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Dehydroepiandrosterone and 7-oxo-dehydroepiandrosterone in male reproductive health: Implications of differential regulation of human Sertoli cells metabolic profile

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ABSTRACT

Dehydroepiandrosterone (DHEA) is a precursor of androgen synthesis whose action is partially exerted through its metabolites. 7-Oxo-dehydroepiandrosterone (7-oxo-DHEA) is a common DHEA metabolite, non-convertible to androgens, which constitutes a promising therapeutic strategy for multiple conditions. Sertoli cells (SCs) are responsible for the support of spermatogenesis, having unique metabolic characteristics strongly modulated by androgens. Consequently, disruptions in androgen synthesis compromise SCs function and hence male fertility. We aimed to evaluate the effects of DHEA and 7-oxo-DHEA in human SCs (hSCs) metabolism and oxidative profile. To do so, hSCs were exposed to increasing concentrations of DHEA and 7-oxo-DHEA (0.025, 1 and 50 μM) that revealed to be non-cytotoxic in these experimental conditions. We measured hSCs metabolites consumption/production by ^{1}H NMR, the protein expression levels of key players of the glycolytic pathway by Western blot as well as the levels of carbonyl groups, nitration and lipid peroxidation by Slot blot. The obtained data demonstrated that 7-oxo-DHEA is a more potent metabolic modulator than DHEA since it increased hSCs glycolytic flux. DHEA seem to redirect hSCs metabolism to the Krebs cycle, while 7-oxo-DHEA has some inhibitory effect in this path. The highest 7-oxo-DHEA concentrations (1 and 50 μM) also increased lactate production, which is of extreme relevance for the successful progression of spermatogenesis *in vivo*. None of these steroids altered the intracellular oxidative profile of hSCs, illustrating that, at the concentrations used they do not have pro- nor antioxidant actions in hSCs. Our study represents a further step in the establishment of safe doses of DHEA and 7-oxo-DHEA to hSCs, supporting its possible use in hormonal and non-hormonal therapies against male reproductive problems.

Abbreviations: 4-HNE, 4-hydroxynonenal; 7-OH-DHEA, 7-hydroxy-dehydroepiandrosterone; 7-oxo-DHEA, 7-oxo-dehydroepiandrosterone; AP, alkaline phosphatase; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DNPHZ, 2,4-dinitrophenylhydrazone; DNPH, 2,4-dinitrophenylhydrazine; EDTA, ethylenediaminetetraacetic acid; G6PD, glucose-6-phospate dehydrogenase; GLUTs, glucose transporters; GLUT1, glucose transporter 1; GLUT2, glucose transporter 2; GLUT3, glucose transporter 3; HBSS, Hanks balanced salt solution; hSCs, human Sertoli cells; ITS, insulin–transferrin–sodium selenite; LDH, lactate dehydrogenase; MCT4, monocarboxylate transporter 4; NMR, nuclear magnetic resonance; NO, nitric oxide; OS, oxidative stress; PBS, phosphate buffered saline; PFK1, phosphofructokinase 1; PPP, pentose phosphate pathway; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; SC, Sertoli cell; SEM, standard error of the mean; SRB, sulforhodamine B; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; 17β-HSD, 17β-hydroxysteroid dehydrogenase.

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1. Introduction

In mammals, dehydroepiandrosterone (DHEA) and its sulfated precursor, DHEAS, are mainly produced in the zona reticularis of the adrenal cortex. Interestingly, the testes are responsible for the production of 5% of DHEAS and 10–25% of DHEA [1]. Whereas DHEAS is the hydrophilic storage form found in blood circulation, only the more lipophilic DHEA can be intracellularly converted to androgens, illustrating the importance of the continuous interconversion between DHEA and DHEAS [2]. In men, around 74% of the DHEAS produced is metabolized to DHEA, but only about 13% of DHEA is metabolized back to DHEAS [1]. Moreover, the secretion of DHEA follows a distinctive age-related pattern [3]. In fact, between the sixth and tenth years of age, a phenomenon designed as adrenarche marks the gradual increase in the serum levels of DHEAS, but the intra-individual maximum is only achieved between the second and third decades of life. Then, the circulating levels of DHEAS progressively decline, so that at 70–80 years of age, concentrations are 10–20% of its maximum level, a phenomenon called adrenopause [4].

DHEA is metabolized in several peripheral target tissues, including the gonads [5], being the precursor of about 30–50% of androgen synthesis (testosterone and androstenedione) in adult men [6]. Androgens play a major role in male reproductive function, having important effects on protein, carbohydrate, and fat metabolism [7]. In this context, Sertoli cells (SCs), the "nurse" cells of the testis, rely on androgens action to maintain spermatogenesis and ensure male fertility. The metabolism of these cells is vital for the normal development of germ cells [8]. The SCs take up glucose from the interstitial space by specific glucose transporters (GLUTs) and convert it to pyruvate [9]. The pyruvate produced from glucose can follow three different pathways: (1) enter the mitochondria to form acetyl-CoA; (2) be converted to alanine; or (3) be converted to lactate [10]. The production of lactate in SCs is a crucial event for spermatogenesis since this metabolite is the preferred substrate used by developing germ cells [8]. In SCs, following the production of lactate by lactate dehydrogenase (LDH), the monocarboxylate transporter 4 (MCT4) mediates the transport of lactate to the intratubular fluid, being also capable of transporting pyruvate [11]. Hence, the disruption in any of these highly regulated mechanisms can surely compromise male fertility.

The age-related decline of androgen levels may lead to SCs malfunctioning and spermatogenesis disruption. Thus, testosterone replacement therapy has been considered as a therapeutic approach, which however is not free of health risks, including prostate cancer [7], cardiovascular diseases [12] and testicular atrophy or even infertility [13]. Interestingly, DHEA replacement appeared as a recommended therapy against testosterone deficiency, being also beneficial on several diseases/disorders such as cancer [14] and diabetes mellitus [15], as well as aging symptoms [16]. Some authors have even considered DHEA as the "fountain of youth" [17] and this fact has led to a widespread self-administration of DHEA as an over-the-counter dietary supplement. However, the unawareness of the beneficial/harmful doses of DHEA and the uncertain long-term safety can cause potential health risks. For instance, the administration of DHEA for extended periods increases testosterone above normal levels, especially in women [18], a fact that may cause masculinization.

The broad effect of DHEA itself limits its therapeutic use and one promising way to circumvent the side effects of DHEA, is the usage of DHEA derivatives with a greater specificity [19]. For instance, 7-hydroxy-dehydroepiandrosterone (7-OH-DHEA) epimers, which have been found in human semen at concentrations 5 times lower than DHEA, are reported to act as active immunoprotective agents [20]. A relevant intermediary of the interconversion between 7-OH-DHEA epimers is 7-oxo-DHEA, a biological DHEA metabolite that has proven to be a more potent therapeutic agent than its precursor, because it has no relevant androgenic activity by itself, it is not convertible to androgens [19] and it is more active than DHEA as an inducer of thermogenic enzymes [21]. So far, most of the studies concerning DHEA and its derivatives used rodents, which, physiologically, are not capable of synthetize DHEA [22], making these experiments limited to elucidate DHEA supplementation in humans. We propose to evaluate the effects of different concentrations of DHEA and 7-oxo-DHEA in human SCs (hSCs) metabolism. Moreover, since the high metabolic rates of hSCs are associated with high levels of oxidative stress (OS) [23] and there is controversial data concerning DHEA pro-oxidant [24] and antioxidant properties [25], we also studied the oxidative profile of hSCs after exposure to DHEA and 7-oxo-DHEA.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless specifically stated. The steroid 7-oxo-DHEA was obtained and characterized by a standard allylic oxidation method developed by our research group as previously described [26].

2.2. Patient selection, ethical issues and testicle tissue preparations

The patients clinical study and testicular tissue processing was performed at the Centre for Reproductive Genetics Professor Alberto Barros (Porto, Portugal) according to the Guidelines of the Local, National and European Ethical Committees. Our studies have been performed according to the Declaration of Helsinki. Testicular biopsies were obtained from patients under treatment for recovery of male gametes and used after informed written consent. Only cells left in the tissue culture plates after patient's treatment were used. Human SCs (hSCs) were isolated from six testicular biopsies of men with conserved spermatogenesis, selected from patients with anejaculation (psychological, vascular, neurologic).

2.3. Human Sertoli cell primary culture

Testicle biopsies were washed twice in $HBSS_f$ (Hanks Balanced Salt Solution without Ca^{2+} or Mg^{2+}) through centrifugations at $500 \times g$ at room temperature, as described by Oliveira et al. [27]. hSCs were obtained by a routine method used by our team [28]. The resulting cellular pellet was suspended in hSCs culture medium (DMEM:Ham's F-12 1:1, containing 15 mM HEPES, 50 U/ml penicillin and 50 mg/ml streptomycin sulfate, 0.5 mg/ml fungizone, 50 μg/ml gentamicin and 10% heat inactivated fetal bovine serum) and forced through a 20G needle, in order to disaggregate large cell clusters. Then, cells were plated on Cell+ culture flasks (Sarsted, Nümbrecht, Germany) and incubated at 30–33 °C and 5% CO_2 in air until used. After 96 h, the cultures were examined by phase contrast microscopy and only hSCs cultures with contaminants below 5% were used.

2.4. Experimental groups

hSCs were allowed to grow until reach 90–95% of confluence. Then, cells were fully washed and the culture medium replaced by serum-free medium (DMEM:F12 1:1, pH 7.4) supplemented with insulin, transferrin and sodium selenite (ITS medium; 5 μg/ml, 5.5 μg/ml, 5 ng/ml, respectively). In order to evaluate the effect of DHEA and 7-oxo-DHEA on hSCs glycolytic and oxidative profile, seven different groups were defined: a control group and other six groups containing ITS medium supplemented with increasing