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Transkarbams as transdermal permeation enhancers: Effects of ester position and ammonium carbamate formation

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ABSTRACT

Transkarbam 12, an ammonium carbamate formed by the reaction of dodecyl 6-aminohexanoate with carbon dioxide, is a highly active, broad-spectrum, nontoxic, and nonirritant transdermal permeation enhancer. It probably acts by a dual mechanism: a part of its activity is associated with the carbamic acid salt and/or its decomposition in the acidic stratum corneum. The ammonium ester thereby released is an active enhancer species as well, and its activity highly depends on the position of the ester group.

Transdermal delivery of drugs through the skin to the systemic circulation offers numerous advantages over the conventional routes of drug administration.[1] A disadvantage to development however, stems from the fact that the skin is a remarkably efficient barrier, designed to keep 'our insides in and the outsides out'.[2] To overcome this limitation, substances temporarily diminishing the barrier properties of the skin known as permeation enhancers or penetration/absorption promoters have been used.[2–8] These chemicals interact with constituents of the major skin barrier, stratum corneum, to promote drug flux. Although hundreds of compounds have been evaluated as permeation enhancers, to-date their use in transdermal formulations is limited since the mechanisms of action of these agents are seldom defined.[2]

Transkarbam 12 (Fig. 1) is an ammonium carbamate formed by the reaction of 6-aminohexanoic acid dodecyl ester with carbon dioxide.[9] This amphiphilic compound is a highly potent broad-spectrum enhancer with low toxicity and minimal skin irritation.[9,10] In our effort to obtain enhancers with higher activity and/or to elucidate the mechanism of action of Transkarbam 12, numerous synthetic modifications have been studied.

The structural requirements are given in Figure 1. The hydrophobic chain(s) should be 10–12 carbons long and linear because both branching and cyclization had negative effects on the enhancing activity.[11–13] The linking chain between the nitrogen and ester carbonyl should be flexible.[14] The ester group is important as well; its replacement by carbonate, carbamate, amide, ketone, and methylene group led to a marked decrease or loss of activity.[15,16] The most unusual structural feature of this enhancer is the ammonium carbamate polar head. It was suggested to be essential because the parent amino ester was inactive.[9] However, its importance for the mechanism of action of transkarbams is still unknown. In general, salts of carbamic acids are unstable in an acidic environment releasing carbon dioxide and the respective ammonium salt[17] (Fig. 1). It was suggested that such carbon dioxide release can take place in the acidic stratum corneum, and may be connected with the mechanism of action of transkarbams.[9,14] In accordance with this hypothesis, stable carbon dioxide derivatives of similar structure were inactive.[18]

The purpose of this study was to evaluate the most important structural features of Transkarbam 12 further. Since the ester group was found necessary for the activity of this enhancer, we aimed to prepare and study a series of Transkarbam 12 isomers with varying distance of the ester group from the carbamate polar

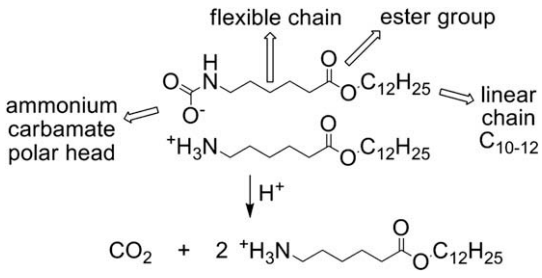

Figure 1. Transkarbam 12, its decomposition in an acidic environment, and the structural features important for its transdermal permeation-enhancing activity.

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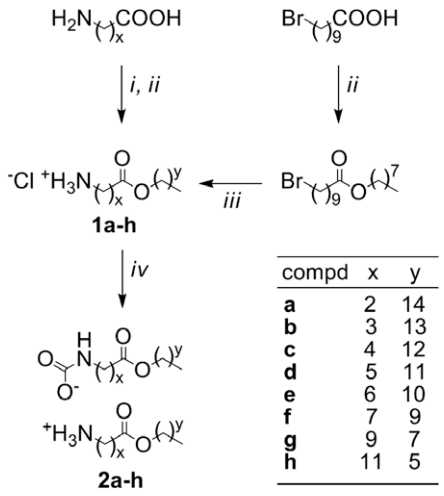

compd	x	y
a	2	14
b	3	13
c	4	12
d	5	11
e	6	10
f	7	9
g	9	7
h	11	5

Scheme 1. Synthesis of Transkarbam 12 (**2d**) and its isomers **2a**–**c** and **2e**–**h**. Reagents and conditions: (i) HCl, rt, 1h; (ii) (1) $SOCl_2$, rt, 1 h, (2) $CH_3(CH_2)_yOH$, $CHCl_3$, 60 °C, 2 h; (iii) (1) potassium phthalimide, DMF, 40 °C, 4 h, (2) $N_2H_4.H_2O$, EtOH, reflux, 4 h, (3) HCl(g), Et_2O, 30 min; (iv) (1) Et_3N, H_2O/Et_2O, 15 min, (2) CO_2, rt, 15 min.

head **2a–h** (Scheme 1). Moreover, the activities of these ammonium carbamate salts were compared to those of the respective ammonium chlorides **1a–h** to confirm the suggestion that the carbamate polar head was essential for the action of Transkarbam 12.

The target ammonium carbamates **2a–h** were prepared from the respective amino acids or bromo acid (Scheme 1). The amino acids were protonized and converted to their acyl chlorides, which immediately reacted with the corresponding alcohols to form the esters **1a–f** and **1h**.[13,19] 10-Aminodecanoic acid ester was prepared from a bromo acid via acyl chloride. The bromoester was then subjected to the Gabriel synthesis with subsequent hydrazinolysis[20] to yield the amino ester, which was stored and characterized as its hydrochloride **1g**. The ammonium chlorides **1a–h** were alkalized and reacted with carbon dioxide to yield the ammonium carbamates **2a–h**.[13,19]

The effects of the prepared enhancers on the permeation of a model drug theophylline through porcine ear skin were evaluated in vitro using Franz diffusion cells. The donor samples contained 5% (w/v) theophylline with/without 1% (w/v) enhancer in propylene glycol/water 6:4 (v/v). At this concentration, maximum thermodynamic activity of the drug was maintained throughout the experiment either with or without the enhancers. For further experimental details, see our previous reports.[21,22]

The transdermal flux values of theophylline either without (control sample) or with the respective enhancers are shown in Figure 2.

Ammonium carbamates versus ammonium chlorides. The first aim of this study was to compare the activities of the ammonium chlorides **1** and transkarbams **2**, that is, the corresponding two-chain ammonium carbamates. Similar experiment was already performed; however, it was a comparison of Transkarbam 12 (**2d**) with a free base of **1d**, not the ammonium salt. In that study, the free base showed no activity.[9] However, when studying the effects of **2d** on adefovir permeation, the maximum activity was found at pH 4.[10] At such pH value, only **1d** but not the carbamate **2d** could be present in the donor sample. Thus, we aimed at revisiting the activity of **1d** by performing a direct comparison with **2d**.

Figure 2 shows that the carbamates **2** were more active enhancers than the ammonium chlorides **1**. Nevertheless, compounds **1d–f** were not inactive as assumed before. Because all the donor samples containing compounds **1** were prepared under nitrogen and the donor compartment of the Franz cell was filled with nitrogen, the reaction with carbon dioxide and partial formation of the carbamates **2** in the donor samples of **1** was excluded. Thus, the ammonium salt formed upon decomposition of the carbamate is an active enhancer species. The previous lack of activity of the free base of **1d** may be explained by an intramolecular aminolysis of the ester by the primary aminogroup yielding caprolactam and dodecanol.

To exclude the possibility that the higher activities of the carbamates **2** compared to **1** were caused only by a different pH of the donor samples and that it may be drug-specific, another experiment was performed. Two drugs, theophylline and hydrocortisone, were used, and the donor samples containing **1d** or **2d** were carefully adjusted at pH 7.0. The effects on the flux of these two drugs are shown in Figure 3.

These results confirmed that the ammonium chlorides **1** possess enhancing activity, but they do not reach that of the carbamates **2**. Thus, a part of the permeation-enhancing potency of carbamates **2** can be attributed to the ammonium carbamate polar head and a part to the simple ammonium ester that is probably released from the carbamate in the stratum corneum.

It should be noted that although the ammonium carbamates are decomposed in an acidic environment and at elevated tempera-

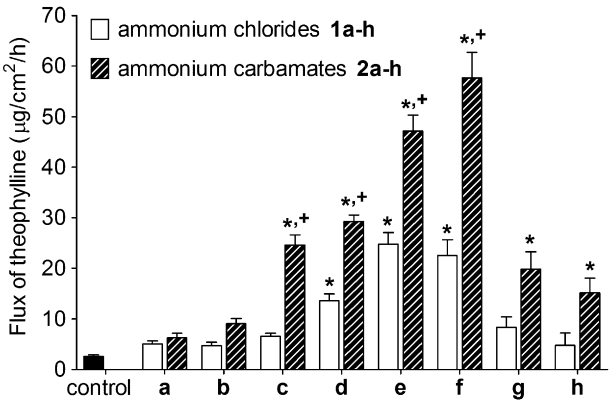

Figure 2. Effects of ammonium chlorides **1a–h** and the corresponding ammonium carbamates **2a–h** on the in vitro transdermal flux of theophylline. Mean ± SEM, $n = 5–20$. *Indicates statistically significant difference against control sample (i.e., without enhancer), +indicates statistically significant difference against the corresponding ammonium chloride.

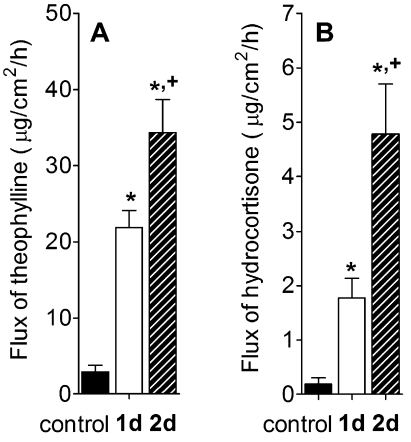

Figure 3. Effects of the ammonium chloride **1d** and the corresponding ammonium carbamate **2d** on the in vitro transdermal flux of theophylline (A) and hydrocortisone (B) at pH 7.0. Mean ± SEM, $n = 5–14$. *Indicates statistically significant difference against control sample (i.e., without enhancer), +indicates statistically significant difference against **1d**.

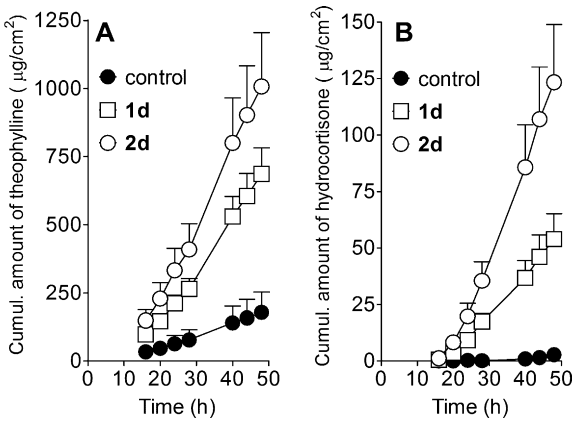

Figure 4. Effects of the ammonium chloride **1d** and the corresponding ammonium carbamate **2d** on the in vitro permeation profiles of theophylline (A) and hydrocortisone (B) at pH 7.0.

tures, they are relatively stable at neutral or slightly alkaline pH or, more precisely, equilibrium between carbamate, bicarbonate and carbonate is formed in aqueous solutions.[17] Thus, the ammonium carbamate concentration in the donor sample did not significantly change over time as reflected by stable flux values (and the ratios of flux values of the carbamates and ammonium salts) during the assay. To illustrate this, Figure 4 shows the permeation profiles of theophylline (Fig. 4A) and hydrocortisone (Fig. 4B) in the presence of **1d** and **2d**.

Ester position. Fig. 2 shows a roughly bell-shaped relationship between the ester position and the enhancing activity of both series of the studied enhancers. Within each series, the compounds are positional isomers; that is, they have the same molecular formula but differ in the position of the ester group. Compounds **1a–c** and **2a–b** having the ester group closest to the nitrogen and longest hydrophobic chain were inactive. When the ester group 'moved' towards the end of the chain, the enhancing activity increased up to compounds **e–f** with 6- to 7-carbon linker and decyl to undecyl chain and decreased thereafter.

The reason for such marked differences between these isomeric compounds is unknown at present. The most likely explanation is that the most potent isomers **1d–f** and **2d–f** possess 10–12 carbon chains, which is the ideal chain length found in many amphiphilic permeation enhancers (for a review, see Refs. 8,23). Such amphiphilic enhancers can insert into the stratum corneum lipid membranes with their polar head and hydrophobic tail into the polar and hydrophobic regions, respectively. The shorter enhancer chains (approximately half of the acyl chain length of ceramides and fatty acids present in the stratum corneum) then disturb the rigid lipid packing and increase the skin permeability for drugs.[24]

This would imply that the ester group is actually located within the polar region of the stratum corneum lipid membranes as well as the ammonium salt. The linker chain between these two groups may be either positioned within the polar head region or form a loop protruding between the lipid chains preventing their tight packing. This would require certain flexibility of the linker, which is in a good agreement with our previous work showing considerably lower activity of the tranexamic acid derivatives having less flexible cyclohexan-1,4-diyl linker.[14]

This hypothesis that both the polar groups are positioned within the polar layer of the lipid membranes and probably close to each other agrees well the previously suggested formation of an intramolecular hydrogen bond between the ester carbonyl and N–H of transkarbams.[15,16] Such interaction would lead to a 'cyclic' polar head similar to Azone, which is a well described permeation enhancer having a dodecyl chain attached to a seven-membered azepan-2-one ring.[25,26]

Another possible explanation of these results, that is, that transkarbams are only pro-enhancers releasing the respective fatty alcohols, was excluded previously.[10,13]

In conclusion, transkarbams, which are highly potent ammonium carbamate transdermal permeation enhancers, probably act by a dual mechanism. First, a part of their activity is associated with the carbamic acid salt and/or with its decomposition in the acidic stratum corneum. Consequently, the ammonium esters thereby released possess transdermal permeation-enhancing activity as well. Further investigation on the mechanism of action of these unusual enhancers is in progress.

Acknowledgements

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