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RESEARCH PAPER

Development of Patches for the Controlled Release of Dehydroepiandrosterone

P. Minghetti,[1,*] F. Cilurzo,[1] A. Casiraghi,[1]
L. Montanari,[1] and A. Santoro[2]

[1]*Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Milano, Viale Abruzzi 42, 20131 Milano, Italy*
[2]*Rotta Research Laboratorium, Via Valosa di Sopra 7/9, 20052 Monza (MI), Italy*

ABSTRACT

Dehydroepiandrosterone (DHEA) and its sulfate conjugate (DHEAS) are the major secretory steroidal products of the adrenal gland. Some epidemiologic studies have found an association between low DHEA serum levels in patients and many important diseases. To prevent all such pathological conditions and, in any case, in aging, a DHEA supplementation has been proposed. DHEA shows a low oral bioavailability; taking the bioavailability obtained by the subcutaneous route as 100%, it was estimated that the potencies of DHEA by the percutaneous and oral routes were approximately 33% and 3%, respectively. Thus, transdermal patches could be considered a promising formulation as a continuous and controlled delivery of DHEA in replacement therapy is desired. With the aim of evaluating the effect of the matrix composition in terms of polymers and enhancers on the DHEA skin permeation flux, 10 types of monolayer self-adhesive patches containing 0.25 mg/cm^2 of active ingredient were designed. The matrices were based on three different acrylic copolymers: an acrylate-vinylacetate copolymer, a polyaminomethylmethacrylate (PAMA), and a polymethylmethacrylate. Transcutol (TR), mint essential oil, Lauroglycol, Brij 58, and propylene glycol (PG) were evaluated as DHEA skin permeation enhancers. All prepared patches were characterized by drug content, light microscopy, and in vitro skin permeation, performed using a modified Franz-type diffusion cell and human stratum corneum and epidermis as a membrane. The in vitro skin permeation studies are

*Corresponding author. Fax: 0039 02 58357565; E-mail: paola.minghetti@unimi.it

particularly significant in the development studies of DHEA patches as the in vivo determination of DHEA is affected by the fact that the endogen substance in the plasma is not constant over time. Among the tested patches, highest DHEA fluxes were obtained using the formulation based on PAMA. Moreover, the introduction in the matrix of binary mixtures of TR and PG, used also for their plasticizer properties, permitted enhancing DHEA skin permeation. On the basis of these studies, the transdermal administration of DHEA using patches seems feasible.

Key Words: *Acrylic copolymers; Dehydroepiandrosterone; Enhancers; Patch; Skin permeation*

INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulfate conjugate (DHEAS) are the major secretory steroidal products of the adrenal gland and are the most abundant steroid hormones in circulation in humans and mammals, but their physiological importance is still not completely clear, although they seem to play major regulatory effects in the immune system.

Normally, the serum concentration of DHEAS is 300–500 times higher than that of DHEA and 20 times higher than that of any other steroid hormone (1).

Several articles emphasize the decline of DHEA with age; the average serum level of DHEAS in men 25 to 35 years of age ranges around $6.44 \pm 2.29\,\mu mol/L$, and it falls to $1.15 \pm 0.52\,\mu mol/L$ in men aged 75 to 84 years. DHEA serum levels fall from $15.91 \pm 6.05\,\mu mol/L$ to $5.36 \pm 1.68\,\mu mol/L$ (2). The decline rate for both DHEA and DHEAS is relatively constant, about 2% per year. Most studies show that the levels in young women are 10% to 30% lower than in young men, but the sex difference appears to decline with age.

Some epidemiologic studies have found an association between low DHEA serum levels in patients with several major diseases, including cancer, arteriosclerosis, Alzheimer's disease, depression, osteoporosis, diabetes mellitus, and cardiovascular diseases. To prevent all such pathological conditions and, in any case, for treatment of aging, a DHEA supplementation has been proposed by several investigators; such supplementation would be interesting and suggested, provided that it is supported by additional animal and human studies to assess its value in the treatment or prevention of diseases of aging that appear to be due in part to loss of DHEA production.

It is described in the literature that DHEA shows low oral bioavailability; taking the bioavailability obtained by the subcutaneous route as 100%, it was estimated that the potencies of DHEA by the percutaneous and oral routes were approximately 33% and 3%, respectively (3).

Beneficial effects of percutaneous application of DHEA therapy on metabolism in postmenopausal women were evidenced by Diamond et al. (4). Among the other advantages, the transdermal route, compared to oral treatment, could permit avoidance of the first-pass effect, providing the active DHEA directly to the circulation and avoiding hormonal overload and perturbation of liver functions. Furthermore, when administered by oral route, DHEA is sulfoconjugated in the liver as DHEAS, which acts as an inactive reservoir. In particular, the transdermal patches could be considered a promising formulation for continuous and controlled delivery of DHEA in the desired replacement therapy.

With the aim of evaluating the effect of the matrix composition in terms of polymers and enhancers on the DHEA skin permeation flux, 10 types of monolayer self-adhesive patches were designed. Polymers affect the drug diffusibility, while enhancers may increase the permeability of the stratum corneum by one or more of these three mechanisms: (1) intercellular lipid disruption, (2) interaction with proteins, and (3) improved partitioning of the drug into the stratum corneum.

The polymeric systems used for matrix preparation were based on three acrylic copolymers characterized by having different functional groups: Durotak (neutral copolymer), Eudragit E (cationic copolymer), and Eudragit L (anionic copolymer).

Two solubilizing agents (Transcutol and Lauroglycol), an essential oil (mint essential oil), and a nonionic surfactant (Brij 58) were used as enhancers to improve DHEA skin permeation. They were chosen considering the above-mentioned mechanisms.

Transcutol, a hydrophilic compound, is thought to act by modifying the solubility parameter of the skin in the direction of that of the permeant. The solubility of the permeant in the outer layers of the skin will be increased; this, in turn, improves the flux (5).

Even if the mechanism by which the lipophilic Lauroglycol increases the permeability through the stratum corneum is not well known, it is widely used, especially in combination with Transcutol (6).

Terpenes contained in the mint essential oil act as enhancers by altering the barrier function of the stratum corneum (7) or by increasing partitioning of the drug into the stratum corneum (8).

Nonionic surfactants are known to have effects on the permeability of biological membranes as they may intercalate into the structured lipids of the skin, where they can disrupt the packaging, altering membrane permeability (9). The activity and the low toxicity and skin irritation power make these compounds good candidates as enhancers.

The effect of the ratio of the enhancer to the drug was also investigated. All the patches were made with a drug loading of $0.25\,mg/cm^2$. Moreover, knowing the influence of the amount of active ingredient loaded per square centimeter in determining the flux rate, we increased the drug loaded in the matrix from which the highest DHEA flux through the human skin was obtained.

The designed patches were prepared by using an occlusive backing layer, polyvinyl chloride (PVC) film, as occlusion determines a higher level of hydration of the skin, increasing the permeability of a drug through the stratum corneum.

All the prepared patches were characterized by drug content, light microscopy, and in vitro skin permeation. The skin permeation test was performed using a modified Franz-type diffusion cell and human stratum corneum and epidermis (SCE) as a membrane.

The in vitro skin permeation studies are particularly significant in the development studies of DHEA patches as in vivo the determination of DHEA is affected by the fact that the endogen substance in the plasma is not constant over time.

MATERIALS

Dehydroepiandrosterone (DHEA) was from Sigma (St. Louis, MO). Durotak 387-2287 (DK), an acrylate-vinylacetate copolymer, was supplied as a solution in ethylacetate (EA) containing 50% w/w of the copolymer (National Starch and Chemical, Zupthen, The Netherlands). Eudragit E 100 (EUE), which is poly(butyl methacrylate, (2-dimethylaminoethyl)methacrylate methyl methacrylate) with molar proportions of the monomer units 1 : 2 : 1 and molecular weight 150,000 Daltons; Eudragit L 100 (EUL), which is poly(methacrylic acid, methyl methacrylate) with molar proportions of the monomer units 1 : 1 and a molecular weight of 135,000 Daltons; Eudragit NE 40 D (EUNE), which is poly(ethyl acrylate, methyl methacrylate) with molar proportions of the monomer units 1 : 2 : 1 and a molecular weight of 800,000 Daltons, came from Röhm (Darmstadt, Germany). This material was supplied as an aqueous dispersion containing 40% w/w of the copolymer.

Brij 58 (BJ) was supplied by Sigma. TR and LG came from Gattefossè (Saint Priest, France). Also used were polyethylene glycol 400 (PEG) and propylene glycol (PG) (ACEF, Piacenza, Italy); glycerol (GLI) and adipic acid (AA) (Carlo Erba, Milan, Italy); lauric acid (AL) and succinic acid (AS) (Fluka, Buchs, Switzerland); acetyltributylcitrate (ATBC) (Röhm); and mint essential oil (MO) (Laury, Milan, Italy).

PVC film with the following specifications was used: thickness $90 \pm 1\,\mu m$, weight $90 \pm 9\,g/m^2$, elongation at break longitudinal $300\% \pm 15\%$ and transversal $400\% \pm 15\%$, tensile strength longitudinal $2.3 \pm 0.23\,Kg/cm$ and transversal 2.0 ± 0.20 Kg/cm (Bouty).

All substances were used as received. All solvents were analytical grade.

METHODS

Preparation of Polymeric Mixtures

The compositions of the prepared polymeric mixtures are shown in Table 1. For preparation of patches, four different methods were used.

Method 1 (formulation 1): The active ingredient was added to the DK; the mixture was stirred for 30 min at room temperature.

Table 1

Polymeric Mixture Compositions (% w/w)

Formulation	DHEA	EUE	EUL	DK	TR	PG	LG	BJ	AL	AA	AS	AC	IP	ET	EA	GLI	PEG	MO	ATBC
1	1.14			76.05											22.81				
2	1.00			76.20	4.80										18.00				
3	1.06	13.90			2.65	6.62			6.29	1.32	2.65	39.11	4.68	21.72					
4	1.48		14.78		3.94	7.69						31.00	3.71	17.20		9.66	10.54		
5	1.97	41.76			4.93							20.64	2.47	11.45					16.78
6	1.06	13.86			2.64	6.60		0.30	6.27	1.32	2.64	38.99	4.67	21.66					
7	2.05	27.40			5.21	13.05	1.91		12.39	2.64	5.21	17.95	2.22	9.97					
8	1.04	13.69			2.61	6.52			6.20	1.30	2.61	38.78	4.68	21.48				1.08	
9	1.03	13.91			2.65	6.62			6.29	1.32	2.65	39.15	4.64	21.73					
10	5.66	13.26			2.53	6.31			6.00	1.26	2.53	37.32	4.42	20.71					

See text for definitions of abbreviations.

Method 2 (formulation 2): The active ingredient, previously solubilized in the mixture TR and EA, was added to the DK; the mixture was stirred for 30 min at room temperature.

Method 3 (formulations 3, 5–10): The EUE was solubilized in the mixture of the organic solvents (acetone, AC; isopropanol, IP; and ethanol, ET) and stirred at 200 rpm at room temperature. When the solubilization was complete, all other components were added at the same conditions.

Method 4 (formulation 4): The EUL was solubilized in the mixture of the organic solvents (AC, IP, ET) and stirred at 200 rpm at room temperature. At complete solubilization, all other components were added in the same conditions.

Preparation of Patches

The patches were prepared using a laboratory coating unit, Mathis LTE-S (M) (Switzerland). The mixture was spread on the backing layer at a constant rate of 1 m/min and with a thickness of 300 μm (formulations 1, 7, 8, 10; Table 1), 350 μm (formulations 2–6; Table 1), or 450 μm (formulation 9; Table 1). The systems were dried at 60°C for 15 min, covered with the protective foil, and stored in an airtight container until used. The matrix compositions of the prepared patches are shown in Table 2.

Drug Content

A sample of 2.54 cm^2 of each patch was dissolved in 10 ml methanol (high-performance liquid chromatography [HPLC] grade). The sample was filtered through a Millipore filter (Millex HV, 0.45-μm pore size), and the solution was assayed by HPLC with the method reported below. Each value represents the average of triplicate injections of three different samples.

Microscopy

The presence of solid particles in the matrices was evaluated using a Zeiss model Axioscope microscope (Oberkochen, Germany) fitted with a polarizing objective.

Permeation Test

Human abdominal skin was used for the skin permeation studies. Skin samples, obtained by surgical operation, were harvested from the abdomen and used within a few hours of removal. The epidermal layer (comprising the stratum corneum and viable epidermis) was separated by immersing the skin in distilled water at 60°C for 1 min and peeling it from the derma. The heat-separated SCE membranes were dried in a desiccator at approximately 25% RH, wrapped in aluminum foil, and stored at −20°C until use. Dried SCE samples were

Table 2

Patch Dry Matrix Compositions (% w/w)

Patch	DHEA	EUE	EUL	DK	TR	GP	LG	BJ	AL	AA	AS	GLI	PEG	MO	ATBC
1	2.91			97.09											
2	2.28			86.79	10.93										
3	3.07	40.30			7.68	19.19			18.24	3.83	7.68				
4	3.08		30.73		8.19	15.99						20.09	21.92		
5	3.01	63.81			7.53										25.64
6	3.05	39.95			7.62	19.03		0.86	18.08	3.79	7.62				
7	2.93	39.22			7.46	18.68	2.73		17.74	3.78	7.46				
8	2.98	39.06			7.45	18.60			17.68	3.71	7.45			3.07	
9	2.58	40.34			7.78	19.51			18.25	3.84	7.68				
10	15.07	35.32			6.73	16.82			15.98	3.36	6.73				

See text for definitions of abbreviations.

rehydrated at room temperature by immersion in saline solution for about 16 h before use. The SCE membranes were placed on the diffusion cell with the dermal side in contact with the receiver solution and the stratum corneum side in contact with the patch. Each membrane was carefully mounted on the modified Franz-type diffusion cell, sealed with parafilm, and fastened with a rigid clamp. These cells, with respect to the original Franz-type diffusion cell, had a wider vertical column, and the bowl shape was removed. The cells had a diffusion area of 0.636 cm^2 and a receiver compartment of approximately 5 ml. Each cell was individually calibrated with respect to its diffusion area and receiver volume.

The receiver compartments were filled with water : PG (80 : 20 v/v) solution containing streptomycin as a preservative (100 g/ml, Sigma Chemical Co., St. Louis, MO).

The solutions were degassed by sonication. The receiver solution was continuously stirred with a small magnetic stir bar. The receiver compartment was kept at $32^\circ \pm 0.5^\circ$C. Then, 0.2-ml samples were withdrawn from the receiver compartment at predetermined times (1, 3, 5, 7, and 24 h) and immediately replaced with fresh medium. Sink conditions were maintained throughout the experiment. Samples were assayed for DHEA content with the HPLC method described below. Each value represents the average of three sample readings.

Drug Assay

The concentrations of DHEA in the medium were determined by HPLC assay (HP 1100, Chemstations, Hewlett Packard, USA).

A 50-µl sample was injected at room temperature on a C reverse-phase column (C_{18} 5-µm Spherisorb ODS2, 20 cm, Waters HPLC, UK). The flow rate was 1.5 ml/min; water : acetonitrile (45 : 55 v/v) was used as the mobile phase and was degassed before use. The ultraviolet (UV) detector was set at 205 nm. The retention time of DHEA was 5.35 min.

The standard curves were obtained by plotting the peak area as a function of drug concentration of six known concentrations of substances, ranging from 1 to 50 µg/ml and prepared using HPLC-grade methanol. Calibration curves demonstrated linearity over the concentration range encountered in the samples ($r^2 = 0.9999$).

Data Analysis

The cumulative amount permeated through the SCE per unit area was calculated from the concentration of each substance in the receiver medium and plotted as a function of time. Each data point on the plot represents a mean of triplicate permeation experiments. The flux J was determined as the slope of the linear portion of the plot.

The tests for significant differences between means were performed by Student t test. Differences were considered significant at the $P < p$ .05 level.

RESULTS AND DISCUSSION

The DHEA patch contents are shown in Table 3. The drug contents of tested patches were in the range 75% to 125% of the mean value; consequently, the DHEA content can be considered equivalent in patches 1–9 (10).

The enhancement effect of TR on DHEA skin permeation was proved by comparing the fluxes obtained with patch 1 ($J = 0.77 \pm 0.01\ \mu g/cm^2/h$) and patch 2 ($J = 1.02 \pm 0.09\ \mu g/cm^2/h$). As a consequence, TR, which is a powerful solubilizing agent, was introduced in all the other tested formulations.

The comparison of the flux obtained using patches made of different polymers showed that the use of EUE, the cationic methacrylic copolymer, for the matrix preparation (patch 3) permitted obtaining higher flux than the neutral copolymer DK (patch 2) or the anionic copolymer EUL (patch 4). On the basis of these preliminary results, patch 3 was selected as the reference.

The mean flux of patch 3, obtained from permeation studies performed using skin from six different donors, was $1.15 \pm 0.27\ \mu g/cm^2/h$, and the coefficient of variation (CV) was 23.6%. These data were even lower than the CV data obtained by other authors using different skin donors and other substances (11,12).

Table 3

Drug Content and Skin Permeation Flux Expressed as Ratio of the Considered Patch on the Reference Patch (No. 3)

Patch. No.	Drug Content ($\mu g/cm^2 \pm SD$)	J Ratio
1	247 ± 34	0.51
2	207 ± 8	0.71
3	277 ± 14	1
4	294 ± 8	0.61
5	325 ± 4	0.37
6	227 ± 22	0.84
7	291 ± 9	0.68
8	299 ± 4	1.1
9	285 ± 21	1.45
10	1424 ± 49	0.74

Considering the interindividual variability, the comparison of DHEA fluxes was possible by normalizing the mean of fluxes of each formulation with those of patch 3, obtained using the same skin donor.

Patch 3 contained PG as the main plasticizer. PG is also a well-known enhancer; it has a slight effect on the membrane integrity, producing a change in the hydration level of the skin (13). Thus, to evaluate its effect on DHEA flux, it was substituted with another plasticizer, ATBC (patch 5). The substitution of PG with ATBC caused very significant decrease of the flux, proving the efficacy of the PG as a percutaneous enhancer even in the case of DHEA (patch 3, $J = 1.48 \pm 0.07\ \mu g/cm^2/h$; patch 5, $J = 0.52 \pm 0.04\ \mu g/cm^2/h$).

Among the other added enhancers (patches 6, 7, and 8), only the use of MO (patch 8) induced a slight increase in DHEA flux with respect to the reference patch. Nevertheless, the difference was not statistically significant ($P = .245$).

In patch 9, a slight increase of TR : DHEA ratio and PG : DHEA ratio from 2.5 to 3.0 and from 6.2 to 7.5, respectively, caused a significant increase of the flux (patch 3, $J = 1.48 \pm 0.07\ \mu g/cm^2/h$; patch 9, $J = 2.01 \pm 0.15\ \mu g/cm^2/h$; $P = .017$).

Increasing of the amount of drug loaded over its saturation with the presence of visible solid particles in the matrix (patch 10) did not improve the DHEA flux; on the contrary, the flux was lower than that obtained in the absence of crystals (patch 3, $J = 1.48 \pm 0.07\ \mu g/cm^2/h$; patch 10, $J = 1.06 \pm 0.25\ \mu g/cm^2/h$). This behavior could be explained if we supposed that patch 3 was a supersaturated system. This hypothesis was confirmed as we detected visible crystals after 18 months of storage at 20°C. The problem of crystallization of a supersaturated system is well known when the drug substances bear a pronounced crystallization tendency. This is especially true for a matrix containing high concentrations of sex steroids and has been reported several times in the recent past (14,15). DHEA could have the same tendency as it is a steroid hormone as well.

The microscopic evaluation of patch 9 after 18 months of storage at 20°C did not show evidence of any crystals. This is justified because of the lower percentage of drug loaded with respect to patch 3 and with the presence of a higher ratio of enhancer (TR and PG) to drug as they can act as solubilizing agents.

CONCLUSION

Organic polymeric systems based on EUE laminated on PVC film were suitable for the preparation of DHEA transdermal therapeutic systems able to control in vitro skin permeation at least for 24 h.

The use of a mixture of TR and PG, selected for their plasticizer properties, caused an increase of DHEA skin permeation, proving their enhancer activity. Moreover, as expected, when the ratio of enhancer to drug was higher, the flux increased significantly.

On the basis of the results of in vitro skin permeation studies, the transdermal administration of DHEA using patches seems feasible.

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