo GmbH, Hilden, Germany; buffer substances, dodecanol, octanol and formic acid: Merck, Darmstadt, Germany; light liquid paraffin: courtesy of Hansen & Rosenthal KG, Hamburg, Germany; hydrophilic cream (Unguentum emulsificans aquosum, German Pharmacopoeia) and lipophilic cream (Unguentum alcoholum lanae aquosum, German Pharmacopoeia) [14]: Bombastus-Werke AG, Freital, Germany; ethanol: BfB, Wittenberg, Germany. Water was of bidistilled quality. HPLC grade methanol was obtained from Baker, Deventer, The Netherlands.

2.2. Formulations

The hydrophilic cream (HC) consisted of emulsifying cetostearyl alcohol Type A 9.0%, liquid paraffin 10.5%, white soft paraffin 10.5% and purified water 70.0%. The formulation was preserved with 0.14% potassium sorbate and was modified by the addition of 2.0% of either Sym Calmin (BuG/PeG-HC), PeG (PeG-HC), BuG (BuG-HC) or PrG (PrG-HC). For the release experiments, all the creams were prepared with 0.1% DHAvD. For analytical reasons, formulations having 0.2% DHAvD and 4.0% of SymCalmin, respectively, were used in the penetration study.

The lipophilic cream (LC) contained wool alcohols 3.0%, cetostearyl alcohol 0.25%, white soft paraffin 46.75% and purified water 50.0%, partly under addition of 2.0% SymCalmin (BuG/PeG-LC).

The hydrodispersion gel was made of PEG-9 tridecyl ether/PEG-5 octanoate 1.0%, SymCalmin 2.0% (release) or 4.0% (penetration), cetearyl octanoate 3.0%, stearic acid 2.0%, acrylates/C10-30 alkyl acrylate crosspolymer 0.25%, liquid paraffin 3.0%, octyldodecanol 4.0%, dimeticone 0.5%, sodium hydroxide 0.5% for pH adjustment (pH 6.1) and purified water up to 100.0%.

The percentage-values given represent % (w/w).

2.3. Solubilization studies

DHAvD was added in excess to the respective media and shaken for 24 h at room temperature. After filtration using a 0.45 μm pore size filter and appropriate dilution, the samples were analyzed by HPLC. The experiments were performed in triplicate.

2.4. Partition coefficient

Mixed equal volumes of buffer-saturated octanol and a stock solution of DHAvD in octanol-saturated citric acid-phosphate buffer (pH 3.1, 5.5, 6.1, and 7.4, respectively) were shaken at room temperature for 24 h. After phase separation, both phases were analyzed for DHAvD content by HPLC. The experiments were carried out in triplicate.

2.5. In vitro release studies

The release experiments were performed by means of the multilayer membrane system described in [15]. In short, three collodion-stabilized vertically arranged 4% dodecanol membranes acted as acceptor and a defined amount of 10 ± 2 mg of each formulation was applied to a 4 cm^2 area on the top membrane. After an incubation time of 10, 30, 100, and 300 min, respectively, at 32 °C, the remaining cream was wiped by a swab, the membranes were separated, and the DHAvD content of each membrane was analyzed by HPLC after extraction with 2.0 ml of ethanol. For the extraction following HC and HDG application, the membranes were shaken with the solvent for 30 min. In the case of LC, a preceding extraction step took place in an ultrasonic bath at 60 °C for 15 min. If necessary, the samples were filtered through a 0.45 μm membrane filter. A control experiment without wiping the remaining cream was carried out in order to assure of a quantitative extraction process. Due to sufficient solubility of DHAvD in dodecanol/octanol (see Table 2) sink-conditions were maintained.

For preparation of the membranes, 120 ml of the respective solution (4% (m/m) dodecanol/octanol 90/10 (m/m), 46% (m/m) ether/ethanol 85/15 (v/v) and 50% (m/m) collodion 4% DAC) was spread on a glass plate. The organic solvents were left to evaporate and from the resulting 30×50 cm membrane round pieces (4 cm diameter) were cut out.

The following formulations were tested: HC, BuG/PeG-HC, PeG-HC, BuG-HC, PrG-HC, LC, BuG/PeG-LC, and HDG. To control the DHAvD content, 10 ± 2 mg of each formulation (exactly weighted) was extracted with 3.0 mL ethanol under the same conditions as described above.

The release studies and the DHAvD content analysis were performed in triplicate.

Table 1
Number of sections and thickness of skin samples from the penetration experiments

Skin sample	Number of sections and thickness
Stratum corneum (SC)	1×10 μm
Viable epidermis (EP)	4×20 μm
Dermis 1-3 (DR1-3)	5×40 μm
Dermis 4 (DR4)	15×40 μm
Remaining dermis	

2.6. Ex vivo penetration studies

Penetration experiments were carried out using Franz type diffusion cells (Crown Glass Company, Somerville, NJ, US) under finite dose conditions [16]. The studies were performed in triplicate using full thickness human skin samples from three different female patients (44, 61, and 68 years). Breast skin was obtained after cosmetic surgery. After cleaning with 0.9% sodium chloride solution and removal of the subcutaneous fat, the skin samples were stored at −20 °C until use. Before the experiments, the skin samples were thawed and placed onto filter gauze in the diffusion cells. The dermal side of the skin was in contact with the acceptor solution (phosphate buffered saline, pH 7.4, 20.0 mL) which was stirred continuously. A defined amount of the formulation (15 ± 2 mg) was applied onto the skin surface (3.14 cm^2). After incubation (30, 300 and 1000 min) at 32 °C under semi-occlusive conditions the remaining formulation was wiped by a cotton wool tip. Three punch biopsies (each 0.2827 cm^2) were excised from each skin sample and cut in horizontal sections using a cryomicrotome (Jung, Heidelberg, Germany) as described in Table 1. Several sections were pooled to one sample to guarantee the detection of small DHAvD amounts in the skin. The collected cuts were extracted with definite amounts of methanol (150 μL for SC – DR3, 200μL for DR4 and the remaining dermis). They were vortexed for 1.5 h at the beginning and for 1 h at the end of the extraction period of 22 h and stored in a refrigerator in between. The supernatant was centrifuged for 10 min if necessary. The cotton wool tips were extracted for 8 h with 2.0 mL methanol. Since the acceptor solution contained low amounts of DHAvD, it needed to be concentrated. 2.0 mL was evaporated at 45 °C in a drying chamber and the residue was dissolved in 500 μL methanol/water 80/20 (v/v). DHAvD concentration in the different skin layers, in the cotton wool tips and in the acceptor solution, was analyzed by HPLC. The mean recovery from all experiments (calculated from the skin biopsies, acceptor and cotton wool tip, but without the filter gauze) was 77.29 ± 7.70% of the applied dose.

2.7. Analytical method

HPLC was performed on a HP 1100 (Agilent Technologies, Waldbronn, Germany) equipped with a vacuum

Table 2
Solubility of DHAvD in different media (data given as means ± standard deviation, $n = 3$; n.d.: not detectable)

Medium	c_s [mg mL^{-1}]
1,2-Propylene glycol	28.86 ± 0.69
1,2-Butylene glycol	45.99 ± 1.07
1,2-Pentylene glycol	45.79 ± 0.67
BuG/PeG 1:1 (m/m)	53.73 ± 1.08
Buffer pH 6.1	1.40 ± 0.03
Buffer pH 7.4	12.46 ± 0.29
Dodecanol/octanol	11.37 ± 0.11
Light liquid paraffin	n.d.

degasser, a binary pump, an autosampler and a diode array detector. 10 μL of each sample was loaded onto a YMC-Pack ODS-AQ column (150 × 4.0 i.d.; S-5 μm; 200 Å; YMC Europe, Dinslaken, Germany) and DHAvD was eluted by methanol/water/formic acid 60/40/0.1 (v/v) as mobile phase at a flow rate of 0.8 mL min^{-1} and a column temperature of 30 °C. Detection occurred at 224.4 nm with a reference wavelength at 360.1 nm. The limit of quantification was 0.05 μg mL^{-1}. Standard solutions were prepared similar to the samples. This means either a dodecanol-collodion membrane or a methanolic skin extract was added to prepare the respective standards.

2.8. Statistics

Statistical significance was tested by one-way ANOVA followed by Tukey's multiple comparison. The level of significance is given in the text.

3. Results and discussion

3.1. Determination of physico-chemical parameters

For physico-chemical characterization of DHAvD, solubility (c_s) in different media (Table 2) and partition coefficients at different pH values (Table 3) have been determined.

As displayed in Table 2 solubility in water is generally low and shows a pH-dependence which is a consequence of the occurring deprotonation of the carbonic acid group as well as the amide structure, which can be protonated at an appropriate pH value. Besides physiological pH of 7.4, pH 6.1 was chosen exemplarily because it represented the conditions of the model drug in one of the vehicles tested (HDG).

Glycols are known to act as co-solvents. Therefore, the solubilization capacity for DHAvD of propylene glycol, butylene glycol, pentylene glycol and a 1:1 (m/m) mixture of pentylene and butylene glycol (PeG/BuG) was investigated. Compared to PrG, the saturation solubility was increased by factor 1.5 using the higher molecular derivatives BuG and PeG. Interestingly, there was no difference in this parameter between the two more amphiphilic glycols but a significant increase using the 1:1 (m/m) mixture of them ($p < 0.01$). An optimized arrangement of solvent molecules that surround DHAvD may be assumed due to different chain lengths.

Table 3
Octanol-buffer partition coefficients of DHAvD (data given as means ± standard deviation, $n = 3$)

pH	log D
3.1	2.98 ± 0.03
5.5	1.24 ± 0.00
6.1	0.72 ± 0.01
7.4	−0.24 ± 0.01

In order to estimate the DHAvD partition in the hydrophilic and lipophilic cream, its solubility in the lipophilic phase of both formulations (white soft paraffin) had to be tested. However, soft paraffin has a high viscosity and, therefore, light liquid paraffin, a lipid of similar polarity but lower viscosity as soft paraffin, was used instead. In light liquid paraffin, DHAvD was not detectable which means a solubility of $<<0.007$ mg mL^{-1}. The results suggest that in the cream formulations, DHAvD resides mainly in the water phase. Same can be assumed for the glycols, as has been pointed out by Schnittger et al. [17].

Determination of the solubility in dodecanol/octanol was performed to make sure that sink-conditions are maintained in the release experiments as this lipid mixture acts as acceptor medium.

Knowledge of drug partitioning further helps to estimate the behavior of DHAvD between formulation and skin. The partition coefficient of an octanol-water system was shown to correlate well with the partition coefficient between human stratum corneum and water [18]. For human skin a pH gradient has been reported which is about 5.5 at the surface of the stratum corneum towards 7.4 in the epidermis regions [19]. Therefore, the partition coefficient of DHAvD has been determined at these two pH values. Further pH values were chosen depending on the environment of the substance in the formulations (HC pH 3.1, HDG pH 6.1). They should give an indication of the molecule's affinity to overcome the interface between vehicle and a lipophilic barrier, which are the lipophilic membranes of the releasing model and the skin, respectively. The results are presented in Table 3.

Comparing the log D values at the pH of HC and HDG, it can be suggested that the pH conditions for DHAvD in HC are more beneficial for passive diffusion.

A partition coefficient next to 1 as it was found at pH 5.5 is advantageous for overcoming the stratum corneum because of its bilayer structure [20]. Therefore, it can be assumed that DHAvD will not accumulate in the stratum corneum. At pH 7.4 more molecules are present in the charged than in the uncharged state, which might enhance the partitioning of the model drug towards deeper and more hydrophilic parts of the skin.

3.2. In vitro release studies

In the first part of the study, the influence of different glycols on the release of DHAvD from various emulsion-like formulations was examined. To obtain the information, whether glycols impact DHAvD release at all and if so, whether it is restricted to a certain type of cream (o/w or w/o), Hydrophilic Cream (HC) and Lipophilic Cream (LC) according to the German Pharmacopoeia [14] were chosen. BuG/PeG was used as additive because the glycol mixture showed the highest solubilization capacity for DHAvD (Table 2). The results are plotted in Fig. 2. Although LC and HC exhibited an opposed phase behavior, their releasing profiles were almost comparable and

without significant differences. The addition of BuG/PeG increased releasing rates at 10 and 30 min significantly (twice up to fourfold) for both creams. Maximum effect was detected for BuG/PeG-LC, from which 80% of the applied dose was found in the acceptor membranes after 10 min. The advantage of this vehicle over BuG/PeG-HC was significant at both short-time experiments ($p < 0.01$). Probably, the higher thermodynamic activity of DHAvD in LC due to the lower water content (50% compared to 70% in HC) enables a fast redistribution from the inner, hydrophilic phase into the outer phase of the cream and further into the membranes.

Hydrodispersion gel (HDG) is a technologically and cosmetically optimized vehicle for anti-itching DHAvD which shows a slight cooling effect on the skin due to the high amount of water. The stabilization of the emulsion-like structure is realized by a surface-modified cross-linked polymer that allows for a phase separation following application on the skin [2]. HDG was prepared with the glycol mixture for further comparison. It also demonstrated good release properties but not as excellent as BuG/PeG-LC. The release rates for HDG of 63% at 10 min and 83% at 30 min ranged between the conventional BuG/PeG-containing creams as becomes obvious in Table 4 that summarizes the short-time experiments. Interestingly, the higher pH of HDG than that of HC, which causes a more unfavorable ratio between charged and uncharged DHAvD molecules for passive diffusion (see Table 3), is not the crucial factor of the process. Possibly, the phase separation occurring in the HDG at skin temperature enables the faster release compared to the more stable emulsified system in HC. After 100 min the process was substantially finished for all formulations and can be classified as sufficient.

In the next experimental series it should be evaluated whether different glycols can improve DHAvD release to a different extent. In order to provide a wider scope for modulation, not the fasted releasing formulation but HC was chosen as vehicle. 0.1% DHAvD plus 2% of propylene glycol (PrG-HC), butylene glycol (BuG-HC) or pentylene glycol (PeG-HC) were added. Every modification led to a significant increase in the DHAvD amount that was detected in the acceptor membranes compared to the glycol-free vehicle ($p < 0.05$). After 100 min of incubation more than 94% of the substance was released from all the creams. Therefore, only the results for the 10 and 30 min experiments are depicted in Fig. 3. It becomes apparent that with increasing carbon chain length of the glycols, the amount released increased linearly. The differences are statistically significant at the second point of time ($p < 0.05$), which is illustrated by the steeper rise of the regression line. BuG/PeG-HC was additionally plotted in the graph at a formal chain length of 4.5 (Fig. 3, open symbols). It showed a slightly better release at 10 min than could be expected from the chain length, but not at 30 min. However, including the statistical spread, the results comply with the general trend.

As discussed before, the glycols are able to increase the solubility of DHAvD. Thus, they probably act as co-solvents in the applied creams. However, the solubilization capacity in the pure glycols did not exhibit the linear

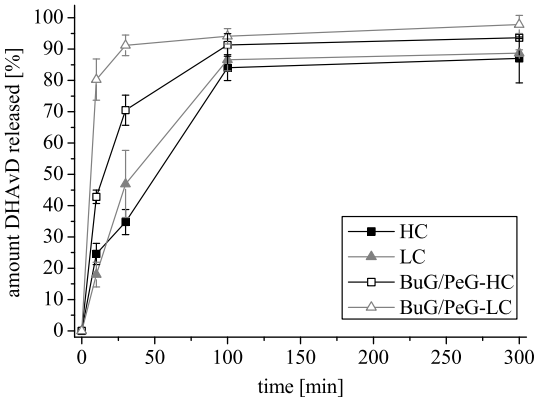

Fig. 2. Comparison of the time dependent *in vitro* release from HC and LC with the respective BuG/PeG-containing formulation (data given as means ± standard deviation, $n = 3$).

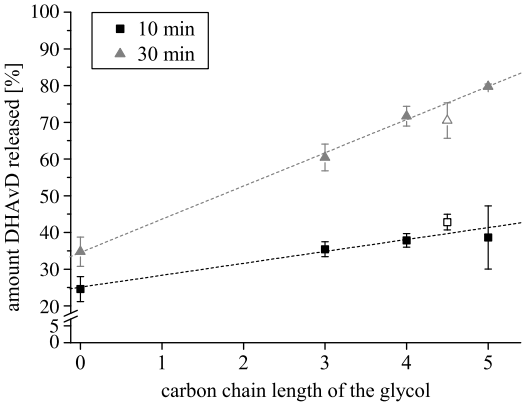

Fig. 3. *In vitro* release of DHAvD at 10 and 30 min from HC, PrG-HC, BuG-HC, PeG-HC (closed symbols), and BuG/PeG-HC (open symbols) dependent on the carbon chain length of the added glycol (data given as means ± standard deviation, $n = 3$). --- linear regression of the results from HC, PrG-HC, BuG-HC, and PeG-HC (10 min: $y = 25.10 + 3.24x$, $R^2 = 0.9907$ and 30 min: $y = 34.57 + 9.04x$, $R^2 = 0.9992$).

Table 4
In vitro release of DHAvD from HC, LC and the respective BuG/PeG-containing formulation as well as from HDG at 10 and 30 min (data given as means ± standard deviation, $n = 3$)

	LC	BuG/PeG-LC	HC	BuG/PeG-HC	HDG
10 min	17.99 ± 3.95	80.26 ± 6.59	24.58 ± 3.39	42.82 ± 2.16	63.19 ± 5.22
30 min	46.92 ± 10.72	91.19 ± 3.31	34.76 ± 4.00	70.48 ± 4.81	82.95 ± 6.24

increase the release did. This might be due to the more complex conditions in two-phase vehicles. As observed microscopically, in HC the model drug occurred partly in the suspended state. This fraction decreased with increasing solubilization capacity of the added glycols and in BuG-HC, PeG-HC and BuG/PeG-HC DHAvD was considered to be completely solubilized since no crystals were found. Hence, in the order of HC–PrG–HC–BuG-HC the concentration gradient for the solubilized fraction of DHAvD between vehicle and acceptor and, consequently, the driving force for drug diffusion increased. However, this does not explain the differences in release rates between BuG-HC and PeG-HC. Another mechanism might be involved as well. According to Bendas, propylene glycol is able to diffuse into the dodecanol-collodion membranes [6]. The homologous glycols are supposed to diffuse as well due to their miscibility with the acceptor lipid. The affinity to dodecanol might increase with the chain length owing to their rising lipophilicity, leading to a higher solvent drag effect for the respective glycol. An accompanied increase in the solubilization capacity of the lipid for DHAvD and, hence, an increase in the concentration gradient on the acceptor side is inconsiderable because sink-conditions are assured anyway. Although BuG/PeG significantly enhanced the solubility of DHAvD compared to its single components, it was not able to further increase the release. Probably, in the complex vehicle the solubility effects are not displayed to the same extent and hence the solvent drag effect is limited.

3.3. Ex vivo penetration studies

Skin penetration of DHAvD was tested by means of Franz type diffusion cells. Due to the limited availability of human skin three formulations had to be selected. By choosing HDG, HC and BuG/PeG-HC, first the effect of glycols on skin penetration can be evaluated by comparing HC and BuG/PeG-HC and second, the influence of the stabilization principle in the two glycol-containing o/w vehicles BuG/PeG-HC and HDG can be examined. The cooling effect of o/w creams is beneficial for DHAvD administration since it might improve the anti-itching effect. Therefore, the lipophilic cream is of less practical relevance.

For analytical reasons, formulations with 0.2% DHAvD and 4.0% the glycol mixture have been used in this finite dose study. By the sufficient solubility in buffer, pH 7.4, the compliance with sink-conditions in the acceptor has been verified. Following 30, 300 and 1000 min of incubation, DHAvD content was analyzed within different skin compartments (stratum corneum, viable epidermis, and dermis) as well as in the acceptor medium.

In Fig. 4 the relative DHAvD amounts achieved at different points of time following application of HC and BuG/PeG-HC within the respective compartments are depicted. Although with pure HC >12% of the applied dose was detected in the living skin layers (viable epidermis and dermis) at 300 min, there is still a need for enhancement, potentially by BuG/PeG. The more so as a strongly increased DHAvD amount within these compartments was found not until the long-term incubation of 1000 min ($p < 0.01$). Comparing both formulations now, a twice up to threefold higher fraction of the applied dose was detected in all the skin layers at any time if the glycol mixture was added. Most of the differences were significant ($p < 0.05$) and therewith the predictions of the *in vitro* release could be confirmed. Of particular importance is the increased availability of DHAvD in the vital skin compartments, because the target structures of the substance are located there. The strongest difference in favor of BuG/PeG-HC became evident at 300 min, where only 4.5% of the applied DHAvD dose was found in the viable epidermis following application of HC compared to about 15% from BuG/PeG-HC. A similar enhancing ratio appeared in the dermis with 7.5% from HC and 26% from BuG/PeG-HC.

Penetration enhancement always is a complex interaction between thermodynamic and structural effects. The increased DHAvD concentration gradient in the case of BuG/PeG-HC compared to HC due to the complete dissolved state in the first accounts for the thermodynamic part. Penetrating glycol molecules might increase the solubility for the model drug in the skin, which also increases the driving force for diffusion. Furthermore, a solvent drag effect can be taken into account. Polyvalent alcohols like propylene glycol exert influence on the SC microstructure, e.g. by solvating of the alpha-keratin and adsorption to hydrogen binding sites which reduces drug/tissue binding [21]. Also a modification of the non-polar route is discussed [22]. Brinkmann and Müller–Goymann assumed an enrichment of PrG in the hydrophilic areas of the SC bilayer structure as well as integration in lateral direction between the lipid head groups [23]. These mechanisms seem conceivable for higher alkanediols and might facilitate DHAvD uptake into the skin.

However, only a few articles are published concerning the comparison of glycol-containing and glycol-free vehicles. Mostly PrG was used. The results are controversial and often restricted to permeation. The amount of flufenamic acid, an anthranilic acid derivative with higher lipophilicity than DHAvD, detected in stratum corneum and deeper skin layers was considerably decreased when 25% PrG was added to the beforehand applied hydrogel [13]. Miller et al. varied the PrG content of a tetracaine solution (0–70%) and did not find a direct relation to the drug flux. Only addition of 40% PrG increased this parameter [24]. On the other hand, PrG enhanced the permeation of acyclovir dependent on its concentration up to an amount of 50–70%. This effect was shown for solvent systems as well as hydrogels [25]. Since in our study small quantities of glycols were incorporated into the cream, the influence of low glycol content was most interesting. Diez-Sales reported that vehicles with 10% PrG facilitated acyclovir permeation 1.5-fold compared to glycol-free formulations. However, for DHAvD permeation rate was generally low, only about

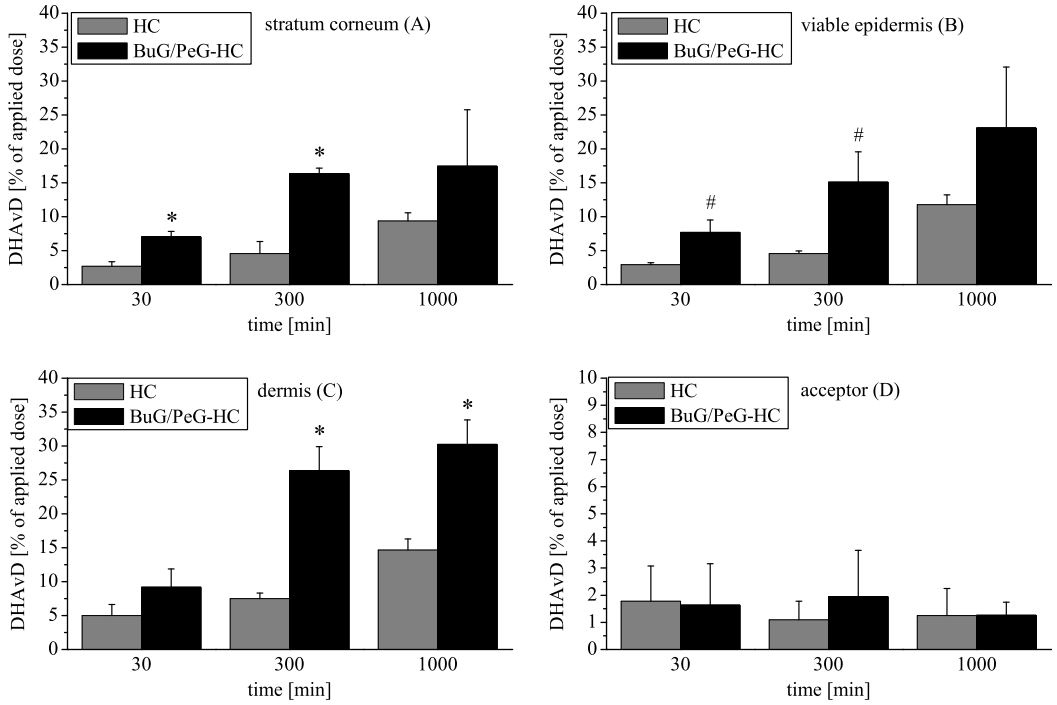

Fig. 4. Comparison of the relative amounts of DHAvD in different skin compartments (A–C) and the acceptor medium (D) following penetration from HC and BuG/PeG-HC at different points of time (data given as means ± standard deviation, $n = 3$; $^{*}p < 0.01$, $^{\#}p < 0.05$).

2% of the applied dose, but the improved penetration due to the addition of BuG/PeG was even more impressive. The distinct increase of the drug concentration in all skin layers argues for a much more potent glycol effect in the case of DHAvD.

However, PrG might not be the best predictor for the enhancing effect of higher alkanediols and their extent, respectively, as one can conclude from the trend in the release studies where PrG had the lowest (but still significant) potential among the tested glycols. Favored partitioning into the skin by the addition of BuG and PeG due to increased solubility of DHAvD in the creams as well as in the skin might play a role. This could explain why the addition of 10% PrG to an ethanolic solution of the DHAvD analogue tranilast did not increase tranilast concentration in skin microdialysate over the PrG-free solution following topical administration on rat skin *in vivo* [26]. In this study, the frequently described enhancing effect of combined oleic acid and propylene glycol occurred.

Butylene glycol has only been tested for its influence on transdermal delivery of a polar model drug before. Addition of 5% BuG did not increase the permeability coefficient of pyridostigmine bromide [27].

Recently, pentane-1,5-diol incorporated into a cream was found to enhance the skin penetration of hydrocortisone over the amount reached by PrG. However, the use of PrG led to a higher permeation rate. Lowest permeation (1% of the applied dose) was achieved without addition of an alkanediol [28]. Whereas in the mentioned study, the creams contained 25% of the enhancer, the present examinations were performed with 4% of the glycol mixture.

Another comparison was carried out between the BuG/PeG-containing o/w vehicles BuG/PeG-HC and HDG. Despite the differences in composition and submicroscopic structure, administration of both formulations resulted in enhanced dermal availability of DHAvD. However, the superior effect of HDG that was already found in the release experiments was observed again. It might result from the collapse of the gel structure (formed by acrylates/C10-30 alkyl acrylate crosspolymer) due to the contact with electrolyte-containing skin surface and, therefore, the fast release – probably of both, DHAvD and the gylcols [2]. In fact, stratum corneum contained similar DHAvD concentrations at the respective incubation times, but the penetrated amounts were increased in viable epidermis and dermis if HDG was used instead of BuG/PeG-HC (Fig. 5). Concentration levels that were obtained by the treatment with HDG at 300 min could not be overcome by BuG/PeG-HC even at 1000 min of incubation. Therefore, the comparison displayed in Fig. 5 was restricted to 30 (Fig. 5a) and 300 min (Fig. 5b). Furthermore, already at 300 min maximum DHAvD uptake in all skin layers was achieved by HDG as the more detailed concentration – depth profiles in Fig. 6 demonstrate. Time dependent dynamics of the penetration process show a steep concentration gradient that kept constant up to the next experimental time of 1000 min. However, the ongoing diffusion process was evident in the slightly increased permeation rate (0.3 μg/cm^2 at 300 min to 0.45 μg/cm^2 at 1000 min), but due to the low DHAvD concentration in the acceptor compartment after 1000 min, it can be reasoned that the diffusion rate is generally low.

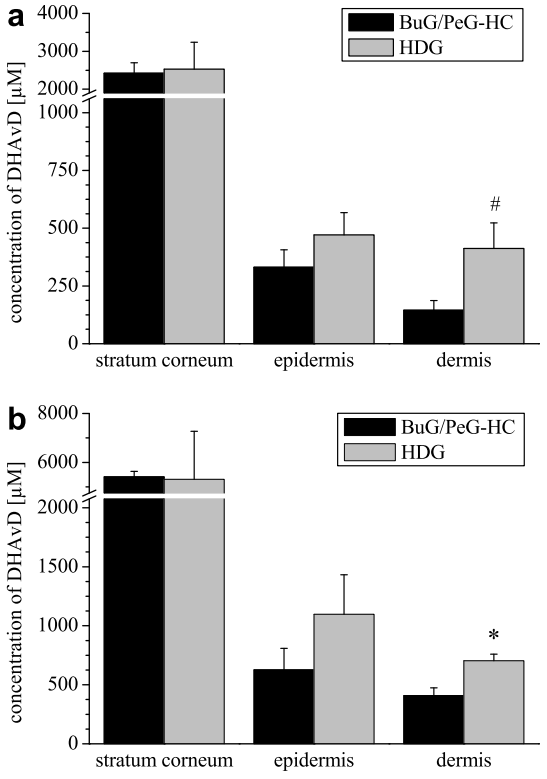

Fig. 5. Comparison of DHAvD concentration within stratum corneum, viable epidermis and dermis following application of BuG/PeG-HC and HDG at 30 min (a) and 300 min (b) (data given as means ± standard deviation, $n = 3$, $^{*}p < 0.01$, $^{\#}p < 0.05$).

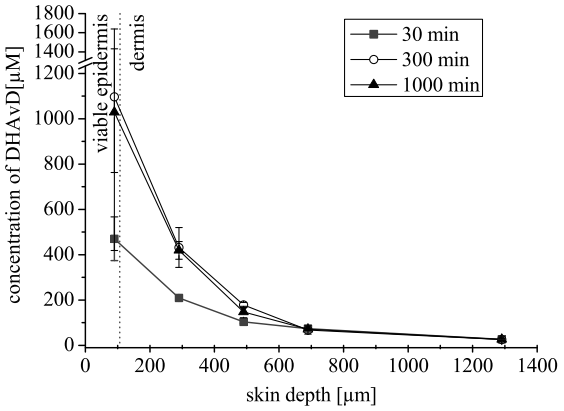

Fig. 6. Depth profiles of DHAvD in the viable skin layers (viable epidermis and dermis) at different points of time following the application of HDG (data given as means ± standard deviation, $n = 3$).

In conclusion, higher 1,2-alkanediols act as powerful enhancer of dermal DHAvD availability, even in low concentrations (4%), and represent a valuable alternative to propylene glycol. Its suitability for other topically applied drugs will have to be tested in the future.

Acknowledgements

The authors thank Marlies Lackner and Ursula Schramm for excellent technical support and Iris Hinneburg for critical revision of this manuscript.

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