Effects of Dehydroepiandrosterone (DHEA) on Ubiquinone and Catalase in the Livers of Male F-344 Rats

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The adrenal steroid, dehydroepiandrosterone (DHEA) acts as a peroxisome proliferator in the rodents. The present study examined the effects on cellular antioxidants ubiquinone and catalase in the liver of DHEA-treated rats. When administered to male F-344 rats for 8 weeks, DHEA produced a significant increase in hepatic ubiquinone-9 and lipid peroxide levels while no change was observed after 2 weeks. Activity of catalase, in contrast, followed an inverse pattern, being significantly induced at 2 weeks with a return to normal levels after 8 weeks. A marked reduction of ubiquinone-10 in DHEA-treated rat livers was only observed after 2 weeks. These findings indicate the potentials of high dose DHEA to modulate ubiquinone in rat hepatic tissue.

Key words: peroxisome; liver; tumor; ubiquinone; oxidative stress

Dehydroepiandrosterone (DHEA) is a C₁₉ steroid produced abundantly by the mammalian adrenal cortex. Although its physiological role remains uncertain, several epidemiological studies have thus far indicated a potential chemo-preventive role of DHEA towards several important human diseases. For example, decreased serum levels of DHEA and its major circulating form DHEAS (dehydroepiandrosterone sulfate) have been positively correlated with increased risks of developing cancer and cardiovascular disease. In experimental animal models, DHEA elicits anti-carcinogenic, anti-diabetic and anti-obesity properties. Contrary to these important beneficial effects, DHEA acts as a peroxisome proliferator and carcinogen in the liver of rodents.

Similar to other peroxisome proliferators, exposure of rodents to high dose levels of DHEA produces hepatomegaly, peroxisomal proliferation, induction of several hepatic enzymes associated with lipid metabolism, and upon prolonged administration, development of hepatocellular carcinoma. A worthwhile goal in DHEA research is, therefore, the dissection of mechanisms of its hepatotoxicity from its potential beneficial effects, and to determine if these findings are relevant to potentially therapeutic doses in man.

Oxidative stress may play a critical role in liver tumor formation caused by peroxisome proliferators. The mechanism emphasizes on the production of excessive cytotoxic free radical as the causative factor in liver carcinogenesis. Hydrogen peroxide-mediated free radicals generation have been related with differential induction of peroxisomal β-oxidation and catalase by DHEA and other peroxisome proliferators, which are responsible for hydrogen peroxide formation and degradation, respectively, in the liver. Interestingly, several cellular detoxifying enzymes including glutathione transferase, glutathione peroxidase and superoxide dismutase were inhibited in the livers of DHEA-treated rats. As a result, there is an excess of un-metabolized hydrogen peroxide molecules that retain the capacity to cause oxidative DNA damage ultimately leading to neoplasm.

It was previously reported that DHEA depletes the endogenous mevalonic acid pool in human colon adenocarcinoma HT-29 cells. Mevalonic acid serves as a precursor for biosynthesis of important isoprenoids in the mammalian cells. Ubiquinone, a mevalonate-derived isoprenoid, plays a critical role in cellular defense by virtue of its antioxidant properties. The aim of the present study was to determine whether mevalonic acid pool depletion following DHEA treatment, resulted in decreased ubiquinone levels in the liver. The findings, however, indicate a temporal change of ubiquinone concentration in DHEA-treated rat hepatocytes along with catalase activity.

MATERIALS AND METHODS

Chemicals DHEA was supplied from AKZO (Basel, Switzerland). Carboxymethyl cellulose, ubiquinone-6, potassium ferricyanide and palmitoyl CoA were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sucrose, HPLC grade methanol and reagent alcohol (ethanol) were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Lithium perchlorate and n-hexane (99% ultrapure) were purchased from Baxter Diagnostics Inc. (McGaw Park, IL, U.S.A.). All other chemicals and reagents used were of the highest grade commercially available.

Animals and Experimental Protocols Male F-344 rats initially weighing 191—224 g (Harlan Sprague Dawley, IN, U.S.A.) were maintained on normal laboratory chow (Purina Mills, St. Louis, MO, U.S.A.) and water ad libitum with a 12 h light/dark cycle and controlled temperature and humidity. After one week of acclimatization, the animals were randomly divided into 2 treatment groups: control or DHEA (300 mg/kg). DHEA suspended in 0.5% carboxymethyl cellulose was administered at 3 ml/kg, while the control animals received the vehicle. The animals were treated once daily by gastric intubation for 2 or 8 weeks where applicable. The duration of the experiment, dosage of DHEA and method of administration were based on previous literature reports. Twenty four hours after the last treatment, the animals were euthanized by CO₂ (100%). Immediately, prior to death, the livers of the rats were perfused in situ with chilled buffered saline (pH 7.4). Liver homogenates were prepared in 20 mM Tris–HCl containing 0.25 M sucrose (pH 7.4) and aliquots were frozen in amber-colored tubes at −70°C until further analyses were performed. Animal experiments were in accordance with the guidelines set by the Animal Care and
Spectrophotometric Assays  Catalase activity of the liver was quantitated spectrophotometrically at 240 nm. Lipid peroxidation was estimated using a colorimetric assay (Calbiochem Novabiochem, CA, U.S.A.). The values were corrected to protein concentration.

HPLC Determination of Hepatic Ubiquinone Content  Total ubiquinone (oxidized and reduced forms) in rat liver homogenates was extracted according to the established methods using the ethanol–hexane solvent system and ubiquinone-6 as an internal standard. HPLC analysis was performed in a mobile phase containing 20 mM lithium perchlorate in ethanol–methanol mixture using an Ultrasphere ODS column (5 μm; 4.6 mm × 2.5 cm; Beckman Instrument, Fullerton, CA, U.S.A.), while the peaks were detected at 275 nm. Extraction recovery was corrected with the internal standard and values normalized to protein concentration.

Statistics  Data are reported as mean ± S.E.M. (standard error of the mean). Student’s t-test was used for statistical analysis using the statistical package GB STAT (version 5.0).

RESULTS AND DISCUSSION

In order to determine DHEA’s effect on ubiquinone, two major isoforms of the isoprenoid namely ubiquinone-9 and -10 were quantitated in rat liver homogenate. Although DHEA did not produce any effect on ubiquinone-9 at 2 weeks, following 8 weeks of DHEA treatment, ubiquinone-9 concentration was increased 1.43 fold compared to control levels (Fig. 1). The levels of ubiquinone-10 followed an opposite time course. There was a significant reduction of ubiquinone-10 at 2 weeks (p<0.05), while no difference was observed after 8 weeks (Fig. 2).

Figure 3 summarizes the results of catalase activity. Similar to previous observations, we observed a marked increase in catalase activity in DHEA-treated rats (p<0.01) at 2 weeks with a return to a basal level at 8 weeks.

Lipid peroxidation serves as a marker of oxidative stress. As shown in Fig. 4, there was a significant increase (p<0.01) in lipid peroxide after chronic exposure to DHEA, although no change was observed at 2 weeks time point. It is worthwhile to note that the basal levels of ubiquinone and catalase in the present study showed an age-related increase as reported previously by other investigators.

Ubiquinone is an essential component of mitochondrial electron transport system. As an antioxidant, it quenches free radicals directly as well as through recycling of Vitamin E, another well-known chain-breaking antioxidant. Ubiquinone-9, the predominant isoform of the coenzyme present in the rat liver contains nine isoprene moieties, whereas ubiquinone-10 is present at much reduced concentrations. Data from the present study indicates a marked re-
duction in hepatic ubiquinone-10 at 2 weeks in DHEA-treated rats. This finding supports the hypothesis of ubiquinone depletion, however, the effect was short lived. In contrast, ubiquinone-9, which remained at basal at 2 weeks was significantly elevated at 8 weeks following DHEA treatment. Thus, DHEA affects hepatic ubiquinone in an isomer-specific and time-dependent manner.

Interestingly, in a separate study from this laboratory, DHEA-sulfate (DHEAS), significantly increased ubiquinone-9 in the liver at 2 weeks, although no effect was observed with ubiquinone-10. It is possible that the modulation of hepatic ubiquinone by DHEAS may be different from that of DHEA.

Catalase reduces cellular levels of hydrogen peroxide, thus serving as a protective mechanism against peroxide associated oxidative damage to cellular macromolecules. As expected, catalase activity was significantly elevated by DHEA at 2 weeks. However, the return of catalase activity to control levels at 8 weeks, suggests the possible recruitment of alternative mechanisms in counteracting oxidative stress. In the present study, we observed a marked elevation of ubiquinone-9 in rat hepatocytes. Since other cellular defense enzymes, i.e. glutathione peroxidase, glutathione S-transferase and superoxide dismutase were inhibited by DHEA, the role of ubiquinone in peroxisome proliferation could be of potentially significant importance, especially after 8 week exposure to DHEA.

Ubiquinone-9 content in the rat liver elicited a pattern exactly opposite to catalase activity. This inverse relationship between catalase and ubiquinone-9 suggests that catalase provides a defense against acute oxidative stress, while ubiquinone-9 replaces catalase as a major antioxidant during chronic oxidative stress. Moreover, increased hepatic ubiquinone-9, resulting after prolonged DHEA treatment, possibly compensate for the inability of the cell to increase catalase activity to keep pace with elevated oxidative stress.

This is the first report of an increase in ubiquinone-9 in DHEA-treated rodent liver. Unlike previous observations in the present study, the contribution of plasma ubiquinone was carefully eliminated by in situ perfusion of liver. Furthermore, a pharmacological dose of DHEA was used rather than incorporating the steroid in the diet which allows variation in dosage.

Increase of hepatic ubiquinone-9 after 8 weeks of DHEA treatment was in concert with a marked increase in lipid peroxidation. These findings provide evidence that the increase in ubiquinone-9 was a compensatory effect in order to protect the hepatocytes from oxidative stress. A lack of increase in lipid peroxidation by DHEA at 2 weeks when ubiquinone-10 was markedly reduced was not surprising as lipid peroxidation might be increased in specific cellular compartments and not detectable in total liver homogenates. In fact, increased lipid peroxidation in mitochondria and microsomes was observed within 3 d of feeding DHEA.

In conclusion, the present suggests that ubiquinone-9 levels can be increased markedly upon chronic exposure to DHEA. Catalase, on the other hand, is induced for a short period. Our previous finding suggests that exogenous ubiquinone significantly reduces the requirement of catalase induction in DHEA-treated rat liver. Further studies are therefore required to understand the relationship between catalase and ubiquinone following exposure to high dose DHEA.

REFERENCES