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Dehydroepiandrosterone sulfate increases hepatic ubiquinone-9 in male F-344 rats

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Received June 5, 2003, accepted July 10, 2003

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Pharmazie 59: 491–492 (2004)

Administration of dehydroepiandrosterone sulfate (DHEAS) for 14 days caused a significant increase in the total ubiquinone-9 level in the hepatic tissue of male F-344 rats ($p < 0.05$). Hepatic ubiquinone-10 level of DHEAS-treated rats however, was found not to be statistically different from control animals. These findings suggest that peroxisome proliferator DHEAS displays typical hepatic response in increasing ubiquinone concentration in the rat.

In man, the circulating level of the adrenal steroid dehydroepiandrosterone sulfate (DHEAS) attains a maximum level between 20–30 years of age, and thereafter markedly declines (Orentreich et al. 1984). Epidemiological studies suggest a possible link between the serum level of DHEAS and development of important human diseases including cardiovascular disease and cancer (Khan and Nyce 1997). Therefore, the age-related decline of DHEAS level in man may increase the susceptibility to serious pathological conditions.

It is important to note that DHEAS belongs to a class of chemicals known as peroxisome proliferators. Peroxisome proliferators have diverse structure and function and are capable of producing a dramatic increase in the number and size of peroxisomes in the liver, induction of lipid metabolism enzymes, and liver tumor in rodents (Khan and Nyce 1997). Imbalance in hydrogen peroxide production and degradation constitutes the oxidative stress model of peroxisome proliferator-induced hepatocarcinogenesis (Rao et al. 1992). Excessive free radicals accumulated in the hepatic tissue interact with DNA and other macromolecules and eventually results in cancer.

The present study examined the role of DHEAS on hepatic ubiquinone in male F-344 rats. Ubiquinone, an intracellular antioxidant is known to play a critical role in maintaining the red-ox balance of the cell. In addition to quenching free radicals directly (Ernster and Forsmark-Andrée 1993), it facilitates proper functioning of another chain breaking antioxidant, vitamin E (Kagan et al. 1990).

Data indicates that hepatic ubiquinone-9, containing nine isoprene units, was significantly increased ($p < 0.05$) following oral administration of 300 mg/kg DHEAS for 14 days (Fig.). In contrast, DHEAS did not produce any appreciable change in hepatic ubiquinone-10 (Fig.). The dose of DHEAS in this study was previously reported to markedly induce peroxisomal β-oxidation of fatty acid (3.6 fold), responsible for hepatotoxic hydrogen peroxide generation (Khan and Nyce 1997). A relatively rapid elevation in ubiquinone-9 indicates a homeostatic response to DHEAS administration.

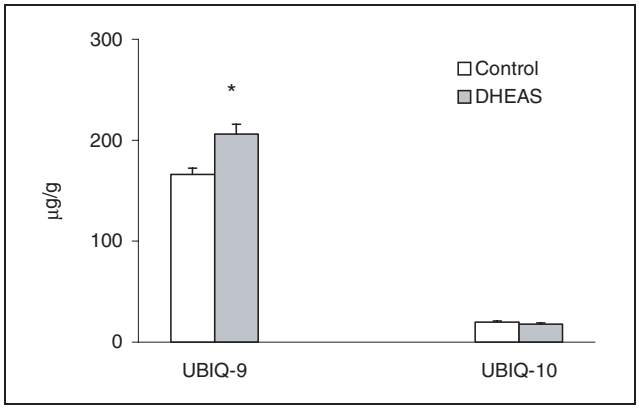

Fig. Effect of DHEAS on hepatic ubiquinone in male F-344 rats. Each bar represents mean ± S.E.M. of 4 rats. * Denotes significantly different from control value ($p < 0.05$).

The present study suggests that DHEAS similar to other peroxisome proliferators increase hepatic ubiquinone levels in rodents. However, this finding contradicts a previous report where peroxisome proliferators caused an increase in ubiquinone-10 in the rat liver (Åberg and Appelkvist 1994) as opposed to ubiquinone-9. This variation could be attributed to the differences in mechanism of action between DHEAS and other peroxisome proliferators in enhancing ubiquinone level.

In essence, DHEAS appears to be a suitable agent against oxidative stress probably by modulating the endogenous levels of ubiquinone and other cellular antioxidants. Further studies are required to elucidate the mechanisms associated with the response.

Experimental

After acclimatization, male F-344 rats initially weighing 191–224 g (Harlan Sprague Dawley, IN) maintained on normal laboratory chow and water *ad libitum* were randomly divided into 2 groups: DHEAS (300 mg/kg; Sigma Chemical Co., St. Louis, MO) and control. DHEAS suspended in 0.5% carboxymethyl cellulose was administered at 3 ml/kg, while the control animals received the vehicle alone. The animals were treated once daily by gastric intubation for 2 weeks as reported earlier (Sakuma et al. 1992; Yamada et al. 1991). Twenty four hours following the last treatment, the animals were euthanized by CO_2 (100%). Immediately, prior to death, the livers of the rats were perfused *in situ* with chilled buffered saline (pH 7.4) (Borges et al. 1993). 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. All other chemicals and reagents used were of the highest grade commercially available.

Total ubiquinone in rat liver homogenates was extracted (Reahal and Wrigglesworth 1992; Okamoto et al. 1985) using an 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) (Lang and Packer 1987), while the peaks were detected at 275 nm. The data was corrected with the internal standard and values normalized to protein concentration. 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).

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Trypanocidal activity of bergenin, the major constituent of *Flueggea virosa*, on *Trypanosoma brucei*

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Received August 21, 2003, accepted November 10, 2003

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Pharmazie 59: 492–494 (2004)

The presence of bergenin in substantial amounts in the methanol leaves extract of *Flueggea virosa* (Euphorbiaceae) was established as a strong chemotaxomic point of differentiation between *Flueggea virosa* and *Securinega virosa*. Bergenin showed an inhibitory effect on the growth of the bloodstream form of *Trypanosoma brucei* with an IC_{50} value of 1 μM.

Despite a reference work (Webster 1984) which should have already resolved problems related to the distinction between *Flueggea* and *Securinega* (Euphorbiaceae) there are still some misleading reports suggesting that *Flueggea virosa* is identical to *Securinega virosa* (Pu 2002; Dehmlow 1999). Moreover, the genus *Securinega* is known to produce a group of tetracyclic compounds classified as the securinega alkaloids (Han 2000; Tatematsu 1991; Golebriewski 1976; Nakano 1963) whereas *Flueggea* mainly produces isocoumarins such as bergenin in substantial amounts (Ahmad 1972).

As a continuation of our programme aimed at the isolation of naturally occurring compounds with potential antiprotozoal activities it was deemed necessary to study the chemical composition of *Flueggea virosa* which is locally used to treat sleeping sickness or related symptoms in order to corrolate its components with the use of plant as a trypanocidal remedy. In contrast to previous results (Pu et al. 2002; Dehmlow et al. 1999), only bergenin (**1**) was isolated in substantial amounts from *Flueggea virosa* and no securinega alkaloid could be detected.

Bergenin (**1**) is an isocoumarin found in a number of plant species including *Flueggea microcarpa*, *Mallotus japonicus*, *Astilbe thunbergii* and *Ardisia japonica*. It has a long history in Asian and Indian natural medicine, where it can be found in various herbal extracts purported