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Effect of Sex Hormones on Lipid Peroxidation in Rat Liver

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The role of sex hormones in hepatic lipid peroxidation, and in hepatic aldehyde oxidase and xanthine oxidase activities were investigated using rat liver homogenates. It was observed that male rat had a significantly greater content of malondialdehyde in liver than female. Among the sex hormones tested, estradiol, one of female hormones, markedly inhibited the formation of lipid peroxides in liver tissues *in vitro*. Especially, the inhibitory effect of estradiol appeared more remarkably in Fe^{+2}-induced lipid peroxidation. The hepatic xanthine oxidase activity was decreased about 15% by 10^{-6} M estradiol, whereas, the aldehyde oxidase activity was almost completely disappeared at the same concentration of estradiol. It implies that sex differences in lipid peroxidation is attributed to the suppression of free radical generating system by estradiol.

Key words: Sex hormones, Lipid peroxidation, Xanthine oxidase, Aldehyde oxidase

INTRODUCTION

The possibility that lipid peroxidation may be a basic mechanism of cell injury and pathogenicity for a wide spectrum of chemicals and of diseases have been clearly suggested from an increasing number of animal and human studies (Yagi, 1987; Dominique, 1992). It is also well known that the initiation and propagation of lipid peroxidation in pathophysiological condition of tissue may be accomplished by reactive oxygen species, which are generated by hypoxanthine or xanthine-xanthine oxidase system(Granger, 1986). This enzyme exists in body predominantly as a NAD^+-dependent dehydrogenase which can be transformed to an oxygen-dependent oxidase by a various diseases conditions. Various physiological sex difference phenomena including biochemical metabolism are largely dependent on male and female sex hormones. Several researchers have recently reported that there are sex differences in biological oxdative reaction as well as resistance against oxidative stress (Hashmi *et al.*, 1986; Mezey *et al.*, 1992; Li *et al.*, 1988).

In this study, we investigated the influences of estradiol, progesterone and testosterone on lipid peroxidation and on *in vitro* enzyme activities of aldehyde oxidase and xanthine oxidase. It was also examined the type conversion of xanthine oxidase which has been proposed as an important source of oxygen species free radical in pathological condition (Hearse, 1986; Bindoli, 1988)

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MATERIALS AND METHODS

Chemicals

Xanthine sodium, hypoxanthine, N-methylnicotine amide, nicotinamide adenine dinucleotide sodium oxidized and reduced form, 2-thiobarbituric acid and bovine albumin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Testosterone propionate, ethynyl estradiol and progesterone were kindly supplied from Dong San Hospital (Taegu, Korea).

Other extra pure chemicals were purchased from reagent commercial company. 2-Pyridon which is a metabolite of N-methyl nicotinamide by aldehyde oxidase, is synthesized according to the method of Holman *et al.* (1948).

Animals

Sprague-Dawley rats about 12 weeks old, were provided with food and water ad lib (products of Life Science Co). The animal room was maintained at 20± 2℃ and with the illumination of a 12 hr light/dark

cycle. The animals were starved overnight before sacrificed in order to reduce the variation of hepatic metabolism.

Preparations of Subcellular Organelles

Animals were killed by exsanguination from the abdominal aorta under light ether anesthesia. The liver was exhaustively perfused with ice-cold 0.15 M sodium chloride through the portal vein until uniformly pale, and immediately removed and weighed. After trimmed and minced, the pieces of liver were homogenized with 4 volumes of ice cold 0.1 M potassium phosphate buffer (pH 7.5) solution. The homogenate was centrifuged at 600×g for 10 min. The pellet was discarded and supernatant was centrifuged at 10,000 ×g for 20 min. The pellet was collected as mitochondrial fraction. The postmitochondrial fraction was further centrifuged at 105,000×g for 60 min. The resultant cytosolic fraction was used as the enzyme sources of xanthine oxidase and aldehyde oxidase.

Enzyme Assay

Xanthine dehydrogenase activity: The xanthine dehydrogenase activity (Stirpe, *et al.*, 1969) was assayed by measuring spectrophotometrically the amount of uric acid formed from xanthine sodium with NAD^+ in the reaction mixture.

Xanthine oxidase activity and type conversion: The xanthine oxidase activity (Corte *et al.*, 1972) was aerobically determined by measuring the rates of uric acid formation without NAD in the reaction mixture from xanthine sodium substrate. The reaction mixture contains 0.1 M potassium phosphate buffer (pH 7.5), 0.1 ml of enzyme, 0.06 mM of the substrate and distilled water in a final vo-lume of 4 ml. The reaction was carried out at 37℃ for 15 min.

Aldehyde oxidase activity: The aldehyde oxidase activity was measured according to the meathod of Branzoli *et al.* (1974), In brief, samples of the supernatant fraction (0.2 ml) were incubated with 1.0 μmol of N^1-methylnicotinamide in 0.2 ml of 0.075 M potassium phosphate buffer, pH 7.5, at 37℃, with shaking. The reaction mixtures were routinely incubated for 30 min and the increase in optical density at 300nm was measured spectrophotometically.

Lipid Peroxidation

Lipid peroxidation of liver tissues was followed by measuring the formation of malondialdehyde according to the method of Ohkawa *et al.* (1979). In brief, lipid peroxidation test was performed at 37℃ in capped tube containing 0.2 ml homogenate of 0.1 M potassium phosphate buffer (pH 7.5), and 0.5 ml distilled water, and 0.5 ml of 30% trichloroacetic acid. The incubated reaction mixture centrifuged at 3000 rpm for 3 min. The supernatant was added to an equal volume of aqueous 0.67% thiobarbituric acid, heated in a boiling water bath for 60 min, and cooled to room temperature. The absorbance measured at 532 nm was expressed as nanomoles of malondialdehyde.

Protein Assay

Protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Statistical Analysis

The differences between the experimental groups were analyzed with student's t-test.

RESULTS AND DISCUSSION

Sex Differences in Lipid Peroxide Content

A pronounced sex difference in the lipid peroxide levels of homogenate was observed, and the average values in the liver tissue homogenate from adult male rat are 50% higher than female rat , as shown in Fig. 1.

In our laboratory, it was also observed that sex difference in lipid peroxide levels of liver tissue was not appeared until 25 days age (unpublished data). The age at which the sex difference of the lipid peroxide was shown, may reflect period in development in which secretion of sex hormone. These results suggested that sex hormones may promote or inhibit the numerous metabolic reaction, and show considerable difference in the speed with which that they act. Above our experimental results tempted us to investigate the effects of sex hormones on lipid peroxidation rate.

Inhibitory Effect of Sex Hormone on Lipid Peroxidation

As shown in Fig. 2 estradiol had a potent antioxidant effect to prevent from malondialdehyde production in hepatic tissue homogenate in vitro. Whereas, progesterone,which is a one of the other female hormones, didn't change the degree of lipid peroxidation reaction rate with the same concentration of estradiol. Testosterone, sex hormone of male, slightly inhibited the malondialdehyde formation compared to the control.

Antioxidant Effect of Estradiol

These experimental results (Fig. 1, Fig. 2) suggested that there were differences in antioxidant among the steroid hormones and that there were some relation-

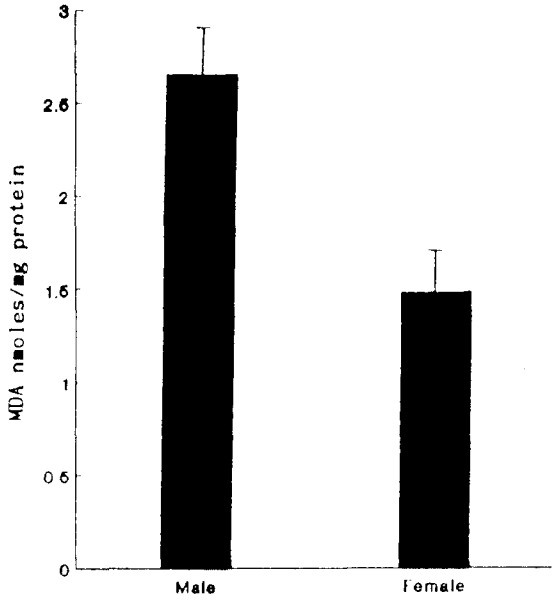

Fig. 1. Sex differences on the hepatic lipid peroxide level in male and female rats. The assay procedure was described in the experimental methods. Values are means± S.E. for 5 animals. *Significantly different from male rat, $p<0.05$. MDA: malondialdehyde

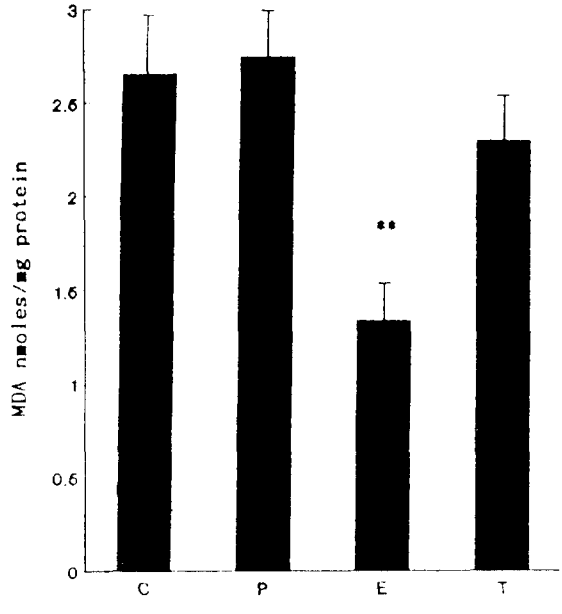

Fig. 2. Effects of sex hormones on the hepatic lipid peroxidation. The assay procedure was described in the experimental methods. Values are means± S.E. for 4 separated experiments. C: control, P: progesterone (10^{-6} M), E: estradiol (10^{-6} M), T: testosterone (10^{-6} M). **Significantly different from the control, $p<0.01$.

ship between the antioxidant effects of sex hormones and chemical structure of hormones. The inhibitory effect of estradiol on lipid peroxidation was substantiated by kinetic investigation, which inhibitory reaction was exhibited with dose dependent manner as shown in Table I.

Table I. Dose response for estradiol on the hepatic lipid peroxidation *in vitro*

Concentration (M)	MDA (nmoles/mg protein)
Control	2.66± 0.29
10^{-10}	2.55± 0.25
10^{-8}	1.87± 0.17
10^{-6}	1.34± 0.14**

The assay procedure was described in the experimental methods. Values are means± S.E. for 4 separated experiments. **Significantly different from the control, $p<0.01$.

Antioxidant Effect of Estradiol on Fe^{2+}-induced Lipid Peroxidation

It is well recognized that iron-mediated reactions are involved in the initiation and/or progression of lipid peroxidation and that it plays an important role in the etiology of number of pathological states (Mizuta *et al.*, 1989).

In order to examine the antioxidant effect of estradiol on pathophysiological model system, it was investigated the inhibitory effect of estradiol on Fe^{2+}-induced lipid peroxidation. As shown in Fig. 3, Fe^{2+}-induced hepatic lipid peroxidation in vitro was decreased in the presence of deferoxamine. It was also found that estradiol in combination with deferoxamine showed remarkable inhibitory effect on hepatic lipid peroxidation than deferoxamine alone did. These results in Fig. 3 indicated that estradiol had more significant inhibitory effect on hepatic lipid peroxidation in the presence of ferrous ions than in the absence of ferrous ions.

Effect of Estradiol on Xanthine Oxidase Activity and Type Conversion

It is widely accepted that oxygen free radical production and their consequences such as lipid peroxidation and protein damage appear to play a major role in the pathogenesis of the injury after ischemia and particulary reperfusion (Hirafuji et al., 1985; Lunec *et al.*, 1990; Mackelvey, 1988). Oxygen free radicals can be produced by different various sources such as oxidizing enzymes (Comporti, 1985; Ross, 1989). McCord (1983) has demonstrated that the xanthine dehydrogenase is converted to the xanthine oxidase in pathophysiological conditions, and has hypothesized that oxygen free radical production of xanthine oxidase is an important mechanism of cellular injury. The effect of estradiol on xanthine oxidase activity and on type conversion was investigated, and the results were illustra-

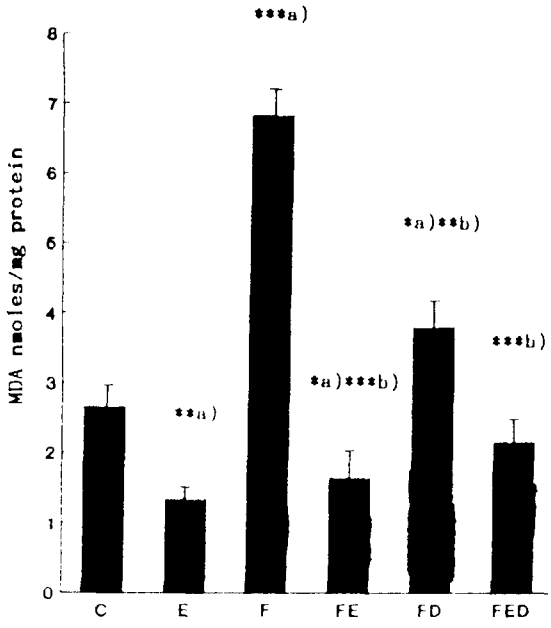

Fig. 3. Effects of estradiol on the Fe^{2+}-induced hepatic lipid peroxidation *in vitro* The assay procedure was described in the experimental methods. Values are means± S.E. for 4 separated experiments. C: control, E: estradiol (10^{-6} M), F: Fe^{2+} (500 μM), FE: estradiol+Fe^{2+} (500 μM), FD: deferoxamine (100 μM)+Fe^{2+} (500 μM), FED: estradiol+deferoxamine+Fe^{2+} (500 μM). a) significantly different from control, b) significantly different from Fe^{2+} added (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$).

Table II. Effect of estradiol on the type conversion of hepatic xanthine oxidase *in vitro*

Concentration (M)	Specific Activity#		Type Conversion Ratio(%)
	Type O	Type D+O	
Control	0.38± 0.02	2.92± 0.11	12.9
10^{-10}	0.37± 0.03	2.91± 0.04	12.7
10^{-8}	0.34± 0.02	2.88± 0.04	11.8
10^{-6}	0.32± 0.01*	3.14± 0.08	10.2

The assay procedure was described in the experimental methods. Values are means± S.E. for 4 separated experiments. Significantly different from control (*: $p<0.05$). Type O: Xanthine oxidase, Type D+O: Xanthine dehydrogenase. Xanthine oxidase activity was expressed as uric acid formed (nmoles/mg protein/min).

ted in Table II. As shown in Table II, the hepatic xanthine oxidase activity was significantly inhibited about 15% compared to the control by the addition of estradiol at the concentration of 10^{-6} M in reaction mixture. It was also observed that the estradiol decreased the type conversion rate of xanthine oxidase in vitro system. However, progesterone could slightly change the xathine oxidase activity and type conversion rate compared with estradiol effect.(data not shown) In the studies of testosterone effects, it was observed that

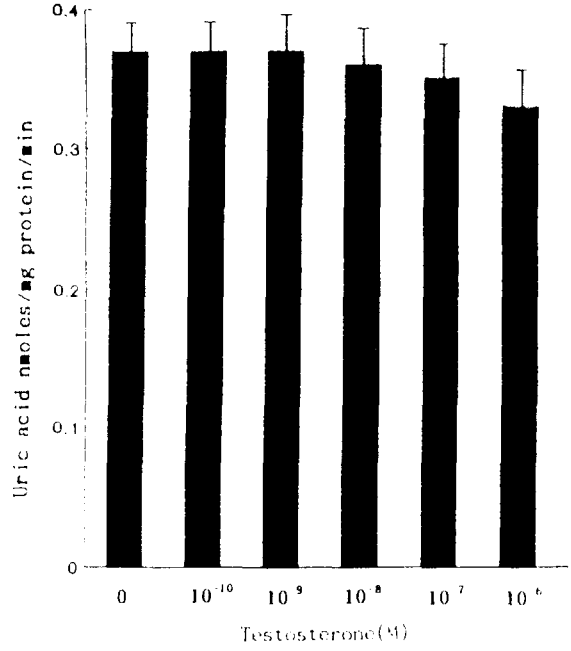

Fig. 4. Effects of testosterone on the hepatic xanthine oxidase activity *in vitro* The assay procedure was described in the experimental methods. Values are means± S.E. for 4 separated experiments.

the testosterone had a mild inhibitory effect on xanthine oxidase activity (Fig. 4). These results indicated that estradiol might inhibit the formation of lipid peroxide in tissue (Fig. 2) through the radical generating enzyme control system and lipid peroxidation ability.

Effect of Sex Hormone on the Hepatic Aldehyde Oxidase Activity

It is well recognized that aldehyde oxidase, one of the other oxidizing enzyme, is concerned in free radical generating system (Stannlovic and Chaykin, 1971). This enzyme has a biochemical function of xanthine oxidase (Beedham, 1985). So, it was observed the influences of both sex hormones on aldehyde oxidase. As shown in Table III, the enzyme activity was markedly inhibited by addition of estradiol. The enzyme activity was almost couldn't detect in 10^{-6} M of estradiol, and the inhibitory effect of estradiol on aldehyde oxidase activity was shown with dose dependent manner. Whereas, the enzyme activity was only slightly changed when testosterone was added in the reaction mixture at the same concentration of estradiol.

In consideration of above inhibitory effect of estradiol on lipid peroxidation, these results suggested that the estradiol might exert at least part of the protection against oxidative stress, and that the effect of estradiol may be due to its ability to act as a free radical scavenger. It is a matter of general common sense that female has longer time of life span than male. The rela-

Table III. Effects of sex hormones on the hepatic aldehyde oxidase activity *in vitro*

Concentration (M)	Specific Activity#	
	Testosterone	Estradiol
Control	1.30± 0.11	1.30± 0.11
10^{-10}	1.29± 0.09	1.29± 0.12
10^{-9}	1.30± 0.10	1.27± 0.09
10^{-8}	1.28± 0.08	1.12± 0.10
10^{-7}	1.28± 0.12	0.72± 0.07**
10^{-6}	1.27± 0.08	0.02± 0.01***

The assay procedure was described in the experimental methods. Values are means± S.E. for 4 separated experiments. Significantly different from control (**: $p<0.01$, ***: $p<0.001$). #: Aldehyde oxidase activity was expressed as 2-Pyridone formed (nmoles/mg protein/min).

tionship between lipid peroxidation and cellular toxicity is recognized, and then it has been widely used lipid peroxidation as an indicator that free radicals has been generated and has persisted long enough to react with other chemical or cellular component (Mottley *et al.*, 1991; Pikul *et al.*, 1989).

According to the experimental results, it could be concluded that the inhibitory effect of estradiol on free radical generating enzyme activity may, to some extent, be concerned with pathophysiological phenomena and life span. Further work is needed to clarify this problem.

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