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Suppression of apoptosis and oxidative stress by deprenyl and estradiol in aged rat liver

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Summary

Aging is accompanied by significant structural and functional transformations of all organs and systems. Age-associated increase in apoptotic behavior may cause disease. Older cells are more susceptible to endogenous oxidative damage, and oxidative stress is a potent inducer of apoptosis. Deprenyl is an irreversible monoamine-oxidase B inhibitor which has anti-oxidant, anti-apoptotic and neuroprotective effects. Estrogen is also a neuroprotective and anti-oxidant hormone. The objectives of this study were to determine whether the anti-oxidative effects of deprenyl can suppress apoptotic activity, with or without estradiol, in aged female rat livers. In this study, ovariectomized female Wistar albino rats were divided into six groups as follows; young (3 months old) saline-treated control, aged (24 months old) saline-treated control, aged deprenyl treated, aged estradiol treated, aged deprenyl plus estradiol treated and aged sham controls. All rats except for the sham group were treated for 21 days. Determination of oxidative stress parameters was performed spectrophotometrically. To detect apoptotic cells, TUNEL staining was performed. The results were analyzed by one-way ANOVA post hoc Bonferroni test. Deprenyl and estradiol administration, alone or in combination, decreased significantly the levels of lipid peroxidation and increased superoxide dismutase activity in the liver relative to aged control and sham rats ($P<0.05$). The number of TUNEL positive cells decreased significantly in deprenyl and estradiol-treated rats compared with aged control and sham rats. The results indicate that deprenyl treatment alone, or in combination with estradiol, may modulate age-related apoptotic changes in rat liver by decreasing oxidative stress.

Abbreviations: CAT, catalase; GPx, glutathione peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick end labeling

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Introduction

Aging is accompanied by significant structural and functional transformations of all organs and systems. The free radical theory of aging states that progressive increase in reactive oxygen species (ROS) and consequent oxidative damage play a major role in aging and age-related degenerative disorders (Harman, 1993; Barja, 2004). Anti-oxidant defenses and repair systems, which help to protect cells against free radical destruction, include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Wickens, 2001). In recent years, a role for apoptosis in the aging process has been suggested (Zhang and Herman, 2002). The cellular damage induced by oxidative stress may trigger the process of apoptosis (Kennedy et al., 2003). Apoptotic cell death is increased at physiological levels during aging in several organs. Misregulation of apoptosis may enhance the aging process and contribute towards the development of age-related diseases. Apoptotic cell death increases with aging in the liver and kidney of laboratory animals (Lee et al., 2004; Tanaka et al., 2004). If free radicals contribute to aging by inducing apoptosis, then it follows that apoptosis is detrimental to aging tissues (Kennedy et al., 2003). Anti-apoptotic or anti-oxidative agents can inhibit free radical-mediated apoptosis by directly eliminating them.

Deprenyl (selegiline) is an irreversible monoamine-oxidase B inhibitor which has anti-oxidant, anti-apoptotic and neuroprotective effects. Deprenyl may protect neurons in age-related neurodegenerative disorders, such as Parkinson's disease. The anti-apoptotic and neuroprotective effects of deprenyl have been shown in experimental studies (Simon et al., 2001; Unal et al., 2001; Kitani et al., 2002; de Lima et al., 2005). Studies examining the effect of deprenyl on apoptosis have concentrated primarily on the brain. In this study, we examined the effect of deprenyl on age-associated apoptosis in aged rat livers.

Estrogen is also a neuroprotective and anti-oxidant hormone. Estrogens function as radical scavengers and inhibit lipid peroxidation *in vivo* and *in vitro* (Miura et al., 1996; Garcia-Segura et al., 2001). Estradiol can inhibit the oxidative stress-induced apoptosis of hepatocytes (Liu et al., 2002).

Several chemicals and drugs have been shown to prevent oxidative stress and apoptosis in the liver of animals. However, the effect of deprenyl, alone and in combination with estradiol supplementation, in aged liver tissue is unclear. There has been no report of an effect of deprenyl combined with estradiol supplementation on apoptosis and oxidative stress in aged liver tissue. The current study was an extension of our previous work in which the effect of deprenyl and estradiol on aged rats has been evaluated. The objectives of this study were to determine whether the anti-oxidative effects of deprenyl could suppress apoptotic activity, with or without estradiol, in aged female rat livers. We examined parameters associated with oxidative stress, lipid peroxidation and SOD enzyme activities, and apoptosis by immunohistochemical assessment in the liver of aged rats.

Materials and methods

Animals and tissues

All experiments were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the Dokuz Eylul University, School of Medicine. In this study, 3 and 24 month-old ovariectomized female Wistar rats were used. Three month-old female rats were used as the young control group and 24 month-old rats were used in all other groups. The animals were maintained under standard colony conditions with a 12 h light/dark cycle and food and water was supplied *ad libitum* throughout the experiments. Rats were divided into six groups, as follows:

(I) young (3 months old) control group ($n = 7$) was injected subcutaneously (sc) with physiological saline
(II) aged (24 months old) control group ($n = 7$) was injected sc with physiological saline
(III) sham group ($n = 4$) rats received no injection
(IV) deprenyl group ($n = 7$) was injected sc with deprenyl (Sigma-Aldrich), 1 mg/kg/day
(V) estradiol group ($n = 5$) was injected sc with β-estradiol (Sigma-Aldrich), 40 μg/kg/day
(VI) deprenyl plus estradiol group ($n = 5$) was injected with deprenyl and estradiol at the same doses as above

All rats, except the sham group, were injected for 21 days. Deprenyl and estradiol were dissolved in physiological saline. Twenty-four hours after the last injection, liver tissue samples were taken immediately from the rats under ether anesthesia and used for estimation of oxidant stress parameters. The remaining liver tissue was used for histological evaluation. Use of ether anesthesia before cervical dislocation is a commonly reported