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7-Hydroxydehydroepiandrosterone – a Natural Antiglucocorticoid and a Candidate for Steroid Replacement Therapy ?

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Summary

7-Hydroxylated metabolites of dehydroepiandrosterone (DHEA) are believed to be responsible for at least some immunomodulatory and antiglucocorticoid effects of DHEA and hence are considered candidates for hormone replacement therapy. Our experiments *in vitro* brought the evidence that 3β, 7β-dihydroxy-5-androsten-3-one (7β-OH-DHEA), but not DHEA and its 7α-hydroxyisomer, could counteract the immunosuppressive effect of dexamethasone on the formation of plaques in culture of murine spleen lymphocytes. In another experiment, DHEA and after a 3-weeks pause 3β-hydroxy-5-androstene-7,17-dione (7-oxo-DHEA) were applied transdermally to 6 male volunteers on 5 consecutive days. Blood levels of DHEA, its 7-hydroxylated metabolites, and in the first case also dehydroepiandrosterone sulphate (DHEAS), were measured before, during and one day after the end of treatment. Application of DHEA increased significantly not only DHEA and DHEAS, but also its both 7-hydroxyisomers. Application of 7-oxo-DHEA also led to a significant increase of both 7-hydroxyisomers of DHEA, with 7β-OH-DHEA being the preferred metabolite the concentration of which was increased more than three times.

Key words

Dehydroepiandrosterone • 7-Hydroxydehydroepiandrosterone • Antiglucocorticoid • Immunoprotection • Hormone replacement

Introduction

In man, a considerable portion of circulating dehydroepiandrosterone (DHEA) is metabolized to its 7α- and 7β-hydroxylated products (Stárka *et al.* 1962, Baulieu 1996). The main site of 7-hydroxylation is in the liver, but this enzymatic reaction has also been shown to proceed in many other tissues including fetal ones (for review see Hampl *et al.* 1997). Both 7-hydroxyisomers of DHEA (7α- and 7β-OH-DHEA), free as well as conjugated with sulphuric acid, circulate in the blood in nanomolar concentrations (Skinner *et al.* 1977), but their

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concentrations may be much higher at the sites where they are produced (Morfin and Courchay 1994).

The physiological role of 7-OH-DHEA has not yet been elucidated. Recently, in connection with the discovery of non-genomic immunomodulatory and antiglucocorticoid effects of DHEA (Kalimi *et al.* 1994), a hypothesis emerged that 7-hydroxylated steroids may be responsible for at least some of the above mentioned effects of DHEA. Evidence based on *in vitro* experiments with various cell cultures as well as on *in vivo* experiments in mice indicates that both 7-hydroxyisomers of DHEA and also its 17β-hydroxymetabolites are able to counteract various immunosuppressive effects of glucocorticoids including dexamethasone-induced apoptosis. These effects differed for both 7-hydroxy-isomers of DHEA (for review see Hampl *et al.* 1997, Loria 1997, Loria and Padgett 1998, Lafaye *et al.* 1999. The addition of dexamethasone led to induction of 7α-hydroxylating enzymes in experiments on human adipose cells, (Khalil *et al.* 1994). This may imply that target cells react by increasing the synthesis of 7-hydroxysteroids, when circulating glucocorticoids rise. Increased 7-hydroxylation of DHEA and also of its precursor pregnenolone occurred at the expense of their oxidation to 4-ene-3-oxosteroids and was reported in cultured rat astrocytes when the cell contact was increased, e.g. during inflammation (Akwa *et al.* 1993).

In this paper, we present further evidence that 7-hydroxylated DHEA metabolite counteracted the effect of dexamethasone in murine spleen lymphocytes.

All these findings, together with the fact that 7-OH-DHEA and its precursor DHEA are endogenous steroids, may list 7-OH-DHEA isomers among the candidates for steroid replacement therapy, similarly as DHEA itself. Primarily, we were interested in ascertaining to what extent 7-hydroxylated metabolites of DHEA appear in the circulation after DHEA administration to humans. Our pilot study with short-term transdermal application of DHEA in the form of a gel to male volunteers is presented here. In a further experiment DHEA was replaced by its 7-oxoderivative supposed to be converted in the periphery to both 7-OH-DHEA isomers.

Methods

Steroids and chemicals

Dehydroepiandrosteone, dexamethasone and chemicals for *in vitro* experiments with cell culture (HEPES, collection of essential amino acids) were purchased from Sigma (St. Louis, Mo, USA). 3β-hydroxy-5-androstene-7,17-dione (7-oxo-DHEA) was from Steraloids Inc. (Wilton, NH, USA), 7α- and 7β-OH-DHEA were synthesized by the method of Stárka (1962). MEM, calf fetal serum and antigen were from USOL (Prague, Czech Republic). Diethyl ether and chemicals used for the radioimmunoassay, all analytical grade, were from Merck (Darmstadt, Germany).

Determination of the number of plaque-forming cells and of cell viability

The number of plaque-forming cells (NPFC), as a measure of primary immune response, was determined in cultured murine spleen lymphocytes following activation with an antigen (sheep erythrocytes, 10^7 cells/tube). Briefly, the cells from BALBc mice (10^7 cells/ml) were cultivated in the Marbrook system consisting of a supplemented MEM medium with 10 % fetal calf serum mixed with the antigen in the absence or presence of various doses of tested steroids, in an atmosphere of 96 % air and 4% CO_2, at 37 °C, for 5 days. The NPFC was then calculated in agarose by a drop modification of the Jerne method (Šterzl and Mandel 1964). The viability of the cells after cultivation was assessed by staining with 1 % nigrosin.

The effect of tested steroids on NPFC was expressed in percentages of NPFC from 28 control experiments, which was 949±58 (mean ± S.D.). Since the parameters of descriptive statistics characterizing the probability distribution of NPFC were asymmetrical, the log-normal distribution was used with the logarithmic mean of NPFC/test chamber being 904 and the 95 % confidence interval 806 to 1016. The probability distribution of the number of surviving cells at the end of experiments was found to be close to the Gaussian distribution, the mean value being 3.18 x 10^6 and the 95 % confidence interval from 2.86 to 3.51 x 10^6. The details of the experiments and statistical evaluation of the results are given elsewhere (Šterzl *et al.* 1999).

Administration of dehydroepiandrosterone and its metabolites to volunteers

Six male volunteers, the personnel of the Institute of Endocrinology aged 27-68 years (mean 45), were included in the experiments. All signed a written, informed consent with the study.

In the first experiment they were given DHEA in the form of an ointment (gel) containing 1 g of the steroid

per 100 g of the gel. The composition of the gel will be patented by one of the authors (A. Nováček). The gel, approximately 5 g containing 50 mg of the substance, was applied each evening on the skin of the abdomen, for five consecutive days. Blood from the cubital vein was collected on the morning of the first day before the application (basal), then on the third day during application (Day 3) and the following morning after the last (fifth) application (Day 6). The sera obtained were stored frozen at –20 °C until analyzed.

In the second experiment, performed 3 weeks after the end of DHEA application, DHEA was replaced with 7-oxo-DHEA in the same form and concentration. The time schedule of the application was the same as in the previous experiment, with the exception that blood was collected only before and after the end of the steroid application.

Steroid determination

DHEA and its sulphate (DHEAS) was determined in the sera by commercial RIA kits from Immunotech (Praha, Czech Republic). Unconjugated 7α-OH-DHEA and its 7β-hydroxyisomer were determined by a recently published radioimmunoassay (Lapčík *et al.* 1998, 1999).

Statistics

Two-factor analysis of variance was applied for determination of the significance between basal steroid levels in individual participants during and after treatment. The phase of the experiment and the name of the patient were chosen as the first and second factor, respectively, to separate the differences resulting from the design of the experiment from individual differences. The significance of the differences between basal levels and the levels after treatment was evaluated by F-test and by Scheffé´s test.

Table 1. The effect of increasing doses of dexamethasone, DHEA and its 7-hydroxylated metabolites on the number of plaque-forming cells (NPFC), and on the cell viability in cultured murine splenic lymphocytes

Steroid dose	0.01 mg/l		0.1 mg/l		1.0 mg/l		10.0 mg/l	
	NPFC	Viability	NPFC	Viability	NPFC	Viability	NPFC	Viability
Dexamethasone	3.5 *	33.5 *	3.4 *	37.7 *	5.1 *	42.7 *	16.5 *	42.9 *
DHEA	47.8 *	64.9	48.7 *	103.0	83.1	126.0	13.6 *	77.6
7α-OH-DHEA	87.7	92.2	20.5 *	90.7	42.1 *	88.6	51.1 *	81.2
7β-OH-DHEA	23.3 *	91.5	22.2 *	103.0	40.1 *	101.0	56.1 *	97.7

The results on NPFC are expressed in percentage of the mean value of NPFC from 28 control experiments, whereas cell viability is given as percentage of surviving cells at the end of the experiment. The data represent the mean values from three measurements for each concentration of steroids. Steroid doses are given in mg/l, the lowest dose 0.01 mg/l corresponds to the concentrations 25, 34.7 and 32.9 nmol/l for dexamethasone, DHEA and 7-OH-DHEA, respectively. Asterisks indicate the significant effect ($p<0.05$) of tested steroid related to the controls.

Results

Effect of steroids on the number of plaque-forming cells (NPFC) and on cell viability

In the first experiment, the effect of increasing doses of dexamethasone, DHEA and of both its 7-hydroxylated metabolites on the NPFC and cell viability was studied separately (Table 1). Dexamethasone almost completely suppressed the formation of plaques even at the lowest concentration (25.5 nmol/l) and it also exerted a significant toxic effect on cell viability, in contrast to other steroids tested. The effect of other steroids on the NPFC differed considerably. While DHEA and also 7α-OH-DHEA decreased the NPFC irrespectively of the dose, the decrease of the NPFC caused by 7β-OH-DHEA was less distinct with increasing doses. Therefore, the simultaneous effect of the latter steroid and dexamethasone on the NPFC as well as on cell viability was tested in the second experiment. The concentration of dexamethasone was kept constant at 255 nmol/l (0.1 mg/l), while three concentrations of 7β-OH-DHEA were tested. The effect of dexamethasone alone (the mean from 9 measurements) was taken as a reference value, to

which the simultaneous effect of 7β-OH-DHEA was related (Table 2). 7β-OH-DHEA counteracted the effect of dexamethasone on NPFC even at the same concentration as dexamethasone, but it did not influence the toxic effect of dexamethasone on cell viability.

Table 2. Simultaneous effect of dexamethasone and of 7β-OH-DHEA on the number of plaque-forming cells (NPFC) and on the cell viability in cultured murine splenic lymphocytes.

Dose of 7β-OH-DHEA	NPFC	Viability
0.1 mg/l (347 nmol/l)	123	94.4
1.0 mg/l (3.47 μmol/l)	156 *	117
10 mg/l (34.7 μmol/l)	151 *	117

The data are expressed in percentage of the effect of dexamethasone alone (255 nmol/l, i.e. 0.1 mg/l, mean from 9 experiments). Each value represents the mean of three parallel measurements. Asterisks show the significant effect ($p<0.05$) of tested steroid as compared to that of dexamethasone alone. The details on calculation of a significance are given elsewhere (Šterzl et al. 1999).

Table 3. Changes of DHEA and DHEA sulphate levels in six male volunteers before, during and one day after transdermal application of DHEA in ointment form (1g of DHEA in 100 g of the gel). Approximately 5 g of the gel was applied each evening on the abdominal skin for five successive days. Blood from the cubital vein was collected on the morning of the first day before application, then on the third day during application (Day 3) and on the next morning after the last (fifth) application (Day 6).

	DHEA (nmol/l)			DHEAS (μmol/l)		
	Basal	Day 3	Day 6	Basal	Day 3	Day 6
Median	13.5	18.8	18.8	4. 90	7.30	5.55
Mean	13.0	26.0 **	19.9 **	4.49	6.43 **	5.82 **
S.E.M.	1.86	5.39	2.08	0.51	0.97	0.72
Range	3.5-22.3	8.24-47.2	9.05-30.6	9.05-30.6	1.7-6.7	2.2-8.3

**, ** Significant differences from the basal level ($p<0.05$ and $p<0.01$, respectively).*

Changes of DHEA, its sulphate and its 7-hydroxylated metabolites in the blood following DHEA application

Free (unconjugated) DHEA, its sulphate and both its 7-hydroxyisomers were measured before, during and one day after application of DHEA in a gel to six male volunteers (Tables 3 and 4). The levels of all the relevant steroids increased significantly during the application and were maintained one day after the end of the treatment. The levels of 7α-OH-DHEA increased to more than twofold values on the average, whereas those of its 7β-hydroxyisomer rose almost three times.

Changes of DHEA and its 7-hydroxylated metabolites in the blood following 7-oxo-DHEA application

The levels of DHEA and its 7-hydroxyisomers before and after transdermal application of 7-oxo-DHEA to male volunteers are shown in Table 5. Significantly higher levels of both 7-OH-DHEA isomers were attained, 7β-OH-DHEA being the preferred metabolite, the levels of which were increased more than three times. Surprisingly, even the levels of DHEA were increased.

Table 4. Changes of 7α- and 7β-OH-DHEA levels in six male volunteers before, during and one day after transdermal application of DHEA in the ointment. The scheme of the application and blood collection is the same as in Table 3.

	7α–OH-DHEA (nmol/l)			7β–OH-DHEA (nmol/l)		
	Basal	**Day 3**	**Day 6**	**Basal**	**Day 3**	**Day 6**
Median	2.02	4.03	4.33	1.33	3.64	3.73
Mean	1.93	4.05 **	4.25 **	1.46	4.44 **	3.99 **
S.E.M.	0.35	0.66	0.56	0.28	1.24	0.57
Range	0.36-3.43	2.07-5.67	2.42-5.87	0.49-3.62	1.70-8.79	2.62-6.60

*, ** *Significant differences from the basal level ($p<0.05$ and $p<0.01$, respectively).*

Table 5. Changes in the levels of DHEA and its 7-hydroxylated form in six male volunteers before and after transdermal application of 7-oxo-DHEA in the ointment (1 g in 100 g of the gel). The scheme of the application is the same as in Table 3. Blood was collected before and one day after the end of the application (Day 6).

	DHEA (nmol/l)		7α-OH-DHEA (nmol/l)		7β-OH-DHEA (nmol/l)	
	Basal	**Day 6**	**Basal**	**Day 6**	**Basal**	**Day 6**
Median	7.99	12.7	0.91	1.36	1.32	4.81
Mean	8.01	19.6 *	1.11	1.77 **	1.35	4.40 **
S.E.M.	1.49	4.81	0.29	0.30	0.28	0.38
Range	3.05-13.5	5.8-41.4	0.33-3.04	0.82-3.34	0.43-3.34	2.47-5.77

*, ** *Significant differences from the basal level ($p<0.05$ and $p<0.01$, respectively).*

Discussion

As was expected, dexamethasone suppressed the primary immune response measured by the NPFC even at the lowest concentration (25.5 nmol/l) and it also had a significant toxic effect on cell viability, in contrast to DHEA and its 7-hydroxylated metabolites. However, the effect of the latter steroids differed because DHEA and 7α-OH-DHEA decreased the NPFC irrespectively of the dose, while the decrease of the NPFC caused by 7β-OH-DHEA was diminished with the increasing dose. Therefore, the simultaneous effect of this steroid and dexamethasone was tested. It has been shown that 7β-OH-DHEA could couteract the dexamethasone-induced suppression of the NPFC. This finding serves as further evidence that 7-hydroxylated DHEA metabolites possess immunostimulatory effects. This is also in agreement with the recent results of Loria and Padgett (1998), who demonstrated that another 7β-hydroxylated DHEA metabolite, namely 5-androstene-3β,7β,17β-triol, acted in a similar way.

The application of DHEA to male subjects clearly demonstrated that its transdermal application significantly increased not only DHEA itself, but also its sulphate form and both its 7-hydroxylated metabolites, which are believed to be in some instances the biologically active compounds responsible for the immunomodulatory effects of DHEA. The application of 7-oxo-DHEA resulted in more than a threefold increase of 7β-OH-DHEA levels and also in a significant increase of 7α-OH-DHEA, indicating that this steroid may also be a candidate for replacement therapy. Further studies are in progress addressing the effect of DHEA and its metabolites on various endocrine and lipid parameters associated with aging.

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References

AKWA Y, SANANES N, GOUEZOU M, ROBEL P, BAULIEU EE, GOASCOGNE C : Astrocytes and neurosteroids: metabolism of pregnenolone and dehydroepiandrosterone. Regulation by cell density. *J Cell Biol* **121**: 135-143, 1993.

BAULIEU EE: Dehydroepiandrosterone (DHEA): a fountain of youth. *J Clin Endocr Metab* **81**: 3147-3151, 1996.

HAMPL R, MORFIN R, STÁRKA L: 7-Hydroxylated derivatives of dehydroepiandrosterone: what are they good for? *Endocr Regul* **31**: 211-218, 1997.

KALIMI M, SHAFAGOJ Y, LORIA RM, PADGETT D, REGELSON W: Antiglucocorticoid effects of dehydroepiandrosterone (DHEA). *Mol Cell Biochem* **131**: 99-104, 1994.

KHALIL MW, STRUTT B, VACHON D, KILLINGER DW: Effect of dexamethasone and cytochrome P450 inhibitors on the formation of 7α-hydroxydehydroepiandrosterone by human adipose stromal cells. *J Steroid Biochem Mol Biol* **48**: 545-552, 1994.

LAFAYE P, CHMIELEWSKI V, NATO F, MAZIE JC, MORFIN R: The 7α-hydroxysteroids produced in human tonsils enhance the immune response to tetanus toxoid and Bordetella pertussis antigens. *Biochim Biophys Acta* **1472**: 215-221, 1999.

LAPČÍK O, HAMPL R, HILL M, BIČÍKOVÁ M, STÁRKA L: Immunoassay of 7-hydroxysteroids: 1. Radioimmunoassay of 7β-hydroxydehydroepiandrosterone. *J Steroid Biochem Mol Biol* **67**: 439-445, 1998.

LAPČÍK O, HAMPL R, HILL M, STÁRKA L: Immunoassay of 7-hydroxysteroids: 2. Radioimmunoassay of 7α-hydroxydehydroepiandrosterone. *J Steroid Biochem Mol Biol* **71:** 231-237, 1999.

LORIA RM: Antiglucocorticoid function of androstenetriol. *Psychoneuroendocrinology* **22** (Suppl 1): S103-S108, 1997.

LORIA RM, PADGETT DA: Control of the immune response by DHEA and its metabolites. *Rinsho Byori* **46**: 505-517, 1998.

MORFIN R, COURCHAY G: Pregnenolone and dehydroepiandrosterone as precursors of native 7-hydroxylated metabolites which increase the immune response in mice. *J Steroid Biochem Mol Biol* **50**: 91-100, 1994.

SKINNER SJM, TOBLER CJP, COUCH RAF: A radioimmunoassay for 7α-hydroxydehydroepiandrosterone in human plasma. *Steroids* **30**: 315-330, 1977.

STÁRKA L: Reaktion der Steroide mit tert-Butylperbenzoat I. Über die 7-Acyloxylierung Δ^5-unsättiger Steroide. *Coll Czech Chem Commun* **26**: 2452-2456, 1962.

STÁRKA L, ŠULCOVÁ J, ŠILINK K: Die Harnausscheidung des 7-Hydroxydehydroepiandrosteronsulfats. *Clin Chim Acta* **7**: 309-316, 1962.

ŠTERZL J, MANDEL R: Estimation of the inductive phase of antibody formation by plaque technique. *Folia Microbiol* **9**: 173-175, 1964.

ŠTERZL I, HAMPL R, ŠTERZL J, VOTRUBA J, STÁRKA L: 7β-OH -DHEA counteracts dexamethasone induced suppression of primary immune response in murine splenocytes. *J Steroid Biochem Mol Biol* **71**: 133-137, 1999.

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