Protective effect of dehydroepiandrosterone against lipid peroxidation in a human liver cell line

Marco Gallo, Manuela Aragno, Valentina Gatto, Elena Tamagno, Enrico Brignardello, Roberta Manti, Oliviero Danni and Giuseppe Boccuzzi

Department of Clinical Pathophysiology, University of Turin, Via Genova 3, 10126 Turin, Italy and Department of Experimental Medicine and Oncology, General Pathology Section, University of Turin, C.so Raffaello 30, 10125 Turin, Italy

(Correspondence should be addressed to G Boccuzzi)

Abstract

Objective: Dehydroepiandrosterone (DHEA) is a widely studied steroid hormone with multi-functional properties. Reports suggest that some of the many activities of DHEA are due to its protective effect against lipid peroxidation. Nevertheless, the antioxidant properties of DHEA are still the subject of debate. The aim was to evaluate whether its two opposed effects on lipid peroxidation reported in the literature may be dependent on schedule and doses used.

Methods: Chang liver cells, a line derived from normal human liver, were grown in media containing either no steroids (control) or DHEA at concentrations ranging from 0.1 µmol/l to 50 µmol/l. At specific times, cultures were halted and cells received a pro-oxidant stimulus (cumene (CuOOH) 0.5 mmol/l), at which time cell viability (by trypan blue staining and lactate dehydrogenase (LDH) release) and thiobarbituric acid reactive substances (TBARS) concentration (spectrophotometrical assay) were evaluated.

Results: At concentrations ranging from 0.1 µmol/l to 1 µmol/l, DHEA protects Chang liver cells against lipid peroxidation and/or death induced by cumene. This effect disappears if the concentration is increased to 10 µmol/l; at higher concentrations (50 µmol/l) a pro-oxidant/cytotoxic effect of DHEA appears.

Conclusions: DHEA exhibits two opposed effects on lipid peroxidation; depending on its concentration it acts either to limit or to induce oxidative stress. The threshold concentration at which the pro-oxidant activity of DHEA prevails is not far in excess of that having an antioxidant effect. Either effect of DHEA on lipid peroxidation is only evident after a 'lag-phase'.

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Introduction

Dehydroepiandrosterone (DHEA), a steroid produced in quantity by the adrenal glands, is a widely studied hormone with multi-functional properties. DHEA has been reported to possess beneficial effects on cancer (1–6), atherosclerosis (7–9), obesity (10), autoimmune diseases (11), infections (12, 13), diabetes (14, 15) and aging (16). These wide-ranging activities cannot fully be explained by activation of the androgen or estrogen receptors. Published data support the hypothesis that DHEA acts to limit lipid peroxidation, in vivo as well as in vitro (17–23). Since many of the diseases on which DHEA has a beneficial effect have been related to repeated oxidative insult (24), the antioxidant properties of DHEA might be expected to explain its effects. However, other studies have reported that DHEA actually induces oxidative stress. In rats, dietary DHEA induces peroxisomal-oxidation enzymes and peroxisome proliferation (25–27). Moreover, levels of cytochrome P450 and lipid peroxidation increase in the liver microsomes of rats fed DHEA (28, 29). The actual effect of DHEA on lipid peroxidation is thus still in doubt.

Since DHEA is now available as an over-the-counter product and is ingested daily by many people throughout the world, it is important to understand its effect on lipid peroxidation. In this study, we used an in vitro model (Chang liver cell line) to investigate this effect. Data show that DHEA, at concentrations slightly above those found in human tissues, protects cells against lipid peroxidation induced by an oxidative stress (cumene, CuOOH). This effect disappears if DHEA is administered at pharmacological doses, which display a pro-oxidant activity. Both effects of DHEA on lipid peroxidation (i.e. antioxidant and pro-oxidant) need a ‘lag-phase’ to become evident.

Materials and methods

Chemicals

DHEA was purchased from Sigma Chemicals Co. (St Louis, MO, USA). The compound was diluted in ethanol;
its final concentration in the culture medium was 0.1%, which has no detectable effect on cell growth. However, ethanol at the same concentration was also added to the control medium. Fetal calf serum (FCS) (Eurobio, Les Ulis, France) was extracted with charcoal dextran solution (10:1) for 60 min at 25°C to remove steroids.

**Cell culture**

Chang liver cells are epithelial-like cells from normal human liver (30, 31). The cells were routinely cultured in 75-cm² plastic flasks (Falcon, Franklin Lakes, NJ, USA), in RPMI 1640 phenol red-free medium (Gibco, Palo Alto, CA, USA) supplemented with 2 mmol/l L-glutamine (Eurobio, France), 100 IU/ml penicillin G, 100 μg/ml streptomycin and with 15% FCS added. The cells were grown in an humidified atmosphere containing 5% (v/v) CO₂ at 37°C. The medium was changed every two days. The cells were passaged by trypsin 0.05% and EDTA 0.02% weekly.

**Experimental protocols**

Cells were plated in 75-cm² plastic flasks at different concentrations (1.3 × 10⁶–7 × 10⁶ cells/flask), depending on the duration of the experimental protocol, so as to attain a similar cell number at the end of the experiment. After plating, cells were allowed to attach for 24 h in the medium supplemented with 15% steroid-stripped FCS; the seeding medium was then replaced with an experimental medium containing either no steroids (control) or DHEA at different concentrations (0.1 μmol/l, 0.5 μmol/l, 1 μmol/l, 10 μmol/l, 50 μmol/l). All experiments were performed in duplicate. The media were renewed every 48 h. At specific times (17 h, 3 days or 6 days) the cells were harvested with trypsin and counted (twice for each well) by Burker’s chamber before determination of lipid peroxidation.

**Lipid peroxidation assay**

Lipid peroxidation was evaluated by measuring the steady state concentration of thiobarbituric acid reactive substances (TBARS) in cell suspension (5 × 10⁶ cells/ml) in a balanced salt buffer (32). For each set of experimental conditions, the cells received the pro-oxidant stimulus (0.5 mmol/l CuOOH) and were then incubated in a shaking water bath at 37°C. After 5-h incubation, aliquots of the cell suspension were taken, mixed with 10% trichloroacetic acid and maintained on ice for 15 min. Cells were then centrifuged at 1500 × g for 10 min and the supernatant mixed with 0.67% thiobarbituric acid and placed for 10 min in boiling water. KOH (2 mol/l) was added to clear the solution and a spectrophotometrical assay at 534 nm was performed. Results are expressed as pmoles TBARS produced/10⁶ cells counted before the pro-oxidant stimulus.

**Viability assay**

Cell viability was evaluated through lactate dehydrogenase (LDH) release and trypan blue exclusion. Aliquots (0.5 ml) of the cell suspension were centrifuged at 200 × g for 5 min. and the supernatant was used for the assay. In a cuvette containing 0.1 mol/l potassium phosphate buffer, pH 7.0, 100 μl 7.6 mmol/l sodium pyruvate (final concentration), and 50 μl 0.2 mmol/l NADH (final concentration), 10 μl of the assay solution were added and spectrophotometrical analysis at 340 nm was performed. LDH release was expressed as percentage of total release, i.e. the release of LDH after cell lysis by 10% Triton X-100.

With the trypan blue method viable cells were counted after staining and results expressed as percentage of total cell number.

**Statistical analysis**

All results are expressed as means ± standard deviation. Statistical comparison between groups was performed by two-tailed paired data Student’s t-test.

**Results**

**Effect of DHEA on lipid peroxidation**

Cumene-induced lipid peroxidation in Chang liver cells, grown for 17 h in the presence of DHEA, did not differ from that of cells cultured in steroid-free medium at any of the concentrations tested (Fig. 1). When the exposure time of Chang liver cells to DHEA was extended to the third day, concentrations ranging from 0.1 μmol/l and 10 μmol/l DHEA had no effect on cumene-induced lipid peroxidation. For example, the concentration of TBARS in the control group (CuOOH alone) was not significantly different from that in the group exposed to DHEA at any concentration tested. This was true for both 3 and 6 days of exposure to DHEA.

**Figure 1** Cumene-induced lipid peroxidation in Chang liver cells, evaluated by measuring the steady-state TBARS concentration after exposure to different DHEA concentrations for 17 h, 3 or 6 days. Bars represent mean values of (n) experiments ± standard deviation. *P < 0.05, **P < 0.01 vs CuOOH alone.
lipid peroxidation, whereas the 50 μmol/l concentration showed a pro-oxidant effect ($P < 0.01$) (Fig. 1).

On further prolonging the exposure of the cells to the steroid (6 days), a protective effect against the lipid peroxidation induced by CuOOH was observed at concentrations of 0.1 μmol/l and 0.5 μmol/l ($P < 0.05$) (Fig. 1), whereas 1 and 10 μmol/l were still ineffective and the 50 μmol/l concentration again showed pro-oxidant activity ($P < 0.01$) (Fig. 1).

**Effect of DHEA on viability of Chang liver cells after cumene-induced lipid peroxidation**

The viability of Chang liver cells, as measured by both LDH release and trypan blue, was dramatically reduced by the pro-oxidant effect of 0.5 mmol/l CuOOH (Figs 2 and 3). Growing cells for 17 h in DHEA-supplemented medium, at concentrations ranging from 0.1 μmol/l to 50 μmol/l, had no effect on their viability after lipid peroxidation triggered by cumene. If the exposure of the cells to the steroid was maintained for 3 days, DHEA concentrations between 0.1 μmol/l and 1 μmol/l protected cell viability, as measured by the trypan blue method ($P < 0.05$) (Fig. 2), whereas 10 μmol/l DHEA had no effect and 50 μmol/l DHEA showed a cytotoxic effect ($P < 0.01$) (Fig. 2). Cumene-induced LDH release, after 3 days of culture, was unaffected by DHEA (Fig. 3).

When the duration of cell culture was extended to the sixth day, the protective effect of DHEA on cumene-induced cell death was still observed at 0.1 and 0.5 μmol/l concentrations, both by trypan blue exclusion (Fig. 2) and by LDH release (Fig. 3). DHEA at a concentration of 1 μmol/l had no effect on cell viability, whereas the higher concentrations (10 and 50 μmol/l) again increased cell death (Figs 2 and 3).

**Discussion**

This study demonstrates that DHEA exhibits two opposed effects on lipid peroxidation; depending on its concentration, it acts either to limit or to induce oxidative stress. At a concentration (0.1 μmol/l) slightly higher than that found in human tissues, DHEA protects Chang liver cells against lipid peroxidation and cell death induced by cumene. By contrast, at pharmacological concentrations (10–50 μmol/l) both lipid peroxidation and cell death after the pro-oxidant stimulus are increased by DHEA.

This result agrees with previous reports. An increase in lipid peroxidation when DHEA is given at pharmacological doses to rodents has regularly been reported (25–29). The pro-oxidant effect of DHEA likely depends on its capability both to induce peroxisome proliferation (25, 26), probably via a peroxisome proliferator-activated receptor (33), and to increase the NADPH-cytochrome P-450 reductase/cytochrome P-450 system (29), thus...
increasing the cell concentration of H$_2$O$_2$. Evidence exists that the increase in hepatic peroxisomal beta-oxidation activity is only induced by high doses of DHEA (25).

Here we show that, at concentrations ranging from 0.1 to 0.5 µmol/l, DHEA exerts an antioxidant effect. At these concentrations, DHEA has been reported to protect both bovine retinal capillary pericytes against glucose toxicity (23) and human low density lipoproteins (LDL) against peroxidation induced by γ radiolysis of ethanol-water mixtures (22).

While the pro-oxidant effect is dependent on peroxisome proliferation, the mechanism underlying the antioxidant effect of DHEA is not obvious. DHEA might modify the composition or the structure of lipid membranes, thus rendering cells more resistant to oxidative stress. In this connection, DHEA has been reported to change the fatty acid composition of mitochondrial membrane phospholipids in rats (34). Mechanisms involving the incorporation of estradiol (35) or DHEA (22) derivatives into LDL have also been postulated to explain the antioxidant protection that these steroids exert on human low density lipoproteins.

Moreover, the intercalation of DHEA into the lipid membranes has been suggested as the mechanism responsible for the change in shape of human red blood cells induced by DHEA in vitro (36). Alternatively, the addition of DHEA to the culture medium might modify the content of some antioxidant species, thus increasing the cell’s defence against free radical damage. DHEA has recently been reported to protect human LDL against lipid peroxidation by regenerating the endogenous vitamin E of LDL (22). However, the content of vitamin E and other antioxidants has been reported to remain unchanged in tissue homogenates of DHEA-treated rats (21).

Both effects of DHEA (i.e. pro-oxidant at high concentrations, and antioxidant) became evident after a lag-phase. After 17-h exposure to DHEA, no effect on cumene-induced lipid peroxidation and Chang liver cells’ death was observed. If exposure to DHEA was prolonged for six days, at concentrations only slightly above those found in human tissues, both TBARS formation and loss of cell viability after pro-oxidant stimulus were significantly reduced. However, at pharmacological doses this protective effect vanished and was replaced by a pro-oxidant and cytotoxic effect.

The lag-phase between DHEA administration and the beginning of the antioxidant or pro-oxidant effect cannot be explained by its intracellular distribution, since DHEA diffuses freely across the plasma membrane, requiring neither a transporter molecule nor an energy sparing mechanism (37). As far as the antioxidant effect is concerned, it is still an open question whether it is due to the DHEA itself, to its metabolites or to a combination of both. It may be speculated that, besides the time which might possibly be needed to modify lipid membranes, DHEA requires some transformation process before becoming active.

Liver cells have the enzymes needed to convert DHEA into a variety of steroids, including androgens, estrogens and 5-en-androsten-3β,17β-diol (ADIOL), the main DHEA metabolite that shows both androgenic and estrogenic properties (38, 39).

We have shown recently that the direct addition of DHEA to liver microsomes isolated from untreated rats does not prevent iron-dependent lipid peroxidation (40). Indeed, most of the effects attributed to DHEA are mediated by one of its metabolites, such as ADIOL for the hormonal effects (39, 41) or 5-en-androsten-3β,7β,17β-triol for its effect on the immune system (42). Nevertheless, a variety of steroids, including androstenedione, 17β-estradiol, ADIOL and dihydrotestosterone, failed to protect bovine retinal capillary pericytes against glucose-induced lipid peroxidation, whereas DHEA was successful (23). The lag-phase before the start of the pro-oxidant effect is probably due to the induction of peroxisome proliferation. Nevertheless, conversion of DHEA to active metabolites would also appear to be needed in this case too. Indeed, studies have shown that in primary cultures of rat hepatocytes DHEA is almost inactive as an inducer of peroxisomal enzymes unless it is first metabolized by sulphotransferase to the 3-beta-sulphate (DHEAS) (33).

Furthermore, both for its pro-oxidant and for its antioxidant effects, the synthesis of accessory proteins, or other nuclear factors that modulate the oxidative processes, cannot be excluded.

Whatever are the mechanism and the active compound, it should be emphasized that the threshold concentration at which the pro-oxidant activity of DHEA prevails is not far in excess of that showing antioxidant effects. This is an important point since DHEA, depicted as the ‘youth hormone’, is taken daily by millions of people throughout the world. The protective effect of low doses of DHEA on lipid peroxidation and cell viability might, however, be of clinical relevance.

References


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