Ionized Prodrugs of Dehydroepiandrosterone for Transdermal Iontophoretic Delivery

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Purpose. The aim of this work was to synthesize ionized dehydroepiandrosterone (DHEA) prodrugs with higher water solubility, useful for iontophoretic transdermal application.

Methods. The synthesized derivatives were characterized and tested for sensitivity to chemical and enzymatic hydrolysis. Solid state and solution stability was also determined. Transdermal iontophoretic anodal transport in vitro was studied using excised rabbit skin.

Results. Two DHEA ionized prodrugs were synthesized: PRO1, a primary amine derivative, and PRO2, a quaternary ammonium salt. The two derivatives possess higher water solubility and lower octanol/saline partition coefficients than DHEA. Prodrugs were sensitive to enzymatic hydrolysis; in particular the primary amine was hydrolyzed faster than the quaternary salt by esterase from porcine liver in vitro. Transdermal flux of the two prodrugs was slightly higher than the parent drug. In the case of passive diffusion, only DHEA was found in the receptor compartment, indicating the complete breakdown of the prodrug in the skin. Current application gave higher drug flux and a significant amount of prodrug was found in the receptor.

Conclusions. The use of ionized prodrugs of DHEA can increase the flux attainable during transdermal anodal iontophoresis by up to 7 times, but they are useful for passive transport as well.

KEY WORDS: transdermal; iontophoresis; prodrug; DHEA; skin.

INTRODUCTION

Dehydroepiandrosterone (DHEA), a naturally occurring adrenal steroid hormone, has received attention after the discovery that its endogenous level declines with aging (1). The restoration of serum levels by exogenous administration has been associated with physical and psychological well-being (2). DHEA administration has also been suggested for post-menopausal diseases (3), AIDS progression in HIV-1 infections (4), cardiovascular diseases (5) and depression (6). The hormonal therapy of age-related diseases using DHEA requires constant or pulsatile long-term administration. DHEA dosage forms suitable for long term therapy still have to be developed, since the only available route of administration is the oral, which suffers for an important first pass effect and low and variable bioavailability (7). The daily oral dose (25–50 mg/day) suggests that transdermal delivery could be an appropriate route for DHEA, in order to maintain plasma levels in the order of 3–5 μg/ml (7), with increased patient acceptability. In addition, transdermal iontophoresis, i.e. the application of electric current to enhance drug transport through the skin, could allow the attainment of high fluxes and/or pulsatile delivery. In order to provide high drug flux through the skin, a high concentration reservoir is required. However, the low water solubility of DHEA makes the preparation of a high concentration aqueous reservoir difficult. Moreover, DHEA is not ionized and this is an obstacle to iontophoresis, even if current application is able to increase to some extent the transdermal flux of un-ionized molecules (8).

A solution for these difficulties linked to reservoir preparation and drug transport could be found by synthesizing ionized prodrugs, that can provide the physico-chemical properties lacking in the parent drug, such as solubility and ionization characteristics.

The aim of this work was to synthesize positively ionized DHEA derivatives, highly water soluble and easily degradable by enzymes in the skin, useful for the enhancement of iontophoretic transdermal delivery. The derivatives were characterized and tested for sensitivity to chemical and enzymatic hydrolysis. Anodal transdermal iontophoretic transport was measured in vitro through excised ear rabbit skin.

MATERIALS AND METHODS

Materials

DHEA (m.w. 288.4, mp 147°–149° C) was obtained from Sigma (Sigma Chemical, St. Louis, MO, USA). For iontophoretic electrode preparation (9), silver wire (diameter 1.0 mm) and silver chloride (Sigma Chemical) were employed.

Saline was used as donor solution. Isotonic phosphate-buffered saline (IPBS) pH 7.4, prepared with 5.98 g of disodium hydrogen phosphate dodecahydrate, 0.19 g of potassium di-hydrogen phosphate and 8.0 g of sodium chloride in water to 1000 ml (ionic strength 0.154 M), was used as receptor solution.

Synthesis and Identification of DHEA Derivatives

Two DHEA derivatives were synthesized according to the following procedure. The course of reaction and purity of products were controlled by TLC, using pre-coated silica gel plates (Merck 60 F254) and chloroform/methanol mixtures as eluents. Preparative separations were performed in columns containing Merck 60 silica gel (70–230 mesh ASTM). Melting points were determined with a Kofler hot stage microscope and are uncorrected.

Elemental analysis results were within ±0.4% of the theoretical values. ^{1}H-NMR spectra were recorded using a Bruker WM-250 spectrometer (Bruker Analytik GmbH, Silberstreifen, Rheinstetten, D) or a Bruker AMX-500 instrument, equipped with a Bruker X-32 computer. Chemical shift values are reported in d units (ppm) relative to TMS used as internal standard. The following symbols were used to indicate the multiplicity of signals: s = singlet, bs = broad singlet, d = doublet, m = multiplet. Reagent grade chemicals and solvents were used.

N-Tert-butoxycarbonyl-glycine-3 β-hydroxy-5-androsten-17-one ester (**3**)

A solution of 1 g (3.5 mmol) of 5-androsten-3b-ol-17-one (**1**) in 17.5 ml of anhydrous dichloromethane was added with

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0.8 g (4.5 mmol) of boc-glycine (**2**) dissolved in dichloromethane and then with 0.54 g (4.5 mmol) 4-dimethylaminopyridine (DMAP). The solution was treated with a dichloromethane solution of N-N'-dicyclohexylcarbodiimide (DCC) (1 g, 5 mmol) and stirred at room temperature for 48 h. The reacted solution was treated with 50% acetic acid aqueous solution (5 ml) and the precipitate was filtered off. The solvent was removed *in vacuo* and the residue dissolved in 100 ml of ethylacetate. The solution was washed twice with water, twice with 1N $KHSO_4$ solution, twice again with water, twice with $NaHCO_3$ saturated solution and, finally, twice with water. The organic solution was dried on anhydrous Na_2SO_4 and then evaporated *in vacuo* to dryness. The crude product was recrystallized from n-hexane to obtain 1.1 g of **3** (yield 69%), m.p. 186–188°C. Anal ($C_{26}H_{39}NO_5$) C,H,N. ^{1}H-NMR ($CDCl_3$): d 6.2 (d, 1H, H-6, J = 13 Hz), 5.7 (bs, 1H, NH), 5.3 (m, 1H, H-3, J = 41 Hz), 4.4 (d, 2H, CH_2), 1.5 (s, 9H, boc), 1.0 (s, 3H, CH_3-18), 0.7 (s, 3H, CH_3-19).

Glycine-3β-hydroxy-5-androsten-17-one ester (**4**)

A solution of 4.2 g (9.5 mmol) of **3** in 200 ml of dichloromethane were added together with 40 ml of 40% trifluoroacetic acid aqueous solution and stirred at room temperature for 3 h. The organic solvent was removed under reduced pressure obtaining an oil which was added with a small amount of diethylether. The precipitate obtained was filtered and dried to afford 2.2 g (yield 67%) of **3**, mp 198–200°C. Anal ($C_{21}H_{31}NO_3$) C,H,N. ^{1}H-NMR ($CDCl_3$): d 8.2 (bs, 2H, NH_2), 5.4 (d, 1H, H-6, J = 11 Hz), 4.6 (m, 1H, H-3, J = 42 Hz), 3.8 (s, 2H, CH_2), 1.05 (s, 3H, CH_3-18), 0.8 (s, 3H, CH_3-19).

Glycine-3β-hydroxy-5-androsten-17-one ester hydrochloride (**5**) - (**PRO1**)

A mixture of 2 g (5 mmol) of **4** and 50 ml of water was added slowly with 6.5 ml of 36% aqueous HCl obtaining a jelly suspension which was extracted three times with chloroform. The organic extract was dried on anhydrous Na_2SO_4 and evaporated *in vacuo* to dryness. The residue was washed with diethylether to obtain the pure product **4** (1.65 g, yield 74%), mp > 200°C dec. Anal ($C_{21}H_{32}$ $NClO_3$) C,H,N,Cl. ^{1}H-NMR ($CDCl_3$): d 8.4 (bs, 3H, NH_3^+), 5.4 (d, 1H, H-6, J = 12 Hz), 4.6 (m, 1H, H-3), 3.9 (s, 2H, CH_2), 1.05 (s, 3H, CH_3-18), 0.9 (s, 3H, CH_3-19).

N,N-Dimethyl glycine-3β-hydroxy-5-androsten-17-one ester (**7**)

A solution of 1 g (3.5 mmol) of 5-androsten-3b-ol-17-one (**1**) in 17.5 ml of anhydrous dichloromethane was added with 0.46 g (4.5 mmol) of N,N-dimethylglycine (**6**) dissolved in dichloromethane and then with 0.54 g (4.5 mmol) of 4-dimethylaminopyridine (DMAP). The solution was then treated with a dichloromethane solution of N,N'-dicyclohexylcarbodiimide (DCC) (1 g, 5 mmol) and stirred at room temperature for 66 h. The reacted solution was treated as for compound **2**, to obtain 0.8 g (yield 60%) of **7**, mp 178–180°C. Anal ($C_{23}H_{34}NO_3$) C,H,N. ^{1}H-NMR ($CDCl_3$): d 5.4 (d, 1H, H-6, J = 13 Hz), 4.8 (s, 2H, CH_2), 4.7 (m, 1H, H-3, J = 41 Hz), 3.6 (s, 6H, $(CH_3)_2$-N), 1.0 (s, 3H, CH_3-18), 0.8 (s, 3H, CH_3-19).

N,N,N-trimethyl-3 β-hydroxy-5-androsten-17-one ester iodide (**8**) - (**PRO2**)

A solution of 0.8 g (2.8 mmol) of **7** in 20 ml of dichloromethane was added with 0.2 ml (3.8 mmol) of CH_3I. The reacted solution was stirred at room temperature for 48 h. We obtained a precipitate that was filtered and dried to afford 0.8 g of **8** (yield 54%), mp > 200°C dec. Anal ($C_{24}H_{37}NIO_3$) C,H,N,I. 1H-NMR ($CDCl_3$): d 5.4 (d, 1H, H-6, J = 13 Hz), 4.7 (s, 2H, CH_2), 4.65 (m, 1H, H-3, J = 41 Hz), 3.7 (s, 9H, $(CH_3)_3$-N^+), 1.0 (s, 3H, CH_3-18), 0.9 (s, 3H,CH_3-19).

HPLC Analysis

A new HPLC method was set up, in order to analyze simultaneously the prodrugs and DHEA, using a reversed phase column (Waters, NovaPak C8 - 3.9 × 150 mm, Millipore Corporation, Milford, MA, USA) and a Perkin Elmer HPLC System (Norwalk, CT, USA). The mobile phase was a mixture of 670 ml of 0.01 M KH_2PO_4 (containing 2 ml of 85% w/w H_3PO_4 per liter) and 330 ml of acetonitrile, at flow rate of 1.5 ml/min.

DHEA, PRO1 and PRO2 peaks were detected at 210 nm, with a retention time of 10, 5 and 7 minutes, respectively. The analytical system suitability was assessed according to the USP23, by checking the reproducibility, tailing factor, theoretical plates and resolution. The reproducibility was expressed as relative standard deviation and resulted <2.0%. The number of theoretical plates was about 4,000, with a resolution factor of 13 for PRO1-DHEA and 4 for PRO2-DHEA. The tailing factor was 1.0 for PRO1, 1.1 for PRO2 and 0.98 for DHEA. The specificity (absence of interfering peaks derived from the skin samples) and sensitivity were assessed as well. The limit of quantification was 3.5 μM.

Stability

Prodrugs stability in the solid state was checked performing accelerated stability tests. Samples of each prodrug, in sealed glass vials, were maintained at 3 different temperatures (37, 55 and 70°C - 60% RH) for 3 weeks. The remaining nondegraded prodrug was determined at appropriate time intervals by HPLC analysis and plotted as a function of time, in order to calculate the first-order rate constant, k. The stability at 20°C was then extrapolated from the log k versus 1/T plot (Arrhenius Plot).

The stability of the prodrugs in the solid state was checked in normal conditions as well, storing samples of the prodrugs at room temperature with 60% RH, in sealed glass vials. The prodrug content was checked every six months for 30 months.

Solution stability was checked as well, in order to know the degradation of prodrugs during the transport experiments.

Solubility, pKa, and Partition Coefficient

The equilibrium solubility was measured by suspending 20 mg of product (either DHEA or its prodrug) in 10 ml of saline at room temperature. After attainment of equilibrium (24 h) the suspension was filtered (0.45 μm nylon filter, Lida, Kenosha, USA) and the solution was analyzed by HPLC.

pKa was determined by potentiometric titration (10).

Octanol/saline apparent partition coefficient was measured according to (11). Briefly, 9.5 ml of a drug solution in saline

(25 μg/ml) was equilibrated with 0.5 ml of octanol at room temperature. After centrifugation and separation, drug concentration in the aqueous phase was determined by HPLC. The drug concentration in the organic phase was calculated from that found in the aqueous phase.

In Vitro Hydrolysis

The in vitro hydrolysis sensitivity of the prodrugs was carried out using esterase from porcine liver (Sigma Chemical). The experiments were conducted in the conditions indicated by the producer as the optimum for esterase activity (room temperature, pH 8.0). A 0.1 mM solution of the prodrug in pH 8.0 buffer (made by mixing 50 ml of 0.2 M potassium dihydrogen phosphate with 46.8 ml of 0.2 M sodium hydroxide and adding water to 200 ml), was used. The experiments were performed at room temperature, using esterase at increasing concentrations, from 0.5 to 16.67 IU/ml. At pre-determined time intervals, 100 μl of the reaction mixture was withdrawn, diluted with 100 μl of distilled water and rapidly frozen, to stop the enzymatic reaction. After thawing and filtration, the concentrations of DHEA and prodrugs were measured by HPLC.

In Vitro Transdermal Iontophoresis

For in vitro transport experiments, Franz-type diffusion cells (0.6 cm^2 area) were used (DISA, Milan, I). Ear rabbit skin was used as barrier. The skin was removed from the inner side of the rabbit ear, obtained from a slaughter house, within 2 h of death, and mounted with the corneal site facing donor chamber (12).

The receptor chamber contained 4.2 ml of isotonic phosphate-buffered saline pH 7.4 and the donor chamber contained 1 ml of saturated solution of DHEA, PRO1 or PRO2 in saline. Receptor compartment content was magnetically stirred to avoid any boundary layer effect. All the experiments were performed at room temperature.

Electric current was applied using AgCl-coated Ag wire for the cathode (receptor compartment) and the same electrodes, electrochemically reduced in sodium chloride solution (9), for the anode (donor compartment). Constant direct current, 0.5 mA/cm^2, was applied for 7 h by a current supply module (Ionol, Cosmic, Pesaro, I). At pre-determined time intervals, 300 μl of the receptor solution were sampled and replaced with the same volume of fresh buffer.

Passive diffusion experiments were also performed.

RESULTS AND DISCUSSION

DHEA prodrugs were synthesized according to the reaction schemes reported in Fig. 1. PRO1 is a primary amine derivative, while PRO2 is a quaternary ammonium salt of DHEA.

Stability

The stability of the two prodrugs was checked both in the solid state and in aqueous solution. Arrhenius plots of the two prodrugs are reported in Fig. 2, where the degradation kinetic constants of solid drugs obtained from the data at three temperatures, are plotted as a function of 1/T. Room temperature degradation kinetics calculated from Arrhenius plot, predicted that the half-life at 20°C was 6 months for PRO1 and 11 months for PRO2. The degradation observed was referred to the hydrolytic generation of DHEA that quantitatively corresponded to the amount of prodrug, which disappeared. No degradation of DHEA was observed.

The solid state stability was also evaluated in normal conditions, in order to confirm the predictions done at higher temperatures. The percentage of hydrolyzed prodrugs after 30 months was 94% for PRO1 and 6% for PRO2. The differences between the results obtained with accelerated and normal conditions suggested that the heat applied to the prodrug started hydrolytic reactions, which do not occur at room temperature. However, PRO1 is confirmed more hydrolytically cleavable than PRO2.

The stability of the two prodrugs in aqueous solution was checked in the presence and absence of electric current. As expected, both the prodrugs showed a lower stability in aqueous solution, compared to the solid state. After 7 hours at room temperature (the experimental conditions used in transport experiments), the percentage of prodrugs still not hydrolyzed was 95% for PRO1 and 99% for PRO2, regardless of the presence of electric current.

The relatively low hydrolytic resistance in aqueous solution suggests that the prodrugs should be stored in the solid state in the reservoir of the transdermal delivery system. This is not an easy task particularly in the case of iontophoretic application, which requires the use of a conductive solution. A possible solution of this problem could be the use of a solid reservoir to be dissolved just prior to current application, as the one previously proposed for calcitonin (13).

Solubility, pKa, and Partition

Solubility and partition characteristics are important parameters affecting transdermal drug transport. High solubility is often related to poor partition properties, so a balance between the two properties must be found. Solubility and partition characteristics of DHEA and the two prodrugs are reported in Table I.

The solubility of DHEA, a non-ionized molecule, in saline was 0.026 ± 0.001 mg/ml. The hydrochloride of the primary amine, PRO1, showed a solubility of 1.38 ± 0.13 mg/ml, 50 times higher than the parent drug (the pH of the solution was 4). Since the prodrug is the hydrochloride of a weak base, its solubility was affected by the pH of the solution. In particular, the solubility decreased quite dramatically as the pH was increased. At pH 6.5 the solubility was 0.7 mg/ml and at pH 8.0 it was 0.057 mg/ml. These values are consistent with the pKa value of 7.8, estimated by the titration method.

PRO2, the quaternary salt, was less soluble than PRO1, but the solubility in saline was still 30 times higher than DHEA. As expected, the solubility of PRO2 was not affected by the pH value of the solution.

The introduction of polar groups in the DHEA molecule reduced the apparent octanol/saline partition coefficient (see Table I): the value for PRO1, obtained in saline solution pH 4, was one third of DHEA partition coefficient value, while the value for PRO2 was 8 times lower. In conclusion, both prodrugs show higher aqueous solubility and lower partition coefficients.

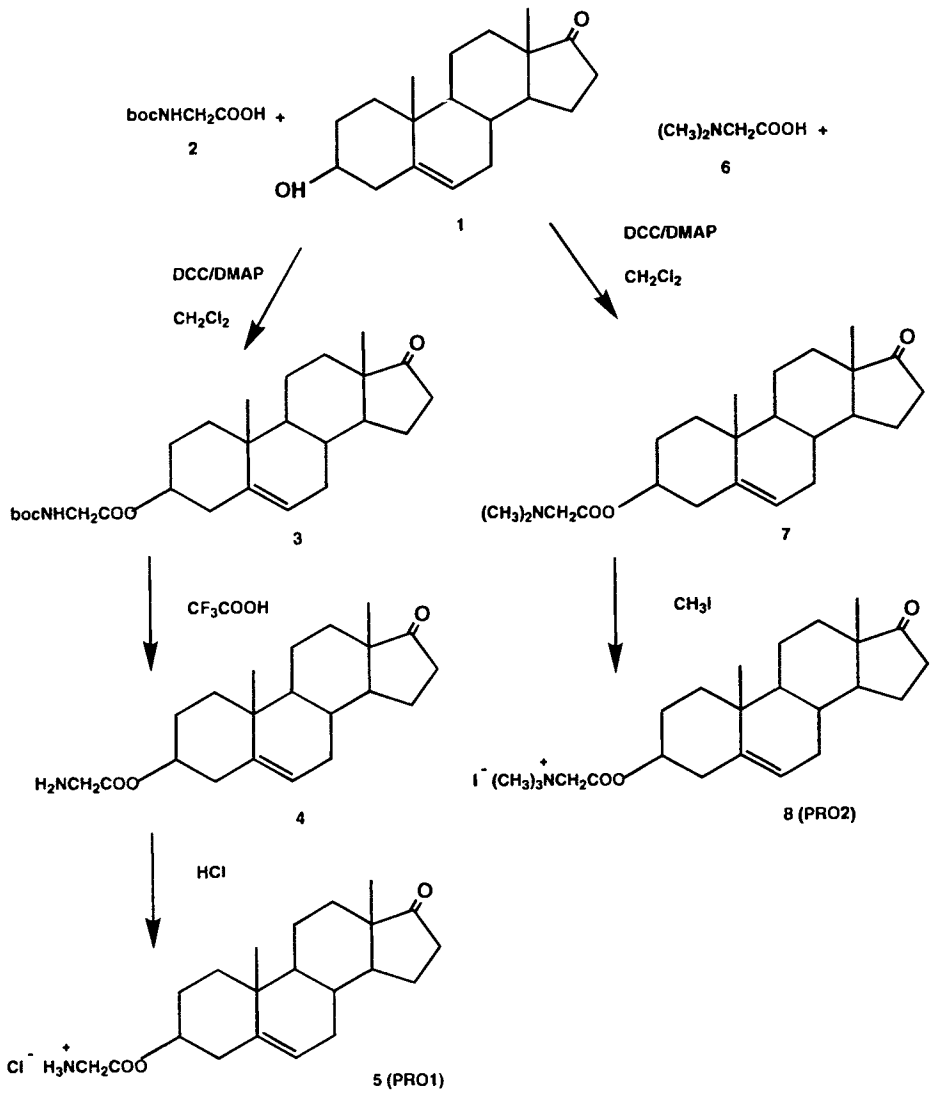

Fig. 1. Synthesis of DHEA ionized prodrugs.

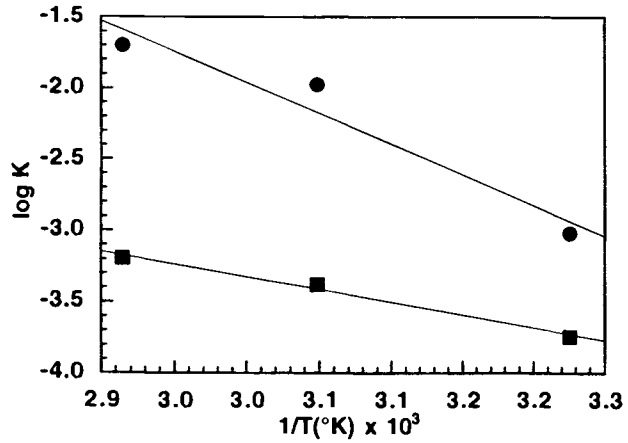

Fig. 2. Arrhenius plot for PRO1 (●) and PRO2 (■) stability as a function of temperature.

Table I. Melting Point, Saline Solubility, and Octanol/Saline Partition Coefficient Values for DHEA and Prodrugs (Mean Value ± Sem; n = 4)

Product	Solubility (mg/ml)	log P[a]	Melting point (°C)
DHEA	0.026 ± 0.001	1.54 ± 0.15	147–149
PRO1	1.38 ± 0.13	1.21 ± 0.06	>200[b]
PRO2	0.85 ± 0.23	0.79 ± 0.04	>200[b]

[a] log of octanol/saline partition coefficient.
[b] Decomposition.

In Vitro Hydrolysis

The two prodrugs synthesized were mainly intended for transdermal iontophoretic delivery, but an important additional objective was to have DHEA in the receptor compartment. It was required that the prodrug generated the parent drug (DHEA) during its passage through the skin. Since both the prodrugs are esters of DHEA, the breakdown in vivo is expected to be enzymatic, caused by esterase present in the skin (14).

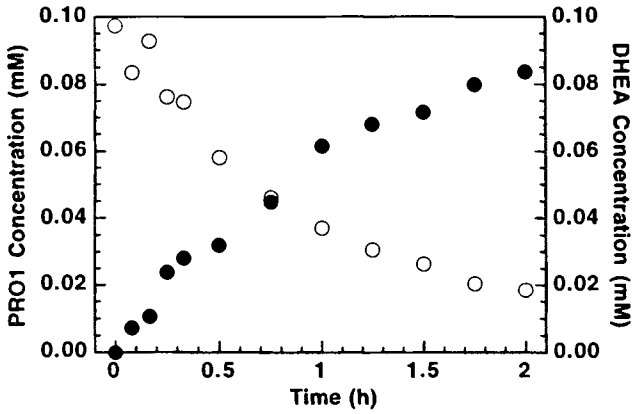

Fig. 3. Time course of PRO1 (○) disappearance and DHEA (●) appearance during a hydrolysis experiment at pH 8.0 (concentration of esterase 1 IU/ml).

Preliminarily, in vitro susceptibility to hydrolysis was tested using esterase from porcine liver. Figure 3 shows a typical profile of prodrug disappearance, obtained using the enzyme at a concentration of 1 IU/ml on a PRO1 solution at pH 8.0. An exponential decay of prodrug concentration, accompanied by an exponential increase in DHEA concentration, was found. Similar patterns were found for all the concentrations of enzyme tested, for both the prodrugs (data not shown).

From the individual disappearance profiles of the prodrugs, the rate constants and the half-lives were calculated, assuming a first order reaction. Figure 4 illustrates the relationship between $t_{1/2}$ and enzyme concentration on a semi-logarithmic scale. Increasing the concentration of esterase, the half-life decrease was very fast. PRO1 was certainly more susceptible to hydrolysis than PRO2, as demonstrated by the variation of half-life values in dependence of enzyme concentration. The value of half-life obtained in the absence of enzyme (113 h for PRO1 and 245 h for PRO2) confirmed the sensitivity of the two prodrugs to chemical hydrolysis in alkaline medium, in particular for the primary amine derivative PRO1.

These results suggest that the two prodrugs are suitable for transdermal iontophoresis, since they are ionized and can be hydrolyzed by esterase in vitro.

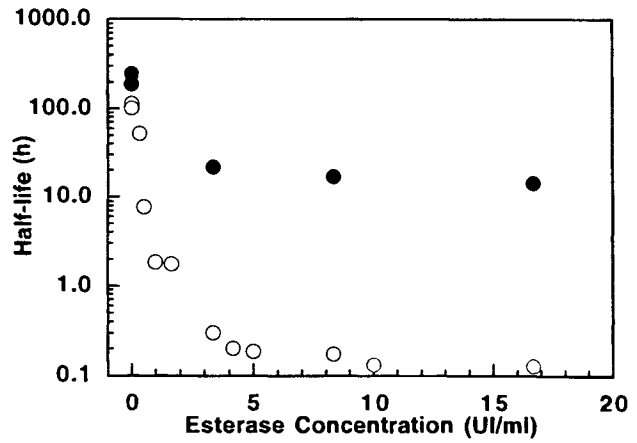

Fig. 4. Half-life values for PRO1 (○) and PRO2 (●) as a function of esterase concentration at pH 8.0.

Transdermal Delivery

Having assessed the susceptibility of the prodrugs to enzymatic hydrolysis, the transdermal permeation of the two prodrugs was checked in comparison with DHEA, to evidentiate an advantage in the use of the prodrugs. Considering the plasma concentration typical of adults (3–5 μg/ml) and the clearance of DHEA (12 l/day), the target input rate to be obtained is about 1–2 mg/h, i.e., 20–40 μg cm^{-2} h^{-1} (0.07–0.14 μmol cm^{-2} h^{-1}) for an area of 50 cm^2. This value can be taken as a reference to discuss the flux data obtained, keeping in mind that the substance is naturally present in the body and then the input rate really required will probably be lower.

The experiments were performed in unbuffered isotonic saline solution, having pH of 4.3 for PRO1, and 6.5 for both DHEA and PRO2.

First of all, the passive transdermal permeation through rabbit ear skin was studied starting from saturated solutions of DHEA (conc. 0.026 mg/ml), PRO1 (1.38 mg/ml) or PRO2 (0.85 mg/ml) in saline. In the case of PRO1 and PRO2 experiments, only DHEA was found in the receptor compartment. The absence of measurable amounts of prodrug in the receptor compartment (the limit of quantification was 3.5 μM) suggests that it was completely hydrolyzed during its passage through the skin.

From the linear portion of the permeation profiles (curves not shown), the substance flux was calculated as DHEA transported, assuming pseudo-steady state conditions. The results, reported in Table II, show limiting fluxes through the skin, i.e., the maximum flux attainable from saturated solution, in the order of 0.47–0.76 μM cm^{-2} h^{-1}, for all three substances studied (the differences were not significant). In the case of DHEA, logP was more favorable for skin permeation, but the water solubility was very low. In the case of the two prodrugs, the presence of electric charge on the molecule substantially increased the solubility, but reduced their affinity for stratum corneum, as manifested also by the lower partition coefficient values. As expected, the use of the synthesized ionized prodrugs gave a non significant advantage over the use of DHEA in terms of transdermal passive flux. Then, the permeation of DHEA from a donor solution containing DHEA in saline was tested in the presence of electric current (anodal iontophoresis 0.5 mA/cm^2). The resulting flux, reported in Table II, is about twice the value obtained during the corresponding passive diffusion experiment, suggesting that electric current is able to promote DHEA transport, even if it is not ionized. This can be attributed to the presence of an electroosmotic contribution, due to the electric charges in the skin (8) and/or to the increased skin permeability, due to current application.

The prodrugs were also tested for their permeation through the skin in the presence of electric current. Figure 5 illustrates the permeation profiles obtained from a donor solution containing PRO1, under the application of electric current 0.5 mA/cm^2. The profiles reported represent the individual species found in the receptor compartment (PRO1 and DHEA derived from the prodrug). The main species found was DHEA, while lower amounts of PRO1 were found in the receptor compartment. The substantial breakdown of the prodrug to the parent drug before reaching the receptor compartment was in agreement with its high susceptibility to enzymatic hydrolysis.

Table II. Limiting Fluxes (μmol cm^{-2} h^{-1}) from DHEA and Its Prodrug Solutions, Expressed as DHEA Transported Through Excised Rabbit Ear Skin

Current Density (mA/cm^2)	Donor: DHEA	Donor: PRO1[a]	Ratio[b]	Donor: PRO2[a]	Ratio[b]
—	$0.47\ 10^{-2} \pm 0.20\ 10^{-2}$	$0.76\ 10^{-2} \pm 0.07\ 10^{-2}$	1.6	$0.68\ 10^{-2} \pm 0.15\ 10^{-2}$	1.5
0.5	$0.92\ 10^{-2} \pm 0.18\ 10^{-2}$	$2.71\ 10^{-2} \pm 0.58\ 10^{-2}$	3.0	$7.14\ 10^{-2} \pm 0.75\ 10^{-2}$	7.8

[a] Prodrug flux was calculated as the sum of DHEA and prodrug fluxes.
[b] Ratio between flux from prodrug and flux from DHEA.

Figure 5 reports also the permeation profiles obtained from a donor solution containing PRO2, in the same experimental conditions as for PRO1. Also in this case, the species found in the receptor compartment were both prodrug and DHEA, but the main amount was given by DHEA.

Comparing these results with the ones obtained in the case of passive diffusion, it is evident that the prodrugs reached the receptor compartment intact in measurable amounts in the case of iontophoresis, but not in the case of passive diffusion. This could be attributed to faster permeation rate than hydrolysis rate and/or to the different penetration pathway. In fact, in the case of passive diffusion, it is assumed that the drug penetrates the skin via the transdermal (inter- or intracellular) route, while in the case of iontophoresis the prevailing route is through the annexes of the skin (15). The two pathways can be quite different as for type and concentration of enzymes present.

The comparison of the iontophoretic permeation profiles obtained with prodrug and DHEA solutions (Fig. 6), in the same experimental conditions, showed that the use of prodrugs significantly increased the permeation of DHEA through the skin. In fact, the calculated total fluxes of DHEA, i.e. the sum of DHEA and prodrug fluxes (see Table II), in the presence of electric current, was higher using the prodrug solutions as donor. In particular, PRO2 gave a DHEA equivalent flux higher than PRO1, even if the corresponding passive fluxes were very similar. This effect could be partially attributed to the different pH value of the donor solutions (4.3 for PRO1 and 6.5 for PRO2), able to modify the intrinsic charge of the skin and, consequently, the contribution of electroosmotic flow to drug transport. Another hypothesis is the difference in the polar groups present on the prodrug molecules. Finally, the ratio between the total flux from each prodrug solution and the flux from DHEA solution were 3.0 and 7.8 for PRO1 and PRO2, respectively.

CONCLUSIONS

The results obtained show that the two ionized prodrugs of DHEA possess higher water solubility and lower octanol/saline partition coefficient. The two prodrugs resulted sensitive to enzymatic hydrolysis; in particular, the primary amine was hydrolyzed faster than the quaternary salt by esterase from porcine liver.

Passive diffusion experiments showed only a small increment in the DHEA flux attainable, due to the reduced lipophilicity of the prodrugs. Using the prodrug in the passive skin transport experiments, only DHEA was found in the receptor compartment, indicating that the prodrugs are easily hydrolyzed in the skin.

The use of quaternary prodrug of DHEA (PRO2) can increase the flux of DHEA attainable up to 7.8 times if transdermal iontophoresis is applied. The primary amine derivative showed only 3 times flux increase. These flux increments are due to the increased water solubility of the derivatives and to the presence of electric charges on the molecule.

During iontophoretic transport experiments, not only DHEA but also the prodrugs were found in the receptor solution, in different amounts (about 30% for PRO1 and 15% for PRO2, with respect to the total amount permeated). This was attributed to a faster permeation rate than hydrolysis rate and/or to the

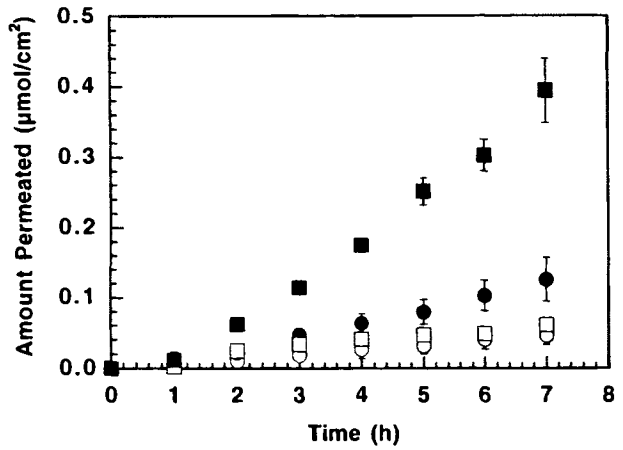

Fig. 5. Permeation profiles of prodrug (open symbols) and DHEA (closed symbols) during transdermal iontophoresis (0.5 mA/cm^2 applied for 7 hours). Circles refer to PRO1 and squares to PRO2.

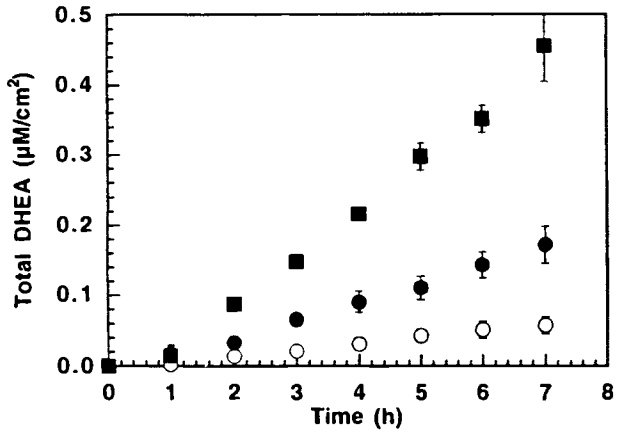

Fig. 6. Permeation profiles obtained during transdermal iontophoresis (0.5 mA/cm^2 applied for 7 hours) from a saturated solution in saline of PRO1 (●), PRO2 (■) and DHEA (○).

transdermal pathway for passive diffusion and annexial pathway for iontophoresis.

Solid state stability of the two prodrugs suggests that the quaternary salt could be used practically, even though some precautions, such as storing it in the solid form, must be adopted.

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