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Research paper

Transdermal and dermal delivery of adefovir: Effects of pH and permeation enhancers

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Abstract

The objective of this work was to investigate feasibility of transdermal and dermal delivery of adefovir (9-(2-phosphonomethoxyethyl)adenine), a broad-spectrum antiviral from the class of acyclic nucleoside phosphonates. Transport of 2% adefovir through and into porcine skin and effects of various solvents, pH, and permeation enhancers were studied *in vitro* using Franz diffusion cell. From aqueous donor samples, adefovir flux through the skin was 0.2–5.4 μg/cm^2/h with greatest permeation rate at pH 7.8. The corresponding adefovir skin concentrations reached values of 120—350 μg/g of tissue. Increased solvent lipophilicity resulted in higher skin concentration but had only minor effect on adefovir flux. A significant influence of counter ions on both transdermal and dermal transport of adefovir zwitterion was observed at pH 3.4. Permeation enhancer dodecanol was ineffective, 1-dodecylazepan-2-one (Azone) and dodecyl 2-(dimethylamino)propionate (DDAIP) showed moderate activity. The highest adefovir flux (11.3 ± 3.6 μg/cm^2/h) and skin concentration (1549 ± 416 μg/g) were achieved with 1% Transkarbam 12 (5-(dodecyloxycarbonyl)pentylammonium 5-(dodecyloxycarbonyl)pentylcarbamate) at pH 4. This study suggests that, despite its hydrophilic and ionizable nature, adefovir can be successfully delivered through the skin.

Keywords: Skin absorption; Transdermal drug delivery; Permeation enhancer; Adefovir; Acyclic nucleoside phosphonates

1. Introduction

Adefovir (9-(2-phosphonomethoxyethyl)adenine, Fig. 1) is a broad-spectrum antiviral from the class of acyclic nucleoside phosphonates highly effective against herpes-, retro-, and hepadnaviruses. Its bis(pivaloyloxymethyl) ester prodrug adefovir dipivoxil has been approved for treatment of hepatitis B; for reviews on adefovir, see Refs. [1,2]. Due to the polar character of the phosphonate moiety, its resorption from gastrointestinal tract is restricted and there is a continuous search for new prodrug types or delivery options to enhance adefovir bioavailability and improve its pharmacokinetic profile.

Transdermal drug delivery offers numerous advantages over conventional routes of administration [3,4]. Considering the chronic nature of adefovir therapy and the requirement for a substantial commitment from the patients due to a risk of severe acute exacerbations of hepatitis on discontinuation of therapy, less frequent application associated with a transdermal patch would be advantageous. Another concern of adefovir is nephrotoxicity, which limits the oral daily dose to 10 mg of adefovir dipivoxil. Adefovir is actively transported by human renal organic ion transporter 1, which plays a critical role in its kidney toxicity [5]. This secretion is concentration-dependent, thus we hypothesize that more stable plasma levels resulting from

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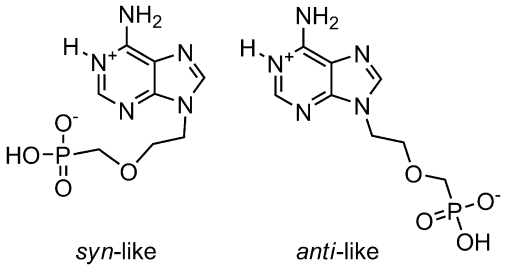

Fig. 1. The *syn*- and *anti*-like structures of zwitterionic adefovir.

transdermal delivery may be beneficial. Moreover, gastrointestinal disturbance, which is another side effect, should be avoided by administration via the skin.

In transdermal systems, variables including drug thermodynamic activity, partitioning, ionization, and ion pairing may be efficiently controlled in contrast to the absorption from gastrointestinal tract. Furthermore, skin barrier properties may be temporarily decreased by chemical substances known as permeation enhancers. These compounds may act by different mechanisms; the most active ones are believed to incorporate into the stratum corneum intercellular lipids and disturb their packing. For recent reviews on permeation enhancers, see Refs. [6–8].

Even though acyclic nucleoside phosphonates are promising drugs for the treatment of numerous viral skin infections, our knowledge on the extent of the absorption of these highly hydrophilic drugs into the skin and their flux through intact skin into the systemic circulation is limited. Currently, only one member of this group, cidofovir, has been studied with regard to dermal and transdermal transport [9–12]; there are no reports on adefovir except for our preliminary study [13].

The objective of this work was to investigate both transdermal flux and local skin concentration of adefovir and the conditions that may influence these processes including various solvents, pH, and permeation enhancers. Identification of adefovir behavior and optimization of the formulation are necessary for prospective improved administration of this potent antiviral and would be also useful for further studies on other acyclic nucleoside phosphonates.

2. Materials and methods

2.1. Chemicals

Adefovir [14] and permeation enhancers 1-dodecylazepan-2-one (Azone) [15], dodecyl 2-dimethylaminopropionate (DDAIP) [16], and 5-(dodecyloxycarbonyl)-pentylammonium 5-(dodecyloxycarbonyl)pentylcarbamate (Transkarbam 12, T12) [17,18] were synthesized as described previously. Their structure and purity was confirmed by IR and NMR spectra. KH_2PO_4, NaH_2PO_4, Na_2HPO_4, and NaCl were purchased from LachNer (Neratovice, Czech Republic). Isopropyl myristate (IPM) was purchased from Kulich (Hradec Králové, Czech Republic). Ultrapure water was obtained using Milli-Q Water Filtration System (Millipore, Bedford, MA). All other chemicals were purchased from Sigma–Aldrich (Schnelldorf, Germany).

2.2. Skin

Porcine skin was selected for the initial evaluation of adefovir delivery as it is easy to obtain and has barrier properties close to human skin [19]. Porcine ears were purchased from a local slaughterhouse. To ensure integrity of the skin barrier, ears were removed post-sacrifice before the carcass was exposed to the high-temperature cleaning procedure. Full-thickness dorsal skin was excised by blunt dissection and hairs were carefully trimmed. The skin was then immersed in 0.05% sodium azide solution in saline for 5 min for preservation. The skin fragments were stored at −20 °C up to 2 months.

2.3. Donor samples for skin permeation experiments

Donor samples were prepared by stirring 20 mg of adefovir in 1 ml of the pertinent solvent either with or without 10 mg of an enhancer. The samples were allowed to equilibrate at 37 °C for 48 h, and were re-dispersed before the application onto the skin if needed. In pH-adjusted samples, adefovir was dissolved in either 100 mM Tris or phosphate buffer (PB) at pH 7.4. pH was adjusted by 2-(hydroxymethyl)-2-aminopropane-1,3-diol (Tris) and HCl, respectively, for Tris samples and by H_3PO_4 and NaOH, respectively, for the PB-based ones, using a microelectrode HC153 (Fisher Scientific, Pardubice, Czech Republic). Samples containing T12 were prepared by dissolving adefovir in Tris, adjusting the pH at approximately 7.5 and then adding T12 to avoid decomposition of the carbamate by acidic media. This stock sample was adjusted at the desired pH.

For the determination of adefovir solubility in the donor solvent, an excess of adefovir was added to the pertinent solvent, pH was adjusted and the suspension was allowed to equilibrate. After 48 h, the suspensions were centrifuged at 10,000*g* for 5 min; the supernatant was withdrawn, diluted with phosphate buffered saline (PBS) at pH 7.4 if needed, and analyzed by HPLC (see below). Three replicates were performed in each solvent.

2.4. Skin permeation experiments

The skin permeability of adefovir was evaluated *in vitro* using the static Franz diffusion cells [20]. Generally, the use of static cells would yield the same results as the flow-through ones when maintaining the skin viability is not necessary. For a comparison of these two diffusion cell types, see Refs. [21–23]. The skin fragments were slowly thawed immediately before use and carefully inspected for any visual damage. They were cut into squares ca

2×2 cm, mounted into the diffusion cells dermal side down and sealed with silicone grease. The diffusion area was 1 cm^2. The acceptor compartment of the cell was filled with PBS at pH 7.4 with 0.03% sodium azide as a preservative and allowed to equilibrate in a 32 °C water bath for 30 min. The precise volume of the acceptor compartment (ca 18 ml) was measured for each cell and included into the calculations. Before application of the donor samples, the skin integrity was checked by measuring the electrical resistance by an LCR meter 4080 (Conrad Electronic, Hirschau, Germany). The donor sample (150 μl) was applied on the skin surface, and the donor compartment was occluded with a cover glass. The acceptor phase was stirred at 32 °C throughout the experiment. Samples of the acceptor phase (600 μl) were withdrawn at predetermined intervals over 48 h, each time being replaced with fresh acceptor phase, and analyzed by HPLC.

2.5. Concentration of adefovir in the skin

At the end of the permeation experiment (48 h), the diffusion cells were dismounted and the skin surface washed three times with 0.5 ml PBS to remove the residual donor samples. 1 cm^2 of the skin exposed to the donor compartment was punched out, blotted dry, and precisely weighed. Then it was placed into a vial with a stirring bar and extracted with 5.0 ml PBS at 32 °C for 48 h. The extract was filtered and analyzed by HPLC. Skin concentration, expressed as μg of adefovir per g of the tissue, was calculated by dividing adefovir amount by the respective skin weight. The efficiency of adefovir extraction from the skin was previously validated and was over 96% [13]. The overall mass balance values were $95 \pm 4\%$.

2.6. Stratum corneum/donor solvent distribution coefficient

The relative stratum corneum/donor solvent distribution coefficient was determined by a modified method of Kiptoo [24]. Stratum corneum sheets were prepared by trypsin treatment as described elsewhere [25] and dried in vacuo. Before the experiment, the stratum corneum sheets of ca 10 mg were precisely weighed and hydrated in 1 ml of saline with 0.03% sodium azide at 32 °C. After 48 h, the stratum corneum was withdrawn and blotted dry on a filter paper. Adefovir solution (10 μg/ml) either with or without T12 (5 μg/ml) in the pertinent solvent was added to each stratum corneum sheet (1 ml of the solution per 10 mg of the stratum corneum) and allowed to equilibrate for 24 h at 32 °C. The sample was centrifuged at 10,000*g* for 5 min, and the concentration of adefovir in the supernatant was determined by HPLC (c_{24}). The same solution without the stratum corneum was treated likewise (c_0). The distribution coefficient was determined as $(c_0 - c_{24})/c_{24}$. The concentration used in this experiment was different from those in the diffusion study. However, irrespective of the drug concentration used, the ratio derived should always be the same since the partitioning (distribution) coefficient measurement is an equilibrium phenomenon [24].

2.7. HPLC conditions

The samples were analyzed with a system consisting of a Shimadzu LC-20AD high-pressure pump, Shimadzu SIL-20AC autosampler (Kyoto, Japan), LCD 2083 UV detector (Ecom, Prague, Czech Republic), and CSW v. 1.7 for Windows integrating software (Data Apex, Prague, Czech Republic). A LiChroCART 250-4 column with Purospher STAR, RP 18e, 5 μm (Merck, Darmstadt, Germany) with a LiChroCART 4-4 guard column with the same sorbent at 40 °C was used for separation of adefovir. The mobile phase consisted of 10 mM KH_2PO_4 and 2 mM Bu_4NHSO_4 at pH 6.0 with 7% acetonitrile at a flow rate of 1.5 ml/min. The detector wavelength was set at 260 nm and the volume of injection was 20 μl. The method was previously validated [13].

2.8. Data treatment

The cumulative amount of adefovir having penetrated the skin, corrected for the acceptor sample replacement, was plotted against time. The steady state flux was calculated from the linear region of the plot and lag time by extrapolation of the linear part to *x*-axis. The skin permeability coefficient was calculated by dividing flux by donor concentration. Enhancement ratio was calculated as a ratio of the permeation characteristics, either flux or skin concentration, with and without the enhancer. The selectivity index *S* was calculated as a ratio of the adefovir amount in the skin and in the acceptor phase at 48 h. The data are presented as means ± SD obtained using 4–8 skin fragments from at least two animals. Statistical significance was determined using *t*-test or Rank Sum Test, where appropriate, for evaluation of the effect of the enhancers. ANOVA or ANOVA on Ranks with Dunn's post test was used for multiple comparisons.

3. Results

3.1. Effect of donor solvent

The permeation characteristics of adefovir formulated in various solvents are summarized in Table 1. Fig. 2 shows an example of adefovir permeation profile to illustrate that 48 h experiment was sufficient to reach steady state. Adefovir solubility in aqueous vehicles was up to 150 mg/ml, which could be associated with a risk of local toxicity. Therefore, a compromise between the maximal thermodynamic activity and therapeutic safety had to be accepted. All donor samples were prepared by dispersing 20 mg adefovir in 1 ml of the solvent. Whether the donor sample was a suspension with maximal thermodynamic activity or a non-saturated solution is indicated in Table 1 and/or in the text below.

Table 1
Influence of donor solvents and 1% permeation enhancers on the permeation characteristics and dermal absorption of 2% adefovir

Preparation (+enhancer)	Donor solubility (mg/ml)	Flux ($\mu g/cm^2/h$)	ER_{Flux}	Permeability coefficient (10^{-3} cm/h)	Skin concn ($\mu g/g$)	$ER_{Skin\ concn}$	S
Water (pH 3.4)[c]	2.56 ± 0.15[a]	1.8 ± 0.5	–	0.70	294 ± 75	–	0.8
60% PG	0.44 ± 0.01[a]	1.2 ± 0.1	–	2.72	445 ± 143	–	1.5
60% EtOH	0.28 ± 0.01[a]	2.9 ± 0.4	–	10.4	897 ± 298	–	1.2
IPM[c]	$11 \pm 1 \times 10^{-5}$[a]	0.6 ± 0.3	–	5450	971 ± 162	–	7.7
PB pH 4.8	70 ± 2[b]	1.4 ± 0.7	–	0.07	219 ± 61	–	0.9
+dodecanol	ND[b]	1.3 ± 0.4	0.9	0.07	206 ± 91	0.9	1.1
+Azone	ND[b]	2.9 ± 1.3	2.1[d]	0.14	411 ± 144	1.9[d]	0.7
+DDAIP	ND[b]	3.9 ± 2.2	2.8[d]	0.20	245 ± 92	1.1	0.4
+T12	73 ± 4[b]	5.1 ± 2.3	3.6[d]	0.27	366 ± 103	1.7	0.3
Tris (pH 4.8)	76 ± 1[b]	1.1 ± 1.1	–	0.06	145 ± 74	–	0.6
+T12	82 ± 1[b]	9.2 ± 3.7	8.4[d]	0.50	1244 ± 372	8.6[d]	0.9
PG/Tris (pH 4.8) 6:4	1.58 ± 0.02[a]	0.2 ± 0.1	–	0.10	552 ± 142	–	11.8
+T12	14.5 ± 0.2[a]	7.8 ± 3.3	49[d]	0.54	737 ± 357	1.3	0.3

Data are presented as means ± S.D. ($n = 4$–8). Enhancement ratio (ER) is a ratio of the permeation characteristics, either flux or skin concentration, with and without the enhancer. The skin permeability coefficient was calculated by dividing the flux by donor concentration. The skin concentration represents the amount of adefovir found in the skin after 48 h divided by the respective skin weight. The dermal/transdermal selectivity index (S) was calculated as a ratio of the absolute adefovir amount in the skin and that in the acceptor phase at 48 h.
ND, not determined.
[a] Suspension.
[b] Non-saturated solution; applied at 20 mg/ml.
[c] Taken from Ref. [13].
[d] Significant enhancement at $p < 0.05$.

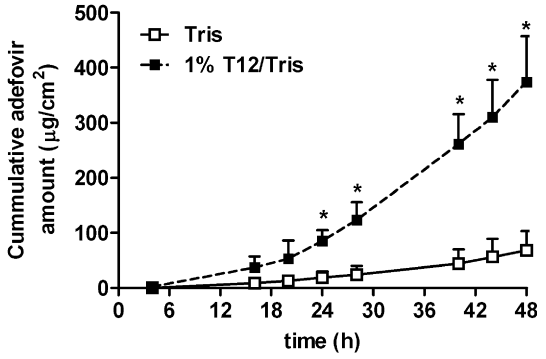

Fig. 2. Skin permeation profile of 2% adefovir from Tris (open squares) and Tris + 1% T12 (filled squares) at pH 4 (*significant difference at $p < 0.05$).

We have previously reported adefovir permeability from water and isopropyl myristate (IPM) [13]; these data are included in Table 1 for comparison. Addition of propylene glycol (PG) into the aqueous suspension increased adefovir permeability coefficient; however, the apparent flux value was slightly lower due to markedly decreased adefovir solubility in the donor solvent. Substitution of PG by ethanol decreased adefovir solubility further. However, the transport rate both through and into the skin was higher. IPM, as a representative lipophilic solvent, showed high skin concentration with low flux value. As the thermodynamic activity of adefovir in all these solvents was equal, i.e. the samples were applied on the skin as saturated suspensions, the difference in flux values may be explained by a change of the barrier properties by these solvents.

3.2. Effect of pH

As adefovir is an ionic compound, we have studied its permeability over a pH range from 3.4 to 7.8. Adefovir pK_a values are 1.2 (loss of a proton from the dihydrogenphosphonate and formation of a zwitterion),

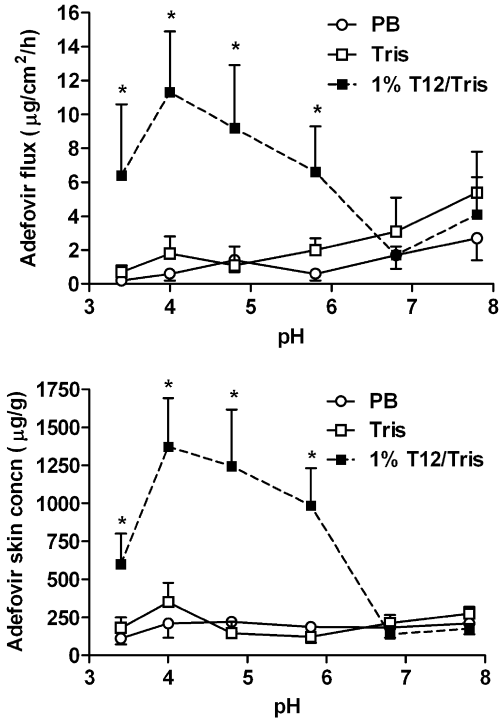

Fig. 3. The *in vitro* delivery of 2% adefovir through (upper panel) and into the porcine skin (lower panel) plotted vs. donor pH, and the influence of 1% permeation enhancer T12 (*different from Tris at $p < 0.05$).

4.2 (release of the free base at N1 and formation of a monoanion) and 6.8 (formation of a phosphonate dianion) [26]. The observed flux values were 0.7–5.4 μg/cm^2/h, and 0.2–2.7 μg/cm^2/h from Tris and PB, respectively (Fig. 3). Similarly shaped curve was observed for the skin content with values up to 350 μg adefovir per g of the tissue.

No significant difference in adefovir solubility was observed between Tris and PB (Fig. 4). The donor samples at pH 4.8 and above were non-saturated solutions with the thermodynamic activity approximately 5–7 times lower than those at pH 4 and 3.4, which seems to contradict the observed pH dependence of flux. The relative distribution coefficient between the stratum corneum and the solvent did not change significantly with pH, reaching the values of 0.060–0.088, and 0.038–0.050 in Tris and PB, respectively (Fig. 4).

Interestingly, significantly higher adefovir flux and skin content were reached from an unbuffered aqueous sample (pH 3.4) than from Tris or PB adjusted at the same pH (Fig. 5). All three samples were saturated suspensions.

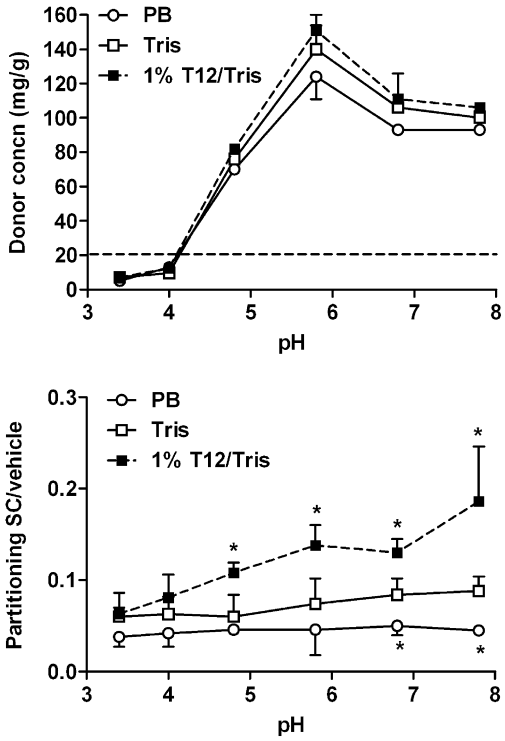

Fig. 4. The effect of T12 on adefovir solubility in the donor solvent (upper panel), and adefovir partitioning between the stratum corneum and the solvent (lower panel). *different from Tris at $p < 0.05$. Dashed line (20 mg/ml) = adefovir amount in the donor sample, i.e. the samples at pH 3.4 and 4 were suspensions and those at pH 4.8 and above were non-saturated solutions.

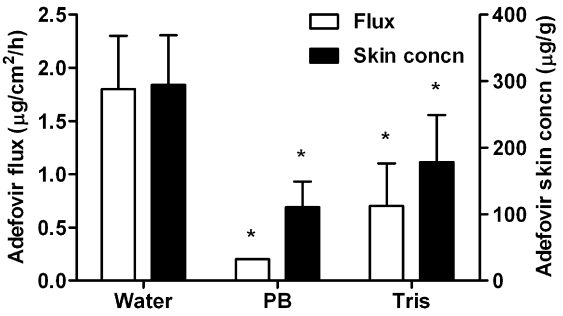

Fig. 5. The effect of counterions in the donor solvent on zwitterionic adefovir flux and skin concentration at pH 3.4 (*different from aqueous suspension at $p < 0.05$).

3.3. Effect of permeation enhancers

The influence of 1% enhancers incorporated in the formulation on the skin permeation characteristics of adefovir is shown in Table 1. Dodecanol was ineffective, Azone and DDAIP showed moderate enhancement. The highest values of both adefovir flux and skin absorption were achieved with 1% T12 in Tris. Therefore, this permeation enhancer was studied further in a greater detail. Fig. 3 shows the characteristics of adefovir permeation both in the presence and absence of T12 plotted versus donor pH. The highest values of both the transdermal permeation and dermal absorption of adefovir were found at pH 4. T12 had no effect on solubility/thermodynamic activity of adefovir in the donor sample (Fig. 4). At higher pH values, this enhancer was able to increase adefovir partitioning into the stratum corneum approximately twice. However, at pH 4, where T12 produced the greatest adefovir flux, no significant effect of T12 on the relative distribution coefficient was found (Fig. 4).

3.4. Dermal/transdermal selectivity

The last column in Table 1 reports the dermal/transdermal selectivity. $S > 1$ implies higher drug retention in the skin than permeation through it, $S < 1$ means that such formulation favors transdermal transport. From this point of view, IPM and pH-adjusted 60% PG appear to be applicable solvents for topical treatment as the adefovir content within the skin was almost 8 and 12 times higher than the amount reaching the acceptor compartment at 48 h, respectively. In other solvents these properties were roughly balanced.

4. Discussion

The purpose of this study was to identify the formulation variables including solvent polarity, pH and permeation enhancers that influence adefovir permeation through and into the skin. Currently, the only member of the acyclic nucleoside phosphonate antivirals studied with regard to the transdermal and dermal delivery is cidofovir [9–12]. The *in vivo* bioavailability of 1% topical cidofovir in rabbits was 0.2% and increased 10-fold with the addition of 10% PG to the formulation [10]. However, this positive effect of PG was not confirmed using mouse skin *in vitro* [9]. Neither this study showed any benefit in adding PG into the donor sample. Actually none of the tested solvents was able to increase adefovir flux more than twice com-

pared to the aqueous sample. Thus we have chosen water as the safest solvent for further study.

The pH dependence of adefovir permeability was studied in Tris and PB. The changes in permeability with pH were only minor, which is surprising regarding the differences in thermodynamic activity and that adefovir was present in the form of a zwitterion, mono- or dianion over this pH range. This is consistent with Aspe who reported no difference in permeation of 0.3% cidofovir at pH 4.5 and 7.0 [9]. These observations may partially be explained by the buffering capacity of the skin, i.e. the ability of the skin to buffer applied acids or bases and resist any potential damage for a certain period of time (reviewed recently by Levin and Maibach [27]). A trend towards higher flux at higher pH was observed in both PB and Tris, which may be explained by the negative influence of an alkaline environment on the skin barrier. Increased pH may, for example, ionize a greater part of the intercellular fatty acids, changing the phase behavior and packing of the barrier lipid mixture. Nevertheless, a long-term application of an alkaline formulation on the skin surface is not suitable for clinical use.

An interesting influence of the counterions was observed at pH 3.4. Adefovir was approximately twice less soluble in water than in Tris and PB at the same pH. Regardless of the solubility difference, all three samples were suspensions with adefovir at its maximal thermodynamic activity. However, the former sample displayed higher transdermal and dermal delivery parameters than the latter ones. At this pH majority of adefovir is in the form of a zwitterion with a cation at N1 of adenine cycle ($pK_a = 4.2$) and a hydrogenphosphonate anion (pK_a of its conjugate acid, i.e. dihydrogenphosphonate is 1.2). Previously, two conformers of adefovir, *syn* and *anti*-like, were identified (Fig. 1) [26,28]. The *syn*-like adefovir with an intramolecular Coulombic interaction between the hydrogenphosphonate anion and protonized N1 was found in solution. This structure might well be associated with lower solubility in aqueous media and greater permeability through the lipophilic skin barrier. The *anti* conformer with the hydrogenphosphonate anion located close to H8 of adenine was found in crystalline zwitterionic adefovir and in adefovir mono- and dianion and mimics the conformation of adenosine monophosphate. As the permeation through the skin barrier of this conformer would be more restricted, we hypothesize that the differences in permeability might be explained by different ratio of both conformers due to absence or presence of counterions.

None of the above-mentioned approaches allowed reaching usable flux values. Previously, microparticles were applied to optimize delivery of cidofovir. However, the flux (3.6 μg/cm^2/h) was lower than that from a solution (5.3 μg/cm^2/h) [11,12]. Significant enhancement of cidofovir permeation was obtained after pretreatment of mouse skin with 30% oleic acid in ethanol [9]. Thus, we focused our attention to a search for suitable permeation enhancer. As PG was ineffective, we selected three model amphiphilic enhancers that are likely to act via a different mechanism of action than PG, probably by disruption of the stratum corneum ceramide packing. The following compounds were evaluated: dodecanol – a fatty alcohol [8], Azone – a seven-membered ring lactam widely used as a standard of enhancement activity [15], and DDAIP – a clinically used biodegradable amino ester [16]. Neither of them showed considerable increase of adefovir flux. T12 is a recently described biodegradable permeation enhancer with high activity towards a variety of drugs with a wide spectrum of physicochemical properties, low toxicity, and no dermal irritability [17,18]. Addition of 1% of this compound to the donor sample allowed for reaching adefovir flux of 11.3 μg/cm^2/h. This equals 5.4 mg of adefovir absorbed from a 20-cm^2 patch in 24 h, while a tablet containing 10 mg of adefovir dipivoxil (equals 5.4 mg adefovir) with 59% bioavailability delivers 3.2 mg adefovir. This simple comparison indicates that adefovir, a zwitterionic highly hydrophilic compound, might be successfully delivered via the skin. Furthermore, only slightly lower adefovir flux values were observed at pH between 4.5 and 5.5, i.e. the physiological pH found in the upper stratum corneum layers in male and female skin, respectively [29].

To understand the nature of T12 action we studied this permeation enhancer further. T12 had no significant effect on adefovir solubility in Tris, i.e. it did not increase its thermodynamic activity. Influence of T12 on adefovir relative distribution coefficient between the stratum corneum and Tris could neither explain its enhancing activity. Thus, according to a Fick's law of diffusion, T12 action on adefovir permeation was connected with pH-dependent increase of diffusion coefficient or decrease of a diffusion path length. This is consistent with the results of Holas using theophylline as a model drug [30].

The pH dependence of T12 enhancing activity, particularly the steep decrease at higher pH values, was surprising. T12 is an ammonium carbamate, and this ionic polar head was found to be essential for its permeation-enhancing activity since the parent amino ester was completely inactive [18]. Such carbamate salts are stable at neutral or slightly alkaline pH [31,32]. During acidification, carbamic acid is released and immediately decomposed. Alkalization, on the other hand, leads to formation of a carbonate. In this study, the permeation-enhancing activity of T12 was completely lost at pH 6.8 and above although care was taken to avoid T12 degradation during the preparation of the sample. Thus, we assume that adefovir hydrogenphosphonate moiety is acidic enough to mediate T12 decomposition. Surprisingly, T12 showed high enhancing activity on adefovir permeation at acidic pH, i.e. in the form of 5-(dodecyloxycarbonyl)pentylammonium salt. This finding warrants further pH-dependence studies with different drugs.

The second part of this study focused on the topical adefovir application for the treatment of viral skin infections. Adefovir skin concentration increased with increasing lipophilicity of the donor solvents, which may have penetrated

into the skin barrier, changing its solvent properties. From the solvents evaluated, IPM and 60% PG adjusted at pH 4.8 seem to be a good starting point for further study. Although adefovir skin concentration was higher after co-application with T12, this composition would allow for considerable systemic exposure. The advantage of the above-mentioned preparations is a selective accumulation of adefovir within the skin tissue. Moreover, the EC_{50} or IC_{50} values of adefovir for e.g. herpes viruses are typically in the range of tens of micrograms per ml [33–35], hence more dilute adefovir preparations might be sufficient to reach effective skin concentrations. It must be noted that only the adefovir concentration in the whole skin was determined in this work with no special attention to its exact localization, which is currently investigated. Moreover, the skin concentrations were determined after the transdermal permeation experiment, i.e. at steady-state flux and using an infinite dose, and represent the maximum (saturation) skin concentrations achievable under the pertinent conditions. Theoretically, the skin concentrations should be similar as the skin was saturated with the drug. The widely differing values observed in this study may be explained by penetration of substantial amounts of the vehicle/enhancer probably involving the pull effect, which has recently been discussed by Bendas et al. [36] and Heard and Screen [37]. Nevertheless, further study focused particularly on dermal absorption of adefovir is necessary.

In conclusion, this preliminary study indicates that clinically relevant transdermal delivery rates and skin concentrations of adefovir can be achieved with the aid of permeation enhancer Transkarbam 12. These results may be applicable also for permeation studies with other acyclic nucleoside phosphonates.

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