Original research article

Effect of vitamin E and C supplements on lipid peroxidation and GSH-dependent antioxidant enzyme status in the blood of women consuming oral contraceptives

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Abstract

Background: Oral contraceptives (OCs) may affect oxidative stress status. We aimed to assess whether supplementation with vitamins E and C reduced this OC effect.

Study Design: One hundred twenty healthy female individuals were divided into three groups: A, control; B, untreated OCU (OC users); and C, treated OCU (OC users with vitamin E and C supplementation). In all cases, plasma glutathione peroxidase (GPx) and glutathione reductase (GR) activities and malondialdehyde (MDA) level were determined.

Results: Significant increases were found in the plasma MDA level, and activities of GPx and GR in plasma were decreased in Group B compared to the control group. Supplementation with vitamin C and E significantly increased the activity of GPx and GR activity, and reduced plasma MDA levels in Group C (p<.05).

Conclusions: These data suggest that low-dose OCs, by enhancing the stress oxidative and lipid peroxidation, may represent a potential cardiovascular risk factor, and the use of vitamins E and C may be beneficial in ameliorating this side effect of OCs.

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Keywords: Oral contraceptives; Vitamin supplementation; Glutathione peroxidase; Glutathione reductase

1. Introduction

Oral contraceptives (OCs) prevent pregnancy in several ways, but primarily by suppressing ovulation. Oral contraceptives contain two active components: estrogen and progestin. Orally active forms of estrogen contain a modified structure of estradiol. Most modern combined OCs (COCs) contain one of nine androgen-derived progestins.

Progestogens are known to have various metabolic effects, including effects on lipid metabolism and, therefore, potentially on the risk for cardiovascular disease [1]. It has been suggested that contraceptive steroids might exert their metabolic effects by changing hepatic enzyme levels related to the synthesis and/or turnover of lipids and lipoproteins [2]. Doring et al. [3] demonstrated that there is a close relationship between women taking third-generation OCs and increasing atherothrombotic risk.

There are some reports that show that synthetic sex steroids may increase the risk of cardiovascular disease by leading to oxidative damage [4,5].

Durand and Blache [6] suggested that platelet hyperactivity, possibly through a stimulated free radical-induced arachidonic acid metabolism, might be involved in the known thrombogenic risk observed in OC users.

Reactive oxygen species (ROS) are metabolic products of the respiratory chain. Under normal circumstances, ROS are eliminated by cellular enzymatic and nonenzymatic
antioxidant defences. Within the enzymatic mechanisms, superoxide dismutase (SOD) converts the superoxide radical (O$_2^-$) into hydrogen peroxide (H$_2$O$_2$), whereas catalase and glutathione peroxidase (GPx) metabolize H$_2$O$_2$ into H$_2$O+O$_2$. If ROS are not effectively eliminated, they can cause oxidative cell injury, such as peroxidation of lipids (membranes and organelles), proteins (receptors and enzymes) and DNA. The end products of lipid peroxidation (lipid damage), especially malondialdehyde (MDA), are usually assessed through the levels of thiobarbituric acid reactive substances (TBARS) [7].

An imbalance between the production of ROS and the antioxidant defenses leads to a state of oxidative stress which is involved in the process of ageing and in various physiopathological processes such as cancer, diabetes and atherosclerosis [8–10].

Nonenzymatic antioxidants are generally small molecules that directly scavenge ROS, preventing them from disfiguring lipids, proteins or nucleic acids. Glutathione (GSH), an important intracellular antioxidant, is converted to its oxidized form (GSSG) by GPx when it is used as a scavenger; it is converted back to its reduced form by glutathione reductase (GR) [7].

Surprisingly, only a few studies have investigated the relationship between OCs containing estrogens and progestins, and oxidative stress. Some studies showed a significant increase of lipid peroxides in women using subdermal implants of levonorgestrel or injectable depo-progesterone acetate [11–13]. Elevated blood lipid peroxides were observed in rats treated with combined OCs [6], and a decrease of the antioxidants coenzyme Q10 and α-tocopherol was shown in women taking OCs compared to a control group not using OCs [14]. Berg et al. [15] described a strong decrease in plasma antioxidant β-carotene among OC users.

The aim of the present study was to examine the effects of OC alone and with vitamin E and C supplementation on the plasma GSH-dependent antioxidant system and also on lipid peroxidation levels in women taking OCs. Therefore, MDA level and GPx and GR activity were measured.

2. Materials and methods

2.1. Materials

All chemicals used in this study were analytical grade and were obtained from Sigma and Merck (Germany). Contraceptives and vitamins were commercially available.

2.2. Subjects

One hundred twenty healthy female individuals participated in the study. The study was approved and performed under the guidelines of the Ethics Committee of Shiraz University of Medical Sciences, and informed consent was obtained from each of the subjects before blood sampling.

The control group (Group A) consisted of 40 noncontraceptive users (NCU) aged 18–40 years with no other hormonal treatments or supplementation.

The test group consisted of 80 women aged 18–40 years who were regular OC users (OCU) and were divided (randomly and using a table with random numbers) into two groups each as Group B (untreated OCU — OC users without vitamin E and C supplementation) and Group C (treated OCU — OC users with vitamin E and C supplementation). The average duration of OC use was 33±26 months (mean±SD), ranging from 1 to 10 years. All the OCU users were taking a contraceptive pill containing 0.03 mg ethinylestradiol and 0.15 mg levonorgestrel 21 days on and 7 days off. All subjects were told to take the supplementation under usual conditions (vitamin C should be consumed after meals to avoid irritation of the stomach, especially in ulcer patients, and vitamin E supplements should be taken with food for optimal absorption), for 4 weeks; the doses of supplementation were 150 mg of vitamin C and 200 IU of vitamin E.

All subjects had normal body weight and were non-smokers with no evidence of chronic diseases. None of them consumed alcohol or was taking other medications, antioxidants or vitamin supplements.

2.3. Samples processing

The trial terminated for each participant 30 days after the vitamin treatment was begun. Blood samples were drawn into EDTA anticoagulant tubes and were centrifuged within 4 h after sampling; plasma samples were immediately frozen and kept at −20°C until tested.

2.4. Estimation of plasma lipid peroxides

Malondialdehyde, an indirect index of lipid peroxidation, was assayed as TBARS using a colorimetric method [16]. Briefly, 0.5 mL of plasma was added to 2 mL of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25 mol/L HCl. The mixture was boiled for 15 min, cooled and centrifuged at 1700×g for 15 min at 4°C. The absorbance of the supernatant was measured at 532 nm. The TBARS concentration was calculated using 1,1,3,3-tetramethoxypropane as a standard. Results are expressed as nanomoles per milliliter of plasma.

2.5. Glutathione peroxidase assay

The GPx activity of samples was measured by continuous monitoring of the regeneration of reduced GSH from oxidized glutathione GSSG upon the action of GR and NADPH according to the method of Fecondo and Augusteyn [17]. To a 750-μL reaction mixture containing 0.3 mmol/L EDTA, 0.1 mmol/L NADPH, 0.5 U GR and 0.5 mmol/L Na$_2$N$_3$ in 50 mmol/L phosphate buffer (pH 7.2), 50 μL of plasma and 100 μL of 2.5 mmol/L GSH were added. Tubes in which distilled water was substituted for GSH were included as controls. Following the addition of 100 μL of
0.4 mmol/L tert-butyl hydroperoxide to each experimental and control tube, the decrease in NADPH absorbance at 340 nm was measured at 37°C for 3 min. The decrease in absorbance, reflecting the oxidation of NADPH which is directly proportional to the GPx activity in the sample, was followed at 340 nm. Results are expressed as units of GPx activity per liter of plasma.

2.6. Glutathione reductase assay

The enzyme, GR, catalyzes the reduction of GSSG to GSH and is essential for the GSH redox cycle in order to maintain adequate levels of reduced cellular GSH.

During the reduction of GSSG by GR, one molecule of NADPH is consumed for reduction of each molecule of GSSG reduced. Therefore, the reduction of GSSG by GR can be determined by the measurement of the consumption of NADPH. The activity of GR was assayed using the method described by Carlberg and Mannervik [18] with minor modifications. The GR assay was performed in a cuvette that contained 1 M Tris–HCl buffer+5 mM EDTA (pH 8.0), 0.033 M GSSG, 2 mM NADPH and a sample in a final volume of 1.0 mL. Decrease in the absorbance, which reflects the oxidation of NADPH during reduction of GSSG by GR present in the sample, was monitored spectrophotometrically at 340 nm. Results are expressed as units of GR activity per milliliter of plasma.

2.7. Statistical analysis

The significance of the data obtained from the study groups was evaluated using the analysis of variance (ANOVA), and the differences between the means were then analyzed using a post-ANOVA test. The level of significance was taken as p<0.05.

3. Results

A total of 120 nonsmoking premenopausal women were enrolled in this study. Baseline characteristics of the subjects are listed in Table 1. All participants completed the study.

The mean (±SD) body mass indexes were, respectively, 23.5±2.8 kg/m² for the NCU group and 23.5±2.9 kg/m² for the OCU group. No significant differences appeared between the groups.

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Untreated OCU</th>
<th>Treated OCU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30.53 (5)</td>
<td>30.42 (5.1)</td>
<td>30.29 (4.6)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.62 (0.8)</td>
<td>23.43 (1.4)</td>
<td>23.54 (1.5)</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>117 (12)</td>
<td>116 (3)</td>
<td>16 (11)</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>64 (6)</td>
<td>62 (6)</td>
<td>66 (7)</td>
</tr>
</tbody>
</table>

Values are means (SD).

Treatment with the contraceptive pill and two vitamins did not cause an increase in blood pressure.

Fig. 1 shows the plasma MDA (mean±SD) in untreated OCU and treated OCU and the control groups. The plasma MDA levels in untreated OCU were increased, and OCU supplemented with vitamins E and C showed a significant reduction of MDA levels compared to the untreated OCU group (p<0.05). In the treated OCU group, the MDA level was very close to the control group, so supplementation with vitamins E and C could significantly reduce the plasma MDA levels (p<0.05). GPx activity (mean±SD) in treated and untreated OCU and the control group is shown in Fig. 2. Decrease in GPx activity was observed in the untreated OCU group. These levels were significantly lower than the GPx activities in treated and control groups; however, vitamin supplementation not only increased the activity of this enzyme but also this increase was higher than that in the control group, too (p<0.05).

GR activity (mean±SD) in the plasma of untreated and treated OCU and the control group is shown in Fig. 3. Supplementation with vitamins E and C significantly increased the activity of GR in the OCU group (p<0.05), but the activity is lower than that in the control group. Untreated OCU showed a marked decrease in the activity of this enzyme.

Table 2 is a summary of the results illustrated in the figures and shows the plasma levels of MDA, GPx and GR activity in the control, untreated OCU and treated OCU group.

4. Discussion

The current study was designed to elucidate the effect of OCs on the plasma levels of lipid peroxidation and on the
two antioxidant enzymes related to glutathione (GSH), GPx and GR, and then to identify the effect of vitamin E and C supplementation in abolishing these OC effects.

Plasma MDA levels (Fig. 1) were increased in untreated OCU. This probably reflects the increase in lipid oxidation due to either an increase in the production of free oxidative radicals or a decrease in the antioxidant defense mechanisms, or both. The same observations have also been reported in humans [19] and in rats [20].

Supplementation with vitamins E and C decreased the level of MDA in this group, confirming the role of the vitamins as powerful antioxidants. Similar observations were also noted with the use of vitamins E and C for lowering MDA levels [21,22].

Fischer-Nielsen et al. [23] found that vitamin C exhibits a protective effect against free radical–induced oxidative damage. Other water-soluble vitamins are crucial for oxidative defense. Reactive oxygen species, from both endogenous and exogenous sources, may be involved in the etiology of diverse human diseases, such as coronary heart disease, stroke, rheumatoid arthritis and cancer [7].

Diets rich in fruits and vegetables are associated with a reduced risk for these pathologies [24], and protection has been attributed to antioxidant vitamins such as vitamins C and E and to β-carotene [25].

Results from the antioxidant enzyme determinations (Figs. 2 and 3, and Table 2) showed that basal GPx and GR levels were lower in OCU, confirming the earlier reports of Vats et al. [26]. Panda et al. [27] and Musalmah et al. [28] proposed that daily oral supplementation with vitamins E and C increase the level of GR and GPx. These enzymes scavenge free radicals and prevent oxidative damage.

The principal defense systems against oxygen-free radicals are SOD, GSH, GPx, GR, catalase and antioxidant nutrients. Vitamins also directly scavenge ROS and up-regulate the activities of antioxidant enzymes. Among them, vitamin E has been recognized as one of the most important antioxidants. Vitamin E inhibits ROS-induced generation of lipid peroxyl radicals, thereby protecting cells from peroxidation of polyunsaturated fatty acid in membrane phospholipids [29]. Consequently, a dietary deficiency of vitamin E reduces the activities of hepatic catalase, GPx and GR [30]; induces liver lipid peroxidation; and causes neurologic and cardiovascular disorders [31,32], all of which can be reversed by dietary vitamin E supplementation. As a reducing agent, vitamin C reacts with the vitamin E radical to yield a vitamin C radical while regenerating vitamin E. Like a vitamin E radical, a vitamin C radical is not a reactive species because its unpaired electron is energetically stable [33]. A vitamin C radical is converted back to vitamin C by GSH.

As a major component of the cellular antioxidant system, GSH has the following characteristics: (a) GSH in diet can be partly absorbed from the small intestine and can be synthesized de novo, so that GSH is an exogenous and endogenous antioxidant; (b) although GSH radical (GS•) formed from the oxidation of GSH is a pro-oxidant radical, GS• can react with another GS• to yield GS-SG, which is then reduced to GSH by the NADPH-dependent GR; (c) GSH can react with a variety of xenobiotic electrophilic compounds in

![Fig. 2. Plasma GPx activity (U/L) in control, untreated OCU and treated OCU groups. Values are mean±SD (n=40 in each group). p<0.05: *, vs. control; θ, vs. untreated OCU.](image)

![Fig. 3. Plasma GR activity (U/L) in control, untreated OCU and treated OCU groups. Values are mean±SD (n=40 in each group). p<0.05: *, vs control; θ, vs. untreated OCU.](image)

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/mL)</th>
<th>GPx (U/L)</th>
<th>GR (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.04 (0.4)</td>
<td>142.58 (5.6)</td>
<td>61.97 (5.4)</td>
</tr>
<tr>
<td>Untreated OCU</td>
<td>4.17 (0.3)</td>
<td>124.8 (3.7)</td>
<td>23.15 (3.9)</td>
</tr>
<tr>
<td>Treated OCU</td>
<td>3.29 (0.3)</td>
<td>158.43 (7.5)</td>
<td>52.36 (6.45)</td>
</tr>
</tbody>
</table>

Values are means (SD).

Table 2
Level of MDA and activity in GPx and GR enzymes in the three groups

the catalytic reaction of glutathione-S-transferase; (d) GSH effectively scavenges ROS (e.g., lipid peroxyl radical, peroxynitrite and H₂O₂) directly and indirectly through enzymatic reactions [34].

However, our results are in contrast with those of Massafra et al. [35] who demonstrated that the use of OCs led to an increase in antioxidant defenses. We concluded from the present investigation that OC can decrease the activity of two antioxidant defense enzymes that are GSH-dependent. Elimination of reduced GSH that plays a central role in the defense against free radicals, peroxides and a wide range of xenobiotics and carcinogens induces stress oxidative and lipid peroxidation. However, use of vitamins E and C may be beneficial in ameliorating this side effect of OCs.

These findings are pilot study-level observations and further research is worthwhile and can define an expected effect size that can be used in power calculations preliminary to a fully powered intervention study. With the data from the present study, we can now consider a preliminary to a fully powered intervention study. With these findings, vitamin supplementation is likely to be beneficial in ameliorating this side effect of OCs.