

Enhancement of Dehydroepiandrosterone Solubility and Bioavailability by Ternary Complexation with α -Cyclodextrin and Glycine

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ABSTRACT: In the present work we investigated the possibility of improving dehydroepiandrosterone (DHEA) solubility and bioavailability by high-energy cogrinding with α -cyclodextrin (α -Cd) in the presence or absence of different auxiliary substances (glycine, biomaltodextrin, poly(vinyl pyrrolidone), poly(ethylene glycol) 400). In all cases, ternary products exhibited higher drug solubilizing properties than the binary DHEA– α -Cd coground system. Glycine was the most effective component. The best combinations, corresponding to 1:1:2 and 1:2:3 drug– α -Cd–glycine molar ratios, were characterized by differential scanning calorimetry and X-ray powder diffractometry and evaluated for dissolution rate. The presence of glycine favored destruction of DHEA crystalline structure during cogrinding, as evidenced by the strong reduction in both time and vibration frequency of milling necessary to obtain total drug amorphization. Both ternary products showed better dissolution properties than the drug alone, affording, respectively, a 40 and 60% increase of dissolution efficiency. The 1:2:3 coground product was then selected for *in vivo* bioavailability studies in women suffering from adrenocortical insufficiency. DHEA and DHEA sulfate blood levels were significantly higher ($p < 0.001$) after oral administration of the coground product than after oral administration of untreated drug, with a 100% increase in the area under the curve (AUC) of concentration versus time. Moreover, the time to reach maximum concentration ($t_{\max}$) decreased from 2.2 with the untreated drug to 0.5 h with the coground product, and the mean permanence time of DHEA within physiological levels was four times longer for the coground product compared with the untreated drug. These results indicate that the developed product is particularly suitable for oral DHEA formulations in hormone replacement therapies.

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Keywords: dehydroepiandrosterone (DHEA); cyclodextrins; complexation; bioavailability; solubility; dissolution

INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are plentiful adrenal secretory

steroid hormones whose serum levels naturally decline in both sexes with ageing, until almost ceasing to be present at senescence.¹ The age-related decline in DHEA production has currently attracted widespread attention because of its possible relevance to the etiology and management of a number of age-related illnesses. In fact, even though the results of epidemiological studies are sometimes conflicting, several reports showed that

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DHEA replacement therapy in elderly people appeared to have beneficial effects on numerous diseases of ageing, such as lipid metabolism, atherosclerosis, and osteoporosis.^{2–6} Other studies suggested that DHEA supplementation may also have favorable antidepressant and promemory effects, thus allowing quality of life enhancement in ageing people with no significant adverse effects.^{7,8} Moreover, numerous experiments in animals and humans indicated DHEA as a multifunctional hormone with anticancer, immune-enhancing, and neurotropic effects.⁹ Finally, it has been demonstrated that DHEA treatment improved mood, well-being, sexuality, and cognition in women with adrenal insufficiency.¹⁰ However, the very limited aqueous solubility and wettability of DHEA can give rise to problems of both formulation and low and variable bioavailability.

Cyclodextrin (Cd) complexation is known as an effective method for enhancing dissolution properties and bioavailability of poorly water-soluble drugs, including several steroids.^{11–14} In particular, techniques of mechanochemical activation by roll mixing or cogrinding with Cds have been successfully employed for improving dissolution properties of drugs.^{15–17} Moreover, recent works have shown that the addition of a suitable third component can often significantly improve both the solubilizing and complexing abilities of Cds demonstrate toward several drugs.^{18,19}

Therefore, in this study, we investigated the possibility of improving DHEA solubility and bioavailability by cogrinding the drug with α -cyclodextrin (α -Cd) in a high-energy vibrational mill in the presence or absence of different auxiliary substances. The ultimate goal of this research was to obtain an effective oral solid dosage form of DHEA.

In a first phase of this study, we evaluated the combined effect of α -Cd and a series of hydrotropic substances [poly(vinyl pyrrolidone K30) (PVP), poly(ethylene glycol 400) (PEG 400), biomaltodextrin, glycine] on DHEA aqueous solubility enhancement. Solid ternary systems, obtained by mechanochemical activation of physical mixtures in a high-energy micromill, were tested for solubilizing efficiency towards the drug in comparison with the simple drug- α -Cd binary complex. The most effective ternary products, suitably characterized by differential scanning calorimetry (DSC) and powder X-ray diffractometry (PXRD), were then compared with untreated, pure drug. The agents were evaluated for *in vitro* dissolution

rate properties (USP paddle method) and subjected to *in vivo* bioavailability studies in women with adrenocortical insufficiency.

EXPERIMENTAL

Materials

Dehydroepiandrosterone (DHEA), glycine, and biomaltodextrin were kindly provided by Euphar Group (Piacenza, Italy). α -Cyclodextrin (α -Cd) was supplied by Roquette (Lestrem, France), and poly(vinyl pyrrolidone K30) (PVP) and poly(ethylene glycol 400) (PEG 400) were from Sigma (St. Louis, MO).

High-Performance Liquid Chromatography (HPLC) Analysis

Analyses of all samples were performed with a Shimadzu Instrument HPLC system (model 5000) equipped with a variable-wavelength ultraviolet (UV) detector. The mobile phase (methanol/water, 85:15, v/v) was pumped at a flow rate of 1.0 mL/min through a C18 Hypersil BDS column (250 $\times$ 4 mm) at room temperature. The injected volume was 100 μ L, and the detection wavelength was 210 nm. Under these conditions, the DHEA retention time was 4.5 min.

Preparation of Solid Systems

Ground products (GR) of DHEA were prepared by grinding 1:1 or 1:2 (mol/mol) drug- α -Cd physical mixtures for 60 min in a high-energy vibrational micromill (Retsch, GmbH, Düsseldorf, Germany) in the presence or absence of 10% (w/w) auxiliary component (glycine, biomaltodextrin, PVP, PEG 400). Ternary coground products with glycine were also prepared at different glycine contents (5, 7, 12.5, and 15%, w/w).

Solubility Studies

Solubility measurements of DHEA and of its 1:1 and 1:2 mol/mol systems with α -Cd in the presence or absence of 10% (w/w) of each examined auxiliary component were performed by adding excess amounts of drug (10 mg) or each product (always equivalent to 10 mg drug) to 10 mL of water in sealed glass containers that were magnetically stirred up to equilibrium (48 h) in a thermostated bath at $25 \pm 0.5^\circ\text{C}$. The solutions were then

filtered (0.45- μ m filter pore size) and assayed for drug concentration by HPLC. Each test was performed in triplicate [coefficient of variation (CV) < 3%].

Dissolution Rate Studies

In vitro dissolution rate studies of the pure drug and of its ternary coground systems with α -Cd and glycine [1:1:2 (GR 1:1:2) and 1:2:3 (GR 1:2:3) molar ratios] were carried out with the USP XXIV paddle apparatus (Sotax AT7). Eight milligrams of DHEA or DHEA equivalent were added to 900 mL of dissolution medium (acetate buffer at pH 5.5), and the solution was thermostated at $37 \pm 0.5^\circ\text{C}$ and stirred at 100 rpm. Sink conditions were maintained throughout the test. Concentration of dissolved drug was assayed at fixed time intervals by the HPLC method already described. Each test was performed at least in triplicate (CV < 1.5%). Dissolution efficiency (DE) was calculated from the area under the curve of dissolution (D) versus time, t (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.²⁰

Differential Scanning Calorimetry (DSC)

DSC analysis was carried out with a Mettler TA 4000 apparatus equipped with a DSC 25 cell on 5–10-mg samples scanned in pierced Al pans at a heating rate of $10^\circ\text{C min}^{-1}$, from 50 to 200°C under static air.

X-ray Powder Diffractometry (PXRD)

PXRD analysis was performed with a Philips PW 1130 diffractometer (Co $K\alpha$ radiation) at a scanning rate of 1°min^{-1} from 10 to $50^\circ 2\theta$ range.

In vivo Studies

Sixteen women, suffering from adrenocortical insufficiency and aged between 18 and 75 years, participated in this study after giving written informed consent. The study was performed according to the tenets of the Declaration of Helsinki (1964) and, before the initiation, the protocol was approved by the ethics committee of the University of Würzburg. On three occasions, either placebo or 25 mg of native DHEA or an equivalent amount of DHEA- α -CD-glycine multicomponent

system (GR 1:2:3) was administered orally as a tablet in a randomized order. The three treatments were randomized and crossed over. Eleven serum samples were drawn over a 24-h period. Commercially available radioimmunoassay kits were used for assay of the serum content of DHEA (Diagnostic Systems laboratories, Sinsheim, Germany) and DHEAS (Diagnostic Products Biemann, Bad Nauheim, Germany). The tests are highly specific, with a very low cross-reactivity. For all assays, the intra- and interassay CVs were <8 and <12%, respectively. All data are reported as the mean $\pm$ standard error of the mean (SEM). The maximum serum concentration measured during a study period for a subject was reported as C_{max} . The time at which C_{max} occurred was reported as t_{max} . The area under the curve of concentration versus time (AUC) was calculated by trapezoidal integration. The AUC from 0 to 10 h ($\text{AUC}_{0-10 \text{ h}}$) was used for data comparison. For both steroids, the mean concentrations, $\text{AUC}_{0-10 \text{ h}}$, as well as C_{max} and t_{max} were calculated and compared by ANOVA and the t test for paired samples. The DHEAS and DHEA mean concentrations were also expressed as the ratio DHEAS/DHEA and analyzed to detect differences in downstream androgenic metabolism after each treatment. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Solubility Studies

Solubility studies showed that the addition of 10% (w/w) of a hydrophilic component to the 1:1 and 1:2 (mol/mol) DHEA- α -CD coground systems resulted in an improvement of the Cd solubilizing power towards the drug in all cases, with the exception of PVP. These results are reported in Figure 1 as the percent increase of DHEA aqueous solubility at equilibrium obtained from the different examined systems. Glycine was primarily the most effective ternary component, resulting in an increase in the initial drug solubility of up to $\sim 200\%$, which represents a 40% increase in comparison with the corresponding drug-Cd binary system. These results account for the formation in solution of multicomponent soluble complexes.

Further solubility studies were subsequently performed to evaluate the effect of varying the percent of added glycine on the dissolution performance of the ternary system (Figure 2). The best results were obtained in the presence of 12.5 and

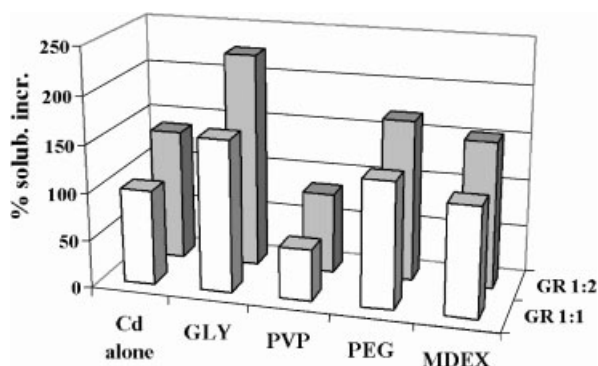


Figure 1. Effects on DHEA aqueous solubility at 25°C of different auxiliary components coground at 10% w/w with 1:1 and 1:2 mol/mol drug- α Cd systems. Key: (GLY) glycine; (PVP) poly(vinyl pyrrolidone K30); (PEG) poly(ethylene glycol 400); (MDEX) biomaltodextrin.

10% (w/w) of the amino acid for 1:1 and 1:2 (mol/mol) DHEA- α Cd systems, respectively. These amounts corresponded approximately to 1:1:2 and 1:2:3 drug- α Cd-glycine molar ratios.

Solid-State Studies

Solid-state studies were then performed to characterize these systems and to investigate the possible interactions between the components. The thermal curves of the pure components and those of some representative binary (drug-Cd at 1:1 and 1:2, mol/mol) or ternary (drug- α Cd-glycine at 1:1:2 or 1:2:3 mol/mol) physical and coground mixtures are shown in Figure 3. The DSC curve of DHEA is typical of an anhydrous pure crystalline substance, characterized by the presence of a single sharp endothermic peak at 150.9°C ($\Delta H_{\text{fus}} = 84.2$ J/g) due to its fusion. The thermal curve of glycine shows a broad endothermic effect

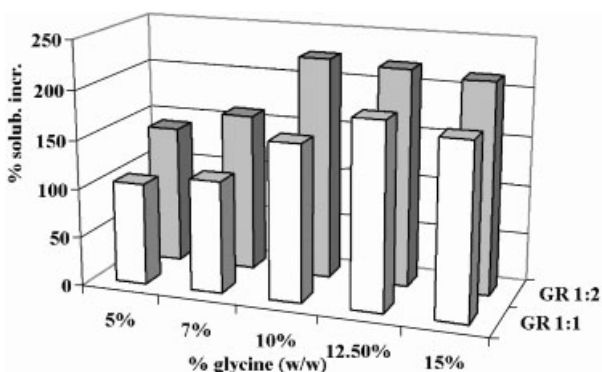


Figure 2. Effects on DHEA aqueous solubility at 25°C of different glycine amounts (% w/w) coground with 1:1 and 1:2 mol/mol drug- α Cd systems.

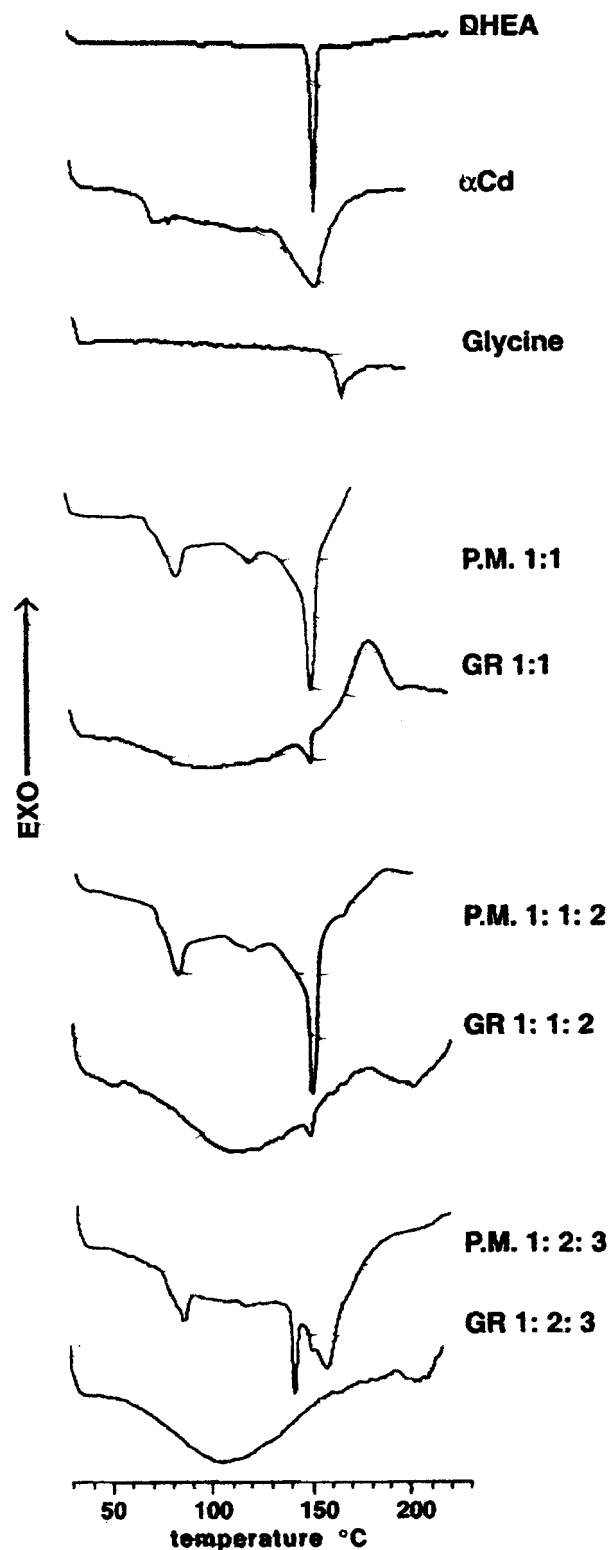


Figure 3. DSC curves of pure components and their binary and ternary physical (PM) and coground (GR) mixtures at different DHEA- α Cd-glycine molar ratios.

peak at 166.9°C, which is attributable to its melting process, whereas that of α -Cd is characterized by a large endothermal band ranging between 60 and 160°C due to its dehydration process ($7.2 \pm 0.2\%$ as mass fraction). DSC curves of binary and ternary physical mixtures almost correspond to the superimposition of the thermal curves of the single components, indicating the absence of strong solid-state interactions, which, in contrast, clearly occur as a consequence of cogrinding. In fact, the strong reduction of intensity of the DHEA melting endotherm observed in the binary coground system and its almost total disappearance in the ternary systems account for the presence of marked interactions between the components that are induced by the mechanochemical activation treatment and that lead to drug amorphization and/or complexation.

The results of XRPD analysis (Figure 4) were consistent with the DSC findings. The spectra of the pure components are typical of crystalline products. The binary and ternary physical mixtures produced patterns that are simply due to the superimposition of those of the crystalline components. On the contrary, a clear decrease of peak number and relative intensity occurred passing from the physical to the corresponding coground mixtures. In particular, drug crystallinity peaks completely disappeared in the 1:2:3 DHEA- α -Cd-glycine coground product (GR 1:2:3), thus confirming complete amorphization of DHEA in this system. The presence of glycine favored DHEA structure destruction and loss of crystallinity during the cogrinding process because it significantly reduced the time and the vibration frequency of milling necessary to obtain total drug amorphization. In fact, by monitoring the progressive drug loss of crystallinity during the cogrinding process by DSC analyses performed every 15 min, it was observed that >3 h at a vibration frequency of 24 Hz were required to achieve total disappearance of the drug melting endotherm in binary DHEA- α -Cd systems. In contrast, in the presence of the amino acid, this same result was obtained after only 60 min. Probably, the amphiphilic nature of glycine lowered the threshold for achieving mechanochemical activation of the system by reducing the interfacial tension between drug and α -Cd particles, thus favoring their interaction and the molecular dispersion of the drug in the carrier phase.

Because amorphous products were obtained, the complex formation in the solid state was not really demonstrated by DSC or XRPD analyses, but only

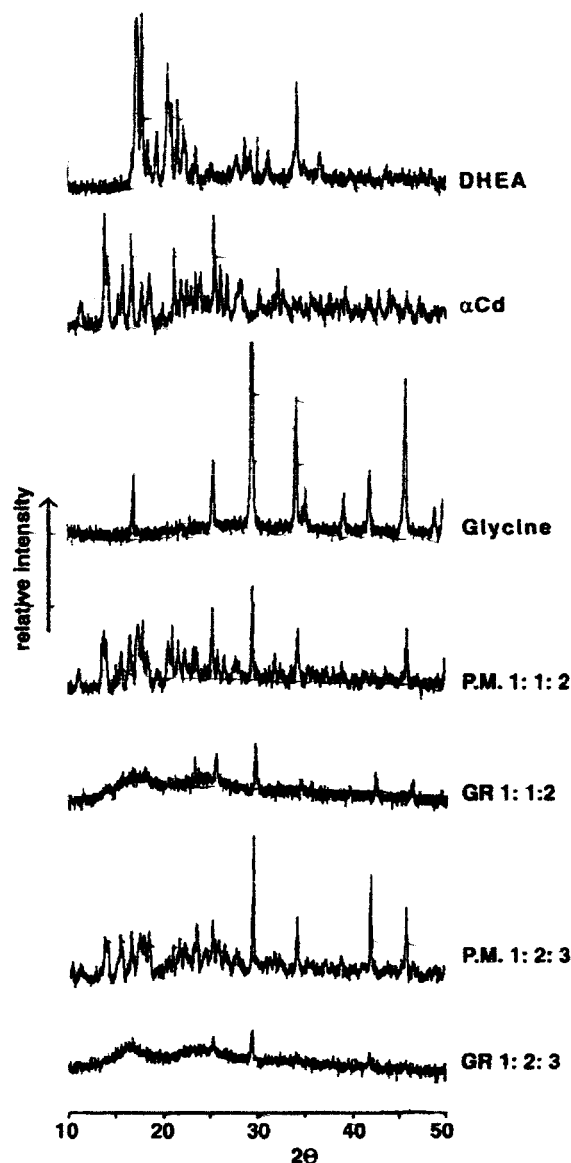


Figure 4. XRD patterns of pure components and their ternary physical (PM) and coground (GR) mixtures at different DHEA- α -Cd-glycine molar ratios.

hypothesized. However, the question as to whether actual inclusion complex formation occurred or simply a molecular dispersion of the drug in the hydrophilic environment created by α -Cd and glycine is only secondary. In fact, it is known that when a drug is administered as a Cd complex, only the free form of the drug, which is in equilibrium with the complexed one in solution, is absorbed.

Dissolution Rate Studies

The mean dissolution curves of DHEA and its 1:1:2 (GR 1:1:2) and 1:2:3 (GR 1:2:3) coground systems are presented in Figure 5. The results in

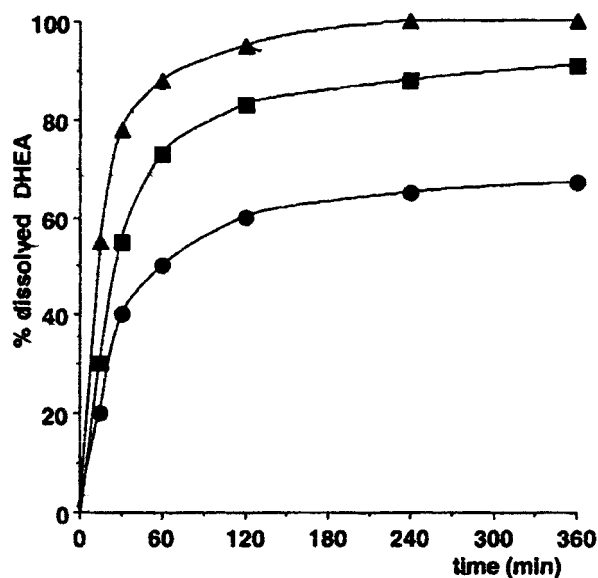


Figure 5. Dissolution curves of DHEA in various forms. Key: (●) untreated; (■) 1:1:2 mol/mol drug- α -Cd-glycine system; (▲) 1:2:3 mol/mol drug- α -Cd-glycine systems.

terms of DE, percent of active ingredient dissolved, time to dissolve 50% of the drug, and relative dissolution rate are collected in Table 1. Both ternary coground products showed better dissolution properties than the drug alone. In particular, GR 1:2:3 was the best product, with a DE at 60 min that is 2.3 times higher than that of the drug alone and 1.6 times higher than that of GR 1:1:2. Moreover, GR 1:2:3 resulted 100% drug dissolution after 240 min, whereas at the end of the test (360 min) only 92% dissolved drug was obtained from GR 1:1:2 and 57% from pure drug. Finally, the marked reduction of $t_{50\%}$ accounted for a strong increase in the initial drug dissolution rate from the GR 1:2:3 system, and demonstrated the importance of the kinetic aspect in the drug

Table 1. Dissolution Efficiency (DE), Percent Dissolved at 15 min (DP), Time to Dissolve 50% Drug ($t_{50\%}$), and Relative Dissolution Rate (RDR) of DHEA Alone or as 1:1:2 (GR 1:1:2) or 1:2:3 (GR 1:2:3) Drug- α -Cyclodextrin-Glycine System

Sample	DE ^a	DP	$t_{50\%}$ (min)	RDR ^b
DHEA	57.4	20.0	60	—
GR 1:1:2	79.2	30.0	<30	1.6
GR 1:2:3	92.0	55.0	<15	2.9

^aCalculated from area under the dissolution curve at $t = 360$ min.

^bRatio between amount of drug dissolved from the complex and that dissolved from DHEA alone at 15 min.

solubility enhancement. The improved drug dissolution properties can be attributed to three main factors: the reduced particle size and consequent increase of surface area of the drug-carrier powder, the improved wettability due to the intimate contact with the hydrophilic carrier, and the drug amorphization and molecular dispersion in the carrier system.^{16,21} Moreover, an additional solubilizing effect due to complex formation in solution can be reasonably hypothesized on the basis of the results of previous solubility studies. Preliminary molecular modelling studies seem to support the possibility of multicomponent complex formation between DHEA, α -Cd, and glycine molecules.²² Further analyses by synchrotron radiation-potentialized techniques, such as small-angle X-ray scattering (SAXS), super electron spectroscopy for chemical analysis (Super ESCA), and X-ray diffractometry (XRD), will be performed to search for a confirmation of these interesting preliminary findings and to investigate in depth the role played by the amino acid in the system performance.

In Vivo Studies

Based on dissolution study results, GR 1:2:3 was selected as the most effective system for *in vivo* experiments. The patterns of serum DHEA and DHEAS levels after oral administration of the drug, formulated in tablets alone or as multicomponent complex, are shown in Figure 6 (A, B) and the related pharmacokinetic parameters are collected in Table 2. After GR 1:2:3 administration, the DHEA and DHEAS blood levels were clearly higher, and $C_{\max}$ and AUC values were more than doubled compared with those after untreated DHEA administration. Moreover, the time to reach maximum DHEA serum levels ($t_{\max}$) was strongly reduced (from >2 h to ~30 min), and the mean total time with DHEA serum levels within physiological range (>3.9 ng/mL) was more than tripled using drug as ternary system compared with the same dosage of native DHEA. In contrast, no significant difference was observed in the DHEAS/DHEA molar ratio throughout the study period between the values obtained after DHEA or GR 1:2:3 administration, indicating that the latter proportionately enhanced the serum circulating levels of both DHEA and DHEAS.

CONCLUSIONS

Multicomponent DHEA- α -Cd-glycine systems, obtained by mechanochemical activation in a high-

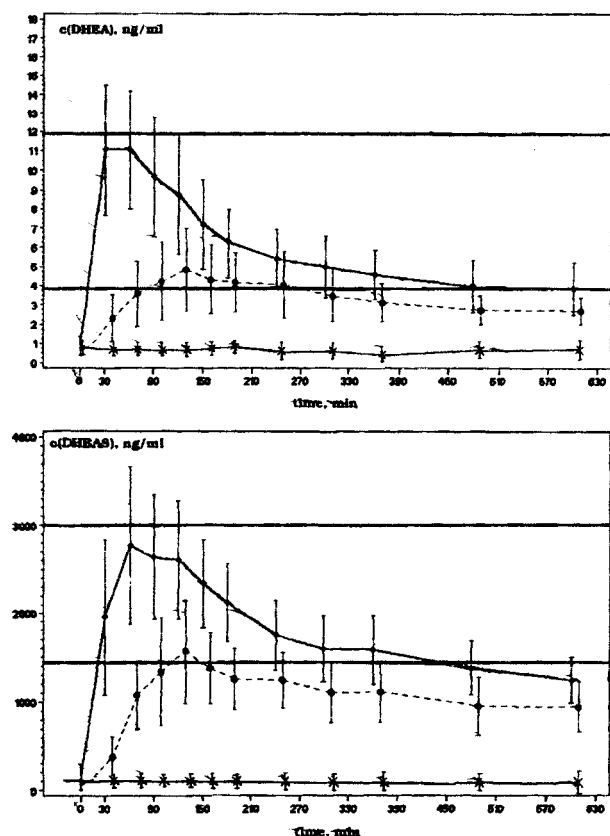


Figure 6. Serum concentrations of (top) DHEA and (bottom) DHEAS (mean $\pm$ SEM) in 16 female volunteers affected by adrenocortical insufficiency after oral administration of (*) placebo, (●) 25 mg of untreated DHEA, or (◆) 25 mg of the 1:2:3 mol/mol drug- α -Cd-glycine (GR 1:2:3) system.

energy mill of the physical mixture of the components in a 1:2:3 molar ratio (GR 1:2:3), significantly improved the drug solubility and dissolution properties. *In vivo* studies on human volunteers showed that oral administration of GR 1:2:3 resulted in a significant enhancement of both

Table 2. Pharmacokinetic Data ($\pm$ SEM) of DHEA after a Single Oral Administration of 25 mg as Native Drug or as Multicomponent System (GR 1:2:3) with α -Cyclodextrin and Glycine

Pharmacokinetic Parameter	Native DHEA	GR 1:2:3
$t_{\max}$ (h)	2.2 ± 0.9	0.5 ± 0.2
$C_{\max}$ (ng/mL)	4.8 ± 3.8	10.9 ± 6.3
AUC (ng/mL $\cdot$ h)	29 ± 17	59 ± 20
t_{res} (h) ^a	2.2 ± 0.7	7.9 ± 1.5

^aMean residence time with DHEA serum levels within physiological range.

DHEA and DHEAS $C_{\max}$ ($p < 0.001$) and AUC ($p < 0.001$) values compared with those determined after untreated DHEA administration, without affecting the DHEAS/DHEA molar ratio. Moreover, the concomitant fourfold increase in hormone mean permanence time within physiological levels will allow reduction of administration frequency of the ternary product. Therefore, oral DHEA administration as a multicomponent system with α -Cd and glycine (GR 1:2:3) seems to be particularly effective for a putative hormone replacement therapy, not only for restoring physiological steroid levels in patients with adrenocortical insufficiency, but also in elderly people for its potential beneficial effects in several age-related diseases.

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