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Amino acid derivatives as transdermal permeation enhancers

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ABSTRACT

Transdermal permeation enhancers are compounds that temporarily decrease skin barrier properties to promote drug flux. In this study, we investigated enhancers with amino acids (proline, sarcosine, alanine, β-alanine, and glycine) attached to hydrophobic chain(s) *via* a biodegradable ester link. The double-chain lipid-like substances displayed no enhancing effect, whereas single-chain substances significantly increased skin permeability. The proline derivative L-Pro2 reached enhancement ratios of up to 40 at 1% concentration, which is higher than that of the well-established and standard enhancers Azone, DDAIP, DDAK, and Transkarbam 12. No stereoselectivity was observed. L-Pro2 acted synergistically with propylene glycol. Infrared studies revealed that L-Pro2 forms a separate liquid ordered phase in the stratum corneum lipids and has no significant effect on proteins. L-Pro2 action was at least partially reversible as measured by skin electrical impedance. Toxicity in keratinocyte (HaCaT) and fibroblast (3T3) cell lines showed IC_{50} values ranging from tens to hundreds of μM, which is comparable with standard enhancers. Furthermore, L-Pro2 was rapidly decomposed in plasma. *In vivo* transdermal absorption studies in rats confirmed the enhancing activity of L-Pro2 and suggested its negligible skin toxicity and minimal effect on transepidermal water loss. These properties make L-Pro2 a promising candidate for potential clinical use.

1. Introduction

Transdermal drug delivery offers several advantages over conventional routes of administration, such as avoidance of the first-pass metabolism, stable plasma levels, lower incidence of side effects, and improved patient compliance. However, due to the remarkable barrier properties of the skin's uppermost layer, the stratum corneum (SC), transdermal administration has not yet achieved its full potential. One approach to enabling this route of administration for a wider range of drugs is the use of chemical compounds that temporarily increase drug flux, known as permeation enhancers or penetration/absorption promoters (for reviews, see refs. [1–6]). Although much effort has gone into the development of these compounds, their wider use in clinical practice is hampered by the fact that their mechanisms of action and their potential toxicity are still not fully understood.

Already in the 1980s, many surfactant-like compounds with C10–C12 chain length have been identified as potent permeation enhancers [7–14]; for reviews, see refs. [1,6,15]. Most of these enhancers, however, affect also viable epidermal cells provoking significant skin irritation. One of the rare exceptions to this rule is an alanine derivative dodecyl 2-(dimethylamino)propanoate (DDAIP, NexAct, [16]), probably because of its biodegradability by epidermal esterases. To identify more enhancers or their combinations with high potency and low irritation risk, Mitragotri's group developed a high-throughput screening tool based on the effect of enhancer on the skin electrical properties [17–20]. They demonstrated that there exist classes of enhancers for which potency and irritation are not particularly well related [17]. One of the compounds which displayed apparent efficacy without noticeable irritation potential was another amino acid derivative, *N*-lauroylsarcosine [21,22].

Thus, amino-acid derivatives seem to be among the most promising class of permeation enhancers, especially those with a hydrophobic "tail" attached to an amino acid "head" *via* a biodegradable linkage, e.g. an ester bond (Fig. 1A). This molecular design is advantageous due to the amphiphilic structure of such enhancer, which could allow it to incorporate into the SC lipid barrier and disrupt the tight arrangement of the membrane lipids. Then, after reaching enzymatically active nucleated epidermis, its labile bond could be hydrolyzed, thus releasing known non-toxic compounds with much lower irritation potential. This approach to designing permeation enhancers resulted in the identification of highly potent enhancers with favorable properties, such as DDAIP [16], Transkarbam 12 (T12, [23,24]), tranexamic acid derivatives [25], and dodecyl 6-(dimethylamino)hexanoate (DDAK, [26–28], Fig. 1B).

Abbreviations: Ala, alanine; Azone, *N*-dodecylazepan-2-one; DDAIP, dodecyl 2-(dimethylaminopropanoate); DDAK, dodecyl 6-(dimethylamino)hexanoate; ER, enhancement ratio; Gly, glycine; HC, hydrocortisone; IR, infrared; PBS, phosphate-buffered saline; PG, propylene glycol; Pro, proline; Sar, sarcosine; SC, stratum corneum; T12, Transkarbam 12; TEWL, transepidermal water loss; TH, theophylline.

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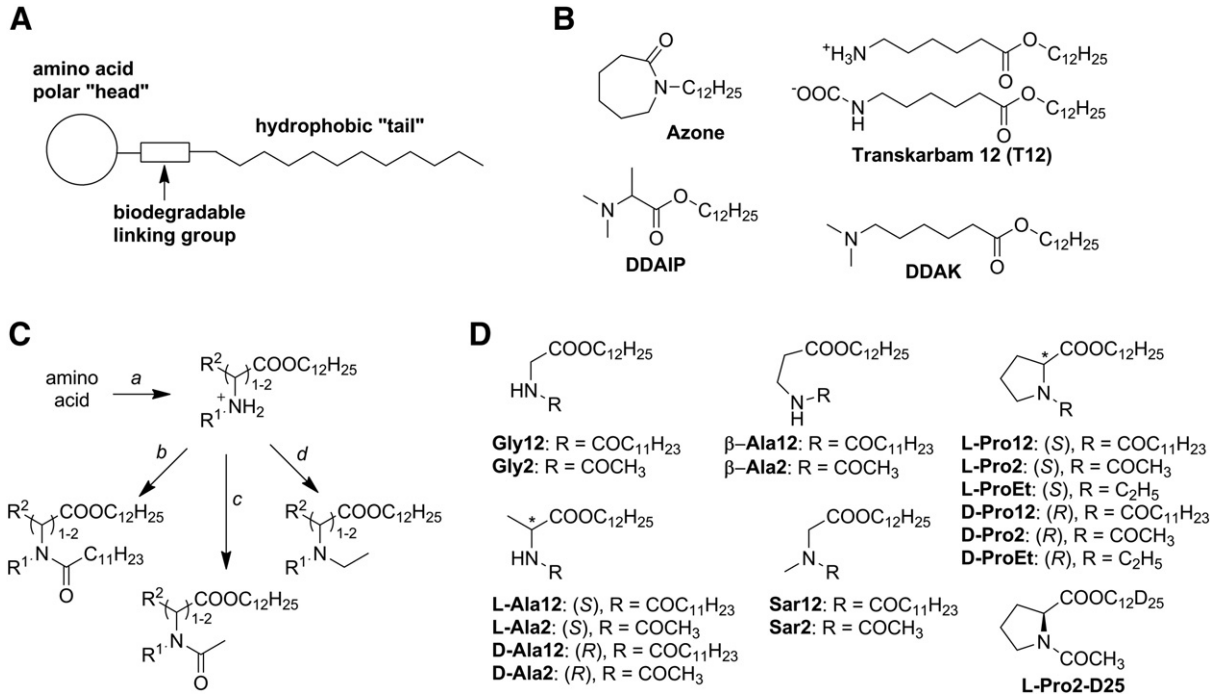

Fig. 1. Schematic representation of the design principles of the amino acid permeation enhancers (panel A), enhancers used as positive standards in this work (panel B), synthesis (panel C), and structures of the studied amino acid permeation enhancers (panel D). Reagents and conditions: *a* — dodecanol, HCl, 120 °C, 7 h; *b* — dodecanoic acid, dicyclohexylcarbodiimide, 4-dimethylaminopyridine, $CHCl_3$, rt, 20 h; *c* — acetic anhydride, 4-dimethylaminopyridine, $CHCl_3$, rt, 5 h; *d* — ethylbromide, triethylamine, tetrahydrofuran, rt, 8 h. R^1 and R^2 = H, CH_3, $-(CH_2)_3-$(Pro).

Here, we explore the use of the amino acids glycine (Gly), L- and D-alanine (L-Ala and D-Ala), β-alanine (β-Ala), sarcosine (Sar), and L- and D-proline (L-Pro and D-Pro) as headgroup components of permeation enhancers (Fig. 1D). Our interest in α-amino acids was originally based on L-serine, a starting amino acid in the biosynthesis of the key skin barrier lipids, ceramides. We hypothesized that enhancers and ceramides must bear a certain structural similarity to ensure the molecular interaction required for their enhancing effect. Thus, in a previous study, we attached two hydrophobic tails to this amino acid to mimic the ceramide structure. We found that the chain length was crucial: L-serine with 12C chains behaved as a moderate permeation enhancer [29,30] while its homolog 14S24, with the same chain lengths as in ceramides, was able to repair skin barrier perturbed by various insults [31,32]. The replacement of L-Ser by Gly, i.e., removal of the hydroxymethyl group, increased its enhancing activity, probably due to its lower ability to form hydrogen bonds [29,30].

In this study, we prepared and studied a series of double-chain enhancers based on the Gly homolog β-Ala, its isomers L-Ala and Sar, and also on the conformationally restricted cyclic amino acid L-Pro. The latter two amino acids were included to test our hypothesis that hydrogen bonding ability negatively influences the enhancing activity, and because Pro [33,34] and Sar [35] derivatives were previously reported to elicit permeation-enhancing activity. Interestingly, Gly, β-Ala, and Pro were also used to prepare prodrugs of 5-OH-DPAT for transdermal iontophoretic delivery [36].

We also prepared a series of single-chain enhancers based on the same amino acids to confirm our previous suggestion that the removal of one long hydrophobic tail increases enhancing activity. The effects of the prepared amino acid derivatives were compared with known standard enhancers including Azone [37], DDAIP, DDAK, and T12 (Fig. 1B). We also studied the reversibility of the effect of L-Pro2, the best enhancer of this group, by electrical impedance measurements, and its interaction with the skin barrier lipids and proteins by infrared spectroscopy. For this purpose, L-Pro2–D25 with perdeuterated alkyl chain was synthesized. The toxicities of selected enhancers and the possible involvement of apoptosis were assessed in keratinocyte HaCaT and fibroblast 3T3 cell cultures and compared to known enhancers. Furthermore, L- and D-enantiomers of selected enhancers were evaluated to address any potential stereoselective action/toxicity. The most potent enhancer, L-Pro2, was also studied *in vivo* in rats to confirm its enhancing properties, toxicity, effect on transepidermal water loss (TEWL) and biodegradability.

2. Materials and methods

2.1. Synthesis of enhancers

The synthetic procedures and properties of the prepared compounds including deuterated L-Pro2–D25 are given in the Supplementary data.

2.2. Donor samples for permeation studies

Control donor samples were prepared as 5% (w/v) suspensions of theophylline (TH) or 2% (w/v) suspensions of hydrocortisone (HC) in distilled water, 60% propylene glycol (PG, v/v), and isopropyl myristate, respectively. TH (mol. weight 180 g/mol, logP ~0) and HC (362 g/mol, logP 1.6) were selected as model permeability markers representing drugs of different physicochemical properties. Enhancer samples for co-application experiments were prepared by adding 1% (w/v) of the studied enhancer to the aforementioned drug suspensions. The samples were stirred at 50 °C for 5 min and then allowed to equilibrate at 37 °C for 24 h. Before application to the skin, the samples were resuspended. The concentrations were selected so that all samples were saturated with both the pertinent model drug and studied enhancer to maintain the same thermodynamic activity throughout the experiments. To determine whether the added enhancers had any effects on the solubility of the drugs in the donor solvent, the samples were prepared in triplicate as described above and allowed to equilibrate. After 24 h, the suspensions