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Ammonium carbamates as highly active transdermal permeation enhancers with a dual mechanism of action

Michal Novotný, Jana Klimentová, Barbora Janůšová, Karel Palát, Alexandr Hrabálek, Kateřina Vávrová *

Centre for New Antivirals and Antineoplastics, Charles University in Prague, Faculty of Pharmacy, Heyrovského 1203, 50005 Hradec Králové, Czech Republic

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ABSTRACT

Transdermal permeation enhancers are compounds that temporarily increase drug flux through the skin by interacting with constituents of the stratum corneum. Transkarbam 12 (T12) is a highly active, broad-spectrum, biodegradable enhancer with low toxicity and low dermal irritation. We show here that T12 acts by a dual mechanism of action. The first part of this activity is associated with its ammonium carbamate polar head as shown by its pH-dependent effects on the permeation of two model drugs. Once this ammonium carbamate penetrates into the stratum corneum intercellular lipids, it rapidly decomposes releasing two molecules of protonated dodecyl 6-aminohexanoate (DDEAC) and carbon dioxide. This was observed by thermogravimetric analysis and infrared spectroscopy. This step of T12 action influences drug permeation through lipidic pathways, not through the aqueous pores (polar pathway) as shown by its effects on various model drugs and electrical impedance. Consequently, protonated DDEAC released in the stratum corneum is also an active enhancer. It broadens the scope of T12 action since it is also able to increase permeation of hydrophilic drugs that prefer the pore pathway. Thus, this dual effect of T12 is likely responsible for its favorable properties, which make it a good candidate for prospective clinical use.

1. Introduction

Transdermal delivery of drugs to the systemic circulation provides a convenient route of administration. However, the application of this technique to a wider range of drugs is limited due to the remarkable barrier properties of the skin. One of the approaches to increase the number of drugs that can be effectively delivered through the skin is the use of permeation enhancers, also known as penetration/absorption promoters. These chemicals temporarily promote drug flux by interacting with protein or lipid constituents in the skin barrier, the stratum corneum (SC), or by changing the drug partitioning equilibria. For reviews on permeation enhancers, see Refs. [1–8].

Transkarbams, which are highly potent transdermal permeation enhancers bearing an unusual ammonium carbamate polar head, were discovered in the 1990s. Originally, they were designed as 6-aminohexanoic acid esters [9], i.e. open and more flexible Azone [10,11] analogs. Later it was found that these aminoesters (from which the dodecyl ester DDEAC was the most promising) trap atmospheric carbon dioxide and form double-chain ammonium carbamates, further referred to as transkarbams [12] (Fig. 1). A direct comparison of the enhancing activity of DDEAC under argon and its corresponding carbamate T12 suggested that the carbamate is, in fact, the active substance and the amino ester is inactive. Moreover, T12 showed broad-spectrum activity, biodegradability by esterase, low toxicity, and low dermal irritation, which is very favorable for its prospective clinical use [13]. Since this discovery, hundreds of synthetic modifications of T12 were performed, further confirming the importance of the labile carbamate salt [14], the presence of an ester bond [15,16] and its position [17], the presence of an unbranched alkyl chain of 10 to 12 carbons [18–20] and a flexible linking chain between nitrogen and the ester group [21].

Although ammonium carbamates have not previously been described as permeation enhancers, such compounds are well known as CO_2 carriers, e.g. in hemoglobin [22]. They have also found utility in various industrial applications, and their behavior has been well described [23]. Thus, carbamates, in general, are stable in neutral and slightly alkaline environments. In aqueous solutions, an equilibrium between carbamate and bicarbonate exists, which is sensitive to pH changes. In the presence of an acid, carbamic acid is released from its salt and immediately decomposed into CO_2 and two molecules of the corresponding amine or, more precisely, ammonium salt [12] (Fig. 1). Similar decomposition occurs at elevated temperatures [24]. Thus, we hypothesized that in SC, which is acidic in nature, decomposition of T12 would occur, releasing CO_2. Such a reaction was

Abbreviations: DDEAC, dodecyl 6-aminohexanoate; FTIR, Fourier transform infrared; HC, hydrocortisone; PA, palmitic acid; PBS, phosphate buffered saline; SC, stratum corneum; T12, 5-(dodecyloxycarbonyl)pentylammonium 5-(dodecyloxycarbonyl)pentylcarbamate (Transkarbam 12); TGA, thermogravimetric analysis; TH, theophylline; Tris, 2-(hydroxymethyl)-2-aminopropane-1,3-diol.

* Corresponding author. Charles University in Prague, Faculty of Pharmacy Hradec Králové, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic. Tel.: +420 495 067 497; fax: +420 495 067 166.
E-mail address: katerina.vavrova@faf.cuni.cz (K. Vávrová).

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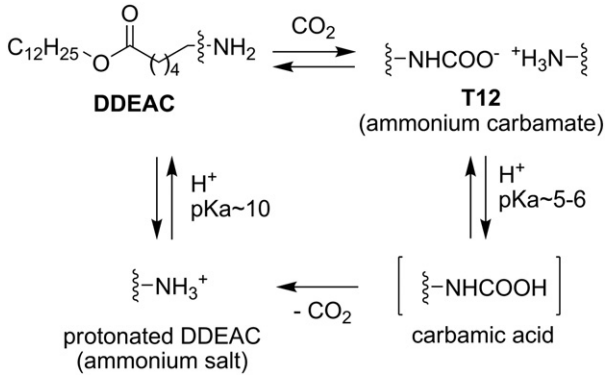

Fig. 1. pH-dependent equilibria between T12 and DDEAC.

expected to be responsible for the exceptional enhancing properties of T12, supported by the observation that stable CO_2 derivatives were inactive [14].

However, when we studied the effect of T12 on adefovir permeation at various pH values, the highest activity was, surprisingly, found at pH 4 and completely disappeared at pH 7 and higher [25]. At such an acidic pH, only protonated amino ester DDEAC and not T12 could have been present in the donor sample. Thus, the hypothesis that T12 was the only active enhancer had to be re-examined. In our recent study, these two species, T12 and protonated DDEAC, were compared [17]. The results showed that protonated DDEAC showed certain enhancing activity as well. Hence, we hypothesized that transkarbams probably act by a dual mechanism: 1) a part of their activity is likely to be associated with the carbamic acid salt and/or with its decomposition in the acidic SC, and 2) the ammonium esters thereby released continue acting as permeation enhancers.

The purpose of this work was to study the effects of T12 on the skin permeability in relation to the proposed dual mechanism of action, particularly the pH-dependence of its action by using two model drugs and skin electrical impedance. Furthermore, the suggested decomposition of the carbamate polar head of T12 in SC was investigated using thermogravimetric analysis and infrared spectroscopy of its mixture with palmitic acid and isolated SC lipids.

2. Materials and methods

2.1. Chemicals and skin

Permeation enhancers DDEAC and T12 were synthesized as described previously [9,12]. Both model drugs and all other chemicals including HPLC solvents were purchased from Sigma-Aldrich (Schnelldorf, Germany). Ultrapure water was obtained using a Milli-Q Water Filtration System (Milipore, Bedford, MA).

Porcine skin was selected for this study as it is easily available and has permeability similar to the human skin [26]. Porcine ears were obtained from a local slaughterhouse. To ensure the 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 fragments were immersed in 0.05% sodium azide solution in saline for 5 min for preservation and stored at −20 °C.

2.2. Donor samples

Control donor samples were prepared as 5% (w/v) suspensions of theophylline (TH) or 2% (w/v) suspension of hydrocortisone (HC) in an inert vehicle (propylene glycol/50 mM 2-(hydroxymethyl)-2-aminopropane-1,3-diol (Tris) 6:4 (v/v)). The vehicle was flushed with nitrogen to remove CO_2 prior to use. Samples with either DDEAC or T12 for co-application experiments were prepared by adding 1% (w/v) of the enhancer to the aforementioned drug suspensions. The sample was then adjusted to pH 3–9. In T12 samples, care was taken to avoid decomposition of the carbamate. Thus, T12 was added to the drug suspension at pH 7.5 and then the sample was carefully adjusted to the desired pH. The samples were allowed to equilibrate at 37 °C for 48 h under nitrogen and were re-suspended before application to the skin. All the samples were saturated with the appropriate model drug. Donor samples for pretreatment experiments containing 1% (w/v) T12 in propylene glycol/Tris 6:4 (v/v) at the pertinent pH without the model drug were prepared likewise.

2.3. Permeation experiments

The effects of the enhancers on the skin permeability was evaluated using Franz diffusion cells with an available diffusion area of 1 cm^2 and an acceptor volume of approximately 17 ml. 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 acceptor compartment of the cell was filled with phosphate-buffered saline (PBS) at pH 7.4 with 0.03% sodium azide as a preservative. The precise volume of the acceptor liquid was measured for each cell and included in the calculation. The Franz diffusion cells with mounted skin samples were placed in a water bath with a constant temperature of 32 °C equipped with a magnetic stirrer. After an equilibration period of 1 h, the skin integrity was checked by measuring the electrical impedance (see later).

For the co-application experiments, 150 µl (i.e. an infinite dose) of the donor sample either with or without the studied enhancers was applied to the SC side of the skin. The donor compartment was then filled with nitrogen to prevent reaction of DDEAC with CO_2 and covered with a glass slide. The acceptor phase was stirred at 32 °C throughout the experiment. Sink conditions were maintained for each drug. Samples of the acceptor phase (0.6 ml) were withdrawn at predetermined time intervals over 48 h (52 h in the case of HC) and replaced with fresh PBS.

In the pretreatment protocol, the skin first received 150 µl of the T12 samples in the inert vehicle at the pertinent pH without the drugs or 150 µl of the vehicle at the same pH as the control. After 2 h, the donors were removed and the skin surface was briefly washed with 0.5 ml of PBS. The electrical impedance was recorded and then the skin received the drug suspensions without T12.

The cumulative amount of the drug penetrated across the skin corrected for the acceptor phase replacement was plotted against time and the steady state flux was calculated from the linear region of the plot. The enhancement ratio (ER) was calculated as a ratio of the flux of the drug with and without the enhancer at the same pH value.

2.4. Electrical impedance measurement

The skin electrical impedance of the skin was recorded using an LCR meter 4080 (Conrad Electronic, Hirschau, Germany, measuring range 20 Ω–10 MΩ, error at kΩ values <0.5%), operated in a parallel mode with an alternating frequency of 120 Hz. These settings should yield the best sensitivity to small impedance changes [27]. The impedance of the skin was obtained by immersing the tip of the stainless-steel probes, one each into PBS in the donor and acceptor compartment of the Franz diffusion cell. The measurements were taken for each cell before and after (2 h) the application of the lipid samples. The data are expressed as relative impedance, i.e. impedance after/impedance before × 100.