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Effects of Low-dose Oral Contraceptive Oestrogen and Progestin on Lipid Peroxidation in Rats

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Groups of six female rats were treated with low-dose oral contraceptive (0.667 mg progestin [norethisterone acetate] and 0.02 mg oestrogen [ethinyloestradiol]/kg body-weight), or its components, separately, at the same doses, for 6 weeks. Changes in liver and kidney levels of lipid peroxides (as indicated by malondialdehyde production), free fatty acids, superoxide dismutase, and catalase liver glutathione and serum ceruloplasmin compared with the untreated control group were studied. Combined oral contraceptive treatment produced a significant increase in the activity of catalase in the kidneys ($P < 0.05$). The levels of lipid peroxides, free fatty acids and glutathione in the liver, and of serum ceruloplasmin increased significantly with oestrogen treatment ($P < 0.05$). Lipid peroxides (in the liver only), and serum ceruloplasmin decreased significantly when progestin was administered ($P < 0.05$). The activities of superoxide dismutase and catalase decreased significantly in the oestrogen group (except for catalase in the kidney) but increased in the progestin group ($P < 0.05$). The results indicate that the components of the low-dose oral contraceptive may alter liver and kidney metabolism.

KEY WORDS: Progestin; Oestrogen; Low-dose oral contraceptive; Lipid peroxidation

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INTRODUCTION

Oral contraceptives have been used and studied extensively for over 30 years. Mishell Jr[1] and Wren[2] have reviewed the impact of oral contraceptives and oestrogen on lipids and lipoprotein profile. Epidemiological studies demonstrate that oral contraceptive administration in female rats increases lipid peroxidation in erythrocyte.[3] It has also been reported that testosterone administration results in increased lipid peroxidation.[4] Elevated levels of free fatty acids in the liver and of serum ceruloplasmin have been reported after oral contraceptive and oestrogen administration.[5-7]

Lipid peroxidation is a complex process and has been implicated in the pathogenesis of a number of diseases; for example, it has been suggested that changes in the levels of free radicals are involved in the atherogenic process[8] and in myocardial infarction.[9] The available reports indicate that not much work has been carried out on the changes taking place in lipid peroxides on administration of oral contraceptives and its components.

The aim of the study reported here was to evaluate the impact of a low-dose oral contraceptive formulation containing 30 mg oestrogen (ethyloestradiol) and 1 mg progestin (norethisterone acetate) and of its components (oestrogen and progestin) at the same dose and potency on lipid peroxidation in female rats.

MATERIALS AND METHODS

ANIMALS

Female Sprague-Dawley albino rats (CFTRI, Mysore), weighing 150 – 170 g, were used for the study. The rats were housed individually in polypropylene cages with wire-mesh floors in a room maintained at 25 °C; they were fed normal laboratory diet (Hindustan Lever rat feed; Kamadhenu Apencies, Bangal-one) and water was provided *ad lib.* The animals were divided into four groups of six rats each.

EXPERIMENTAL PROCEDURE

Rats of one group were given low-dose oral contraceptive containing 0.667 mg of progestin (norethisterone acetate) and 0.02 mg of oestrogen (ethyloestradiol) daily, while two further groups were treated with 0.667 mg norethindrone acetate or 0.02 mg of 17-β oestradiol/kg of body weight, respectively. Oral contraceptives, progestin and oestrogen, were dissolved in 0.1 ml propylene glycol and given orally by tube; the same quantity of propylene glycol was administered orally to control rats. The duration of the experiment was 6 weeks. At the end of this period, all of the animals were killed by decapitation and the liver, kidneys and blood were quickly transferred to ice-cold containers of the various assays.

ASSAY METHODS

Lipids were extracted by the method of Folch *et al.*[10] The concentration of free fatty acids in the lipid extract was measured by the method of Falholt *et al.*[11] Tissues were rinsed in ice cold physiological saline, dried by pressing between the folds of filter paper, weighed and homogenized in chloroform: Methanol (2:1), and the contents extracted in the cold for 24 h. The extract was made up to a known volume and used for estimation. The malondialdehyde level in tissues, homogenized in tris-hydrochloride buffer (0.1 mol/l, pH 7.5), was determined by the thiobarbituric acid method of Buege & Aust.[12]

Superoxide dismutase was assayed by the method of Kakkar *et al.*[13]; tissues were homogenized in 0.25 M sucrose buffer at 1 – 4 °C.

Catalase activity was measured using the method of Aebi[14] in M/150 phosphate buffer (pH 7.0) at 1 – 4 °C and by following the decomposition of hydrogen peroxide at 240 nm. Glutathione was determined according to the procedure of Kumari *et al.*[15]; tissues were homogenized at 1 – 4 °C in phosphate buffer at pH 7.5. Serum ceruloplasmin was estimated by the method of Henry *et al.*[16]

STATISTICAL ANALYSIS

The statistical significance of differences between groups was determined by analysis of variance.

RESULTS

Concentrations of malondialdehyde in the liver and kidney increased significantly in the oestrogen group compared with the control group. Progestin treatment significantly reduced the malondialdehyde concentration in the liver, while no significant changes compared with the controls was observed in the kidney tissue. Administration of the combined oral contraceptive did not alter the levels of malondialdehyde in the liver or kidney compared with control levels (Table 1).

The free fatty-acid concentration in the liver increased significantly in the oestrogen group but remained unchanged in the progestin and oral contraceptive groups. Free fatty-acid levels in the kidney remained unchanged in all the three groups compared with controls. The liver glutathione and serum ceruloplasmin concentrations were increased when oestrogen was administered and decreased in the progestin group compared with the controls. No significant alteration was seen in the oral contraceptive group (Table 2).

The activities of superoxide dismutase in both the liver and the kidney increased markedly in the progestin group but decreased significantly in the oestrogen group. Administration of the oral contraceptive does not affect the activity of superoxide dismutase either in the kidney or in the liver. The activities of catalase in both the liver and kidney were increased in the group given progestin but decreased in the group given oestrogen (only in the liver). In the

TABLE 1

Concentrations of malondialdehyde in the livers and kidneys of groups of six rats given oral contraceptive (containing 0.667 mg progestin and 0.002 mg oestrogen), or 0.667 mg progestin, or 0.002 mg oestrogen, orally, daily for 6 weeks

Treatment	Malondialdehyde concentration (mM/100 g)	
	Liver	Kidney
Control	0.825 ± 0.015	0.942 ± 0.078
Oral contraceptive	0.835 ± 0.018	0.752 ± 0.099
Progestin	0.607 ± 0.010*	0.825 ± 0.077
Oestrogen	1.467 ± 0.080*	1.232 ± 0.105

Values are means ± SE.
*$P < 0.05$ compared with control value.

combined oral contraceptive group, the activity of catalase in the kidney increased significantly while its activity in the liver was unaffected (Table 3).

DISCUSSION

The malondialdehyde concentrations indicate that oestrogen administration resulted

TABLE 2

Concentrations of free fatty acids in the livers and kidneys, glutathione in the liver, and ceruloplasmin in the serum of groups of six rats given oral contraceptive (containing 0.667 mg progestin and 0.002 mg oestrogen), or 0.667 mg progestin, or 0.002 mg oestrogen, orally, daily, for 6 weeks

Treatment	Free fatty-acid concentration (mg/100 g)		Glutathione concentration (mM/100 g)	Ceruloplasmin concentration (mg/100 ml)
	Liver	Kidney	Liver	Serum
Control	658 ± 47.29	712.50 ± 61.15	379.12 ± 29.17	48.33 ± 3.33
Oral contraceptive	666.7 ± 49.44	583.3 ± 33.33	387.50 ± 28.68	51.50 ± 3.60
Progestin	583.3 ± 49.44	616.7 ± 33.33	283.33 ± 32.70	34.27 ± 2.76*
Oestrogen	875.0 ± 76.37*	762.5 ± 54.68	487.58 ± 36.37*	64.33 ± 5.19*

Values are means ± SE.
*$P < 0.05$ compared with control value.

TABLE 3

Activities of superoxide dismutase and catalase in the livers and kidneys of groups of six rats given oral contraceptive (containing 0.667 mg progestin and 0.002 mg oestrogen), or 0.667 mg progestin, or 0.002 mg oestrogen, orally, daily for 6 weeks

Treatment	Superoxide dismutase activity (unit[a]/mg protein)		Catalase activity (values × 10^{-3} unit)[b]/mg protein	
	Liver	Kidney	Liver	Kidney
Control	10.48 ± 0.35	18.88 ± 0.93	71.50 ± 2.29	31.77 ± 1.50
Oral contraceptive	10.33 ± 0.45	21.62 ± 1.02	69.65 ± 3.00	48.07 ± 1.72*
Progestin	12.70 ± 0.72*	22.07 ± 0.84*	90.52 ± 0.65*	68.90 ± 4.70*
Oestrogen	6.66 ± 0.33*	13.82 ± 0.74*	51.58 ± 2.18*	25.61 ± 1.53

Values are means ± SE.
[a]Amount of enzyme needed to inhibit chromogen production (optical density of 560 nm) by 50% in 1 min.
[b]Velocity constant/s.
*$P < 0.05$ compared with control value.

in increased lipid peroxidation in the liver, while progestin significantly decreased the level of lipid peroxides in the liver but not the kidney, and combined oral contraceptive had no significant effect on the levels of lipid peroxides. Roy *et al.*[17] have observed that after 7 days of infusion of oestradiol, fluorescent products of lipid peroxidation were more than doubled in the hamster kidney but remained unchanged in the liver. Studies on erythrocytes in mature female Swiss albino rats have shown that administration of oral contraceptive (ethinylestradiol/norethisteron acetate) for 3 months and 2 years increased the lipid peroxide concentration.[3]

Free fatty acid levels showed a slight but not statistically significant increase in the liver but showed a slight decrease (not statistically) significant in the kidney in the oral contraceptive group. It has been reported that in a similar animal model combined oral contraceptive treatment, for 6 months, increased free fatty acid levels in the liver.[5] Studies with combined oral contraceptive in women indicate that the levels of serum free fatty acids remain unchanged.[18] In the present study, free fatty acid concentration in the liver increased in the oestrogen group but progestin and oral contraceptive treatment did not significantly affect its levels. It has been shown that serum free fatty-acid levels in women decrease during oestrogen therapy.[19]

The increases in lipid peroxides in the oestrogen group were accompanied by reductions in the activities of superoxide dismutase and catalase. The activities of these enzymes showed significant increases in the progestin group. In the oral contraceptive group, the activities of superoxide dismutase and catalase increased in the kidneys but were unchanged in the liver. McCormick *et al.*[20] have reported that, in male hamsters, after 4.4 months of diethylstilbestrol exposure, the activities of catalase and total superoxide dismutase in the kidney fell below untreated levels.

In the present study, oestrogen treatment increased the concentration of glutathione in the liver but progestin administration reduced its levels, while there was no significant alteration when the combined oral contraceptive was administered. Studies using various doses of Navacycline and high doses of Ovral (1/20th of a pill) showed reduced activities of glutathione-S-transferase in the livers of mice.[21] Increased serum ceruloplasmin was observed with oestrogen treatment but the serum ceruloplasmin concentration decreased with progestin; in the oral contraceptive group there was no significant change in the serum ceruloplasmin concentration. Increased levels of serum ceruloplasmin in response to oestrogen treatment have been reported previously.[6,22] It has also been reported that oral contraceptive use in women did not alter the concentration of serum ceruloplasmin.[23] Glutathione is the substrate for glutathione peroxidase, which plays an important role in scavenging toxic intermediates of incomplete oxidation. The increased levels of glutathione in the oestrogen group may be due to decreased activity of glutathione peroxidase, and the reduced levels of glutathione observed in the progestin group may indicate enhanced activity of this enzyme.

Serum ceruloplasmin is an important enzyme which oxidizes iron from the ferrous to the ferric state; it has been demonstrated that iron-catalysed lipid peroxidation requires both Fe(II) and Fe(III) and the maximum rate of peroxidation occurs when the ratio is approximately one. It has also been observed that ceruloplasmin can function as a pro-oxidant or an antioxidant depending on the capacity of the ceruloplasmin to influence the ratio of Fe(II) to Fe(III) by catalyzing Fe(II) oxidation. The mixed-valence iron

model predicts that, in systems containing all Fe(III), lipid peroxidation will be initiated on addition of reductant. Decreased levels of glutathione (a reductant) should therefore result in decreased peroxidation, and increased levels should result in enhanced peroxidation. The elevated levels of lipid peroxides observed in the oestrogen group in the liver and the reduced level in the progestin group (liver only) are consistent with this view.

The present results suggest that oestrogen treatment of rats can activate lipid peroxide formation while progestin can reduce it. The increased tissue concentrations of lipid peroxides may have deleterous effects on the tissue concerned resulting in changes in its properties and function.

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