ϵ-Aminocaproic Acid Esters as Transdermal Penetration Enhancing Agents

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The synthesis of ϵ-aminocaproic acid esters is described. Two representative members from a group of five of the 1-alkyl homologues synthetized as flexible analogues of 1-alkylazacycloheptanone derivatives were evaluated *in vitro* for their effectiveness on the transport of theophylline through the excised human cadaver skin in comparison with Azone. The 1-octyl- and 1-dodecyl-ϵ-aminocaproic acid esters (OCEAC and DDEAC) show excellent penetration enhancement. Donor samples contained 2.5% theophylline and 1% enhancers tested in three different vehicles. Fluxes of theophylline were increased with OCEAC about 19 times from olive oil, 45 times from water, and about 38 times from water–propylene glycol (3:2) vehicle toward controls (with DDEAC about 17, 39, and 35 times, respectively) and they were markedly higher than Azone under the given conditions. Acute LD_{50}'s (i.p. in mice) of OCEAC (DDEAC) were 245 mg/kg (352 mg/kg), with a slightly lower toxicity than Azone. OCEAC and DDEAC did not exhibit acute dermal irritation *in vivo* on rabbits at a 5% concentration in white petrolatum.

KEY WORDS: percutaneous absorption; penetration enhancers; ϵ-aminocaproic esters; Azone; theophylline.

INTRODUCTION

The advantages and limitations of transdermal administration have been reviewed (1,2). Approaches to make the transdermal penetration of drugs feasible include the use of penetration enhancers.

Penetration enhancers can be defined as pharmacologically inert, cosmetically acceptable substances which immediately, specifically, and reversibly lower the barrier resistance of stratum corneum (SC)[3] (3,4). A large number of substances have been tested as penetration enhancers so far. The main chemical classes and basic properties of enhancers have been summarized (5–7); yet more effective and less toxic, nonirritant and nonallergenic enhancers need to be developed. This study focuses on the development of a new group of potent nontoxic biodegradable penetration enhancers.

On the basis of Barry's structural model of SC, and a proposed mechanism of action of enhancers (8), formalized as the lipid-protein-partitioning theory (9), an efficient enhancer should be amphiphilic to interact with both lipophilic and hydrophilic components of SC. Further, we proposed that a flexible enhancer molecule might be advantageous because it may more readily interact with SC, the drug, and the components of the topical vehicle.

We considered the synthesis of such a structural type of an enhancer that would be cleaved into products of low toxicity by enzymes present in both the skin and the systemic circulation. Because of the highly active nonspecific esterases in skin, we focused on the ester-type enhancers group, in parallel to a previous study on substituted amino acetates (10). Azacycloheptane-2-ones (11), azacyclopentane-2-ones, azacyclohexane-2-ones (12), and lysine esters (13), as known enhancer types, served as the structural model for the selection and preparation of ϵ-aminocaproic acid esters (14).

MATERIALS AND METHODS

Synthesis

The hydrochloride of EAC was prepared by reaction of an aqueous solution of EAC with more than an equivalent amount of concentrated hydrochloric acid. The obtained solution was treated with charcoal for 1 hr and evaporated under vacuum. The solid was dried in a desiccator over potassium hydroxide.

Esters of EAC were prepared by reaction of chloride EAC hydrochloride with a small molar excess of alcohols. The crude ester hydrochlorides were purified and treated with triethylamine. Esters with a free primary amine function prepared in this way were allowed to crystallize.

Reagents. ϵ-Aminocaproic acid, thionyl chloride, 1-octanol, 1-nonanol, 1-decanol, and 1-undecanol, all synthesis grade, were from Merck; 1-dodecanol purum, from Koch-Light Laboratories; and triethylamine purum, from Lachema. Thionyl chloride and alcohols were distilled before use. The solvents used were of analytical grade.

Structure Characterization. The esters synthetized were characterized by CHN analysis and infrared and proton nuclear magnetic resonance. CHN analyses were made with a LP CHN1 apparatus (Laboratorní přístroje), infrared spectra were recorded on a Perkin–Elmer 577 spectrophotometer using chloroform as the solvent, and proton nuclear magnetic resonance spectra were recorded on a Tesla BS 497 spectrometer using deuteriochloroform as the solvent and tetramethylsilane as the internal standard.

EAC Octyl Ester. Five grams of EAC hydrochloride (30 mmol) was dissolved in 7 mL of thionyl chloride at room temperature. After 30 min of stirring the solution was evaporated under vacuum until dry. This resultant chloride of EAC hydrochloride was added to a solution of 4.5 g of 1-octanol (35 mmol) in 20 mL of dried chloroform. This mixture was stirred at 50°C for 1 hr and then evaporated under vacuum until dry. After the remainder of hydrochloride was removed, the crude ester hydrochloride was dissolved in 50 mL water. This solution was extracted three times with 15 mL of diethyl ether to remove the remaining 1-octanol. A colorless aqueous solution was treated with 8.3 mL of triethylamine (60 mmol). The obtained emulsion was extracted

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[3] Abbreviations used: EAC, ϵ-aminocaproic acid; OCEAC, ϵ-aminocaproic acid octyl ester; DDEAC, ϵ-aminocaproic acid dodecyl ester; OLV, olive oil; PPG, propylene glycol; W, water; SC, stratum corneum; EF, enhancement factor; ER, enhancement ratio

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five times with 15 mL of diethyl ether, the organic layer was dried with Na_2SO_4 and evaporated under vacuum. The oily product that remained was allowed to crystallize for 24 hr, the yield being 6.5 g (89%). *Anal.* Calc. for $C_{14}H_{29}NO_2$ (C,H,N%): 64.98, 12.01, 5.75. Found: 65.21, 12.32, 5.49. ^1H NMR ($CDCl_3$): δ 0.88t, J = 6, 3H(CH_3); 1.27 qs overlapping 1.2–1.8 m, 18H [$(CH_2)_6+(CH_2)_3$]; 1.86 bs, 2H(NH_2); 2.31t, J = 7, 2H(CH_2CO); 2.70 bs, 2H (CH_2NH_2); 4.05t, J = 6.5, 2H(CH_2O). IR ($CHCl_3$): 2910, 2850 (CH); 1715 (C=O); 1260, 1175 (C–O–C).

Acute Toxicity Study

The acute toxicity tests of ε-aminocaproic octyl ester (OCEAC) and ε-aminocaproic dodecyl ester (DDEAC) were carried out on experimental mice (NMRI) of both sexes. Each of the EAC esters were dispersed in water for injection at three graduated concentrations and administered intraperitoneally. The results were evaluated by probit analysis.

Dermal Irritation Study

The irritation test was performed on male and female grey chinchilla rabbits. The dorsal back hair of the rabbits was shaved in three areas of 2 × 3 cm, separated from each other by 3 cm of intact hairy skin. The esters were applied as 1 or 5% suspensions in white petrolatum, which also served as control. The semisolid formulations (0.5 mL) were administered for a period of 24 hr. Afterward they were not removed. Visual assessment of the skin reaction was made, and the results were evaluated using the modified Draize scoring system (15).

In Vitro Permeation Study

Chemicals. Theophylline, sodium acid phosphate, sodium phosphate, purum grade (Lachema, Brno), sodium azide (Fluka), distilled water (pharmaceutical grade), and laurocaprame (Azone) were kindly provided by Teijin Co., Ltd., Tokyo.

Skin Membranes. Samples of human skin were obtained from the Tissue Bank, Teaching Hospital, Hradec Králové. Strips about 300 μm thick (i.e., the epidermis with the upper part of the pars papillaris corii) were dermatomed from the front side of the thigh of the cadaver and stored at 4°C (expiration time, 4 weeks). The skin donor population included 15 males and 16 females; the average age of skin donors was 56 ± 11 years.

Permeation Experiment. The permeation-accelerating effect of OCEAC and DDEAC was estimated by a study of transdermal permeation of theophylline as a model permeant of intermediate polarity, in comparison with Azone. The permeation of theophylline across the excised human cadaver skin was measured by the "finite-dose" technique with the use of the modified glass diffusion cells based on the Franz design (16). The skin samples of appropriate size were clamped into cells. Before their placement in diffusion chambers, the skin samples were tempered for 10 min in aqueous sodium azide (0.03%) at 37°C to avoid possible wrinkles. The exposed surface of the skin between the donor and the acceptor was 2 cm^2. Skin samples were lined up in such a way that for each donor vehicle containing theophylline and an enhancer, the respective control measurement (without the enhancer) was performed with the use of the skin of about the same thickness (±5 μm) obtained from the same skin donor.

The prethermostated acceptor fluid consisting of isotonic phosphate buffer of pH 7.4 with 0.03% sodium azide was then filled into the acceptor compartment (volume, about 15 mL). Samples (0.5 mL) of the thermostated (37 ± 0.5°C) and stirred acceptor phase were withdrawn at fixed time intervals up to 32 hr and replaced with a fresh thermostated buffer.

Application of Enhancers. The donor samples (1.0 mL) always contained 2.5% of theophylline suspended (partly dissolved) in distilled water (W), in a mixture (3:2) of water–propylene glycol (W-PPG), in olive oil (OLV), or in the vehicles containing 1% of the enhancer studied (after predispersion of the enhancer in a vehicle at a temperature of 50°C). The donor samples stored at 37°C were resuspended immediately before application. The pH values of the hydrophilic donors were 6.8–7.9. Polyethylene caps were used to cover donor samples after application onto skin in order to prevent evaporation (hydrophilic samples) and to maintain the same skin temperature.

Solubility Study. The concentrations of theophylline dissolved in water, in a mixture of water–propylene glycol (3:2), and in 1% dispersions of the enhancers tested in these hydrophilic vehicles were estimated using a common procedure at 37°C, after 48 hr of equilibration of 2.5% theophylline suspensions.

Analysis. Determinations of theophylline were performed using the HPLC method. The analytical system (Laboratorní Přístroje) consisted of a sample injector LCI/30 with a 14-μL loop, a HPP 5001 pump, a LCD 2040 UV detector, and a CI 100 computing integrator.

HPLC was performed under the following conditions: mobile phase, methanol/water (1:1); flow rate, 0.5 mL/min; metal column, Lachema 250 × 4 mm with Silasorb 300 C18, 6 μm; and wavelength, 272 nm.

Data Treatment. Permeation data were evaluated by calculation of theophylline transdermal flux, J (μm/$cm^2 \cdot hr^{-1}$), using linear regression of the straight-line portion of the cumulative amounts of theophylline permeated per unit area versus time data. The level of significance of the linearization was $P \geq 0.05$ for at least four points.

Permeation results were expressed as enhancement factors (EF) calculated as the ratio of the flux value in the presence of the enhancer to the control value obtained with the use of the skin sample of comparable thickness from the same donor individual. The significance of the differences between the compared groups was tested with the use of Student t tests ($P \geq 0.05$). The permeability coefficients K_p (cm/hr) were calculated from the pseudo-steady-state fluxes and the drug solubility values (for hydrophilic vehicles only) in the respective vehicle (with or without the enhancer) at 37°C.

RESULTS AND DISCUSSION

The esters of EAC were prepared by a published method (17) which is five times less time-consuming than previous procedures (18,19). Further, it yields products in

large quantities and purity (see data in Table I) and facilitates their crystallization. The IR spectra of the prepared substances are identical in their principal features to the spectrum of the octyl ester. ^{1}H NMR spectra of these esters differ from each other only in the intensity of the signal in the chemical shift of 1.29 corresponding to the methylene groups of the alcohol moiety of ester.

Evaluation of the penetration-enhancing and biological effects focused on OCEAC and DDEAC, which yielded the best enhancing effect in preliminary experiments. Acute toxicity tests with i.p. administration in mice gave values of LD_{50} = 245 ± 42 mg/kg for OCEAC and 352 ± 76 mg/kg for DDEAC, which is similar to the i.p. toxicity of Azone (LD_{50} = 232 mg/kg) (20). The similar toxicity is interesting because the same enhancing effect occurs at a much lower concentration of evaluated esters in comparison with Azone, as shown below. In addition, it can be expected that EAC can be cleaved by skin enzymes into units of a systemic toxicity substantially lower than that possessed by the parent esters. EAC alone is only slightly toxic (21); in human therapy it is used both orally and intravenously as an antifibrinolytic agent with a maximal dose up to 30 g per day. Octanol, as the more toxic of the alcohol substituents, with some enhancing of their own, has an LD_{50} = 1.79 g/kg in mice in p.o. administration (22). A considerable surface activity of the EAC esters may be the cause of their acute toxicity which may have resulted from interactions of these esters with the peritoneal membrane.

Preliminary results show that the EAC esters tested did not produce any dermal irritation under the given experimental conditions, and therefore, overall toxicity may not limit their therapeutic use.

Typical cumulative permeation profiles of the theophylline amount across the excised skin, Q_t (mg/cm^2), from the water–propylene glycol (3:2) vehicle are illustrated in Fig. 1. Permeation profiles that signaled an interruption of the integrity of the skin membrane were excluded from analysis. With the same composition of donor samples, different skin samples differed strongly in the values of the lag period. Therefore the values of lag time were removed from the next evaluation.

Table I. CHN Data, Melting Points, and Yields of EAC Alkyl Esters

$H_2N\text{-}CH_2\text{-}(CH_2)_4\text{-}COO\text{-}CH_2\text{-}(CH_2)_n\text{-}CH_3$

	CHN (%)				
n	C, calc. found	H, calc, found	N, calc. found	Melting point (°C)	Yield (%)
6	64.98	12.01	5.75	45–49	89
	65.21	12.32	5.49		
7	69.99	12.14	5.44	51–54	85
	70.12	12.00	5.23		
8	70.80	12.25	5.16	54–56	91
	70.98	12.38	4.93		
9	71.53	12.36	4.91	57–61	90
	71.38	12.20	5.06		
10	72.19	12.45	4.68	60–65	83
	72.32	12.30	4.52		

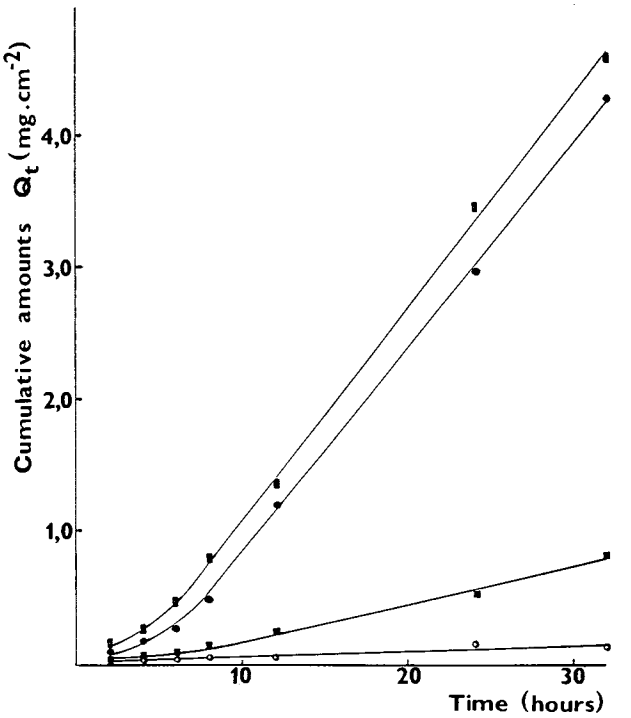

Fig. 1. Example permeation profiles of theophylline through excised human cadaver skin from the water–propylene glycol (3:2) vehicle. Open circles, control; filled squares, Azone; filled circles, DDEAC; filled rectangles, OCEAC.

The results of permeation experiments are summarized in Table II. The flux values at pseudo-steady state varied considerably as a result of interindividual variability of skin samples. This variability could also have resulted from different stretching of skin samples during fixation into permeation cells (in fact, the skin membranes are not planar as declared). The problem of "geometrical" source of variability is partly solved by the use of the enhancement ratio, ER (23,24), for nearly identical membrane samples in this study. A better solution of the problem presumes, however, the expression of the enhancement factors in connection with other permeation techniques [e.g., flow-out techniques following the method of Cooper and Bernet (25)] and/or a different experimental protocol as well.

The mean values of the flux of theophylline from an oil vehicle with the addition of 1% Azone did not differ from the controls at $P > 0.05$) (Table II). This result agrees with previous data showing that a hydrophobic environment (particularly as far as paraffinic hydrocarbons are concerned) inhibits the enhancing properties of Azone (26). As we intended to compare the effect of Azone at the same 1% concentration as the esters tested (equimolar with regard to DDEAC), a mixture of water and propylene glycol, 3:2, was used as the hydrophilic vehicle because the combination of propylene glycol and Azone is known to produce a marked increase in the permeation of different substances (27). The expected effect was achieved; the values of the flux of theophylline from these donor samples significantly differed from controls and the mean value of the EF was increased over 5. In the water–propylene glycol vehicle, OCEAC and DDEAC were 7 times and 6.5 times, respectively, more ef-

Table II. Effect of EAC Octyl Ester (OCEAC), EAC Dodecyl Ester (DDEAC), and Azone on *in Vitro* Theophylline Fluxes Across Human Skin

Vehicle/enhancer	n^a	N^b	Flux ($\mu g/cm^2/hr$)[c]	SE[d]	ER[e]	SE	K_P [(cm/hr) $\times 10^4$][f,g]
OLV	14	12	1.1	0.4			—
OLV/OCEAC	8	6	20.5	2.6	18.6	1.6	—
OLV/DDEAC	8	6	18.4	3.5	16.7	1.6	—
OLV/Azone	7	6	1.2[h]	0.3	1.1	0.2	—
W	15	12	2.2	0.6			2.7
W/OCEAC	8	7	100.2	7.6	45.5	4.2	73.0
W/DDEAC	8	7	86.5	9.6	39.3	3.8	91.0
W-PPG	14	12	2.8	0.6			2.9
W-PPG/OCEAC	8	6	106.0	9.2	37.8	3.3	72.0
W-PPG/DDEAC	8	7	98.9	13.2	35.0	4.0	92.0
W-PPG/Azone	7	6	15.2	3.1	5.4	0.3	1.5

[a] Number of replicates.
[b] Number of different skin donors.
[c] Mean of the fluxes.
[d] Standard error of respective means.
[e] Mean of the enhancement factors obtained by dividing the individual flux values by the respective controls using the same skin donor.
[f] Mean of the permeability coefficients.
[g] Coefficients of variation of K_p values are the same as the analogue values of fluxes.
[h] Not different from control ($P \geq 0.05$).

fective than Azone and increased the mean values of theophylline fluxes approximately 37 and 35 times, respectively, in comparison with the control. Similarly favorable results can be found in tabulated data for the other vehicles also.

In conclusion, both OCEAC and DDEAC under the given conditions enhanced the permeation of theophylline significantly more than Azone. They showed a marked enhancement not only in W-PPG and water vehicles but also, in contrast to Azone, in an oil vehicle.

In the group of EAC esters, two substances were shown to have a penetration-enhancing effect. Their synthesis by the method described in the present paper is relatively easy and provides access to a large number of similar structures.

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