

Oral Contraceptive Therapy Increases Oxidative Stress in Pre-Menopausal Women

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ABSTRACT

Background: Oral contraceptive therapy (OCT) is associated with an increased risk of deep vein thrombosis, venous thromboembolism and stroke. However, the underlying mechanisms have not yet been elucidated. The objective of this study was to investigate the influence of OCT on blood levels of an oxidative stress maker in pre-menopausal women.

Methods: Oxidative stress was determined in 87 pre-menopausal healthy women (24 with and 63 without OCT) using a blood assay for reactive oxygen metabolites (by the d-ROMs test). The subjects with OCT received a triphasic preparation consisting of ethinyl estradiol and norethisterone.

Results: Subjects with OCT showed significantly higher d-ROMs levels (median: 380; interquartile range: 328-502 Carr U) than those without OCT (325 [271-369]; $P < 0.05$). The results remained the same after adjusting for potential confounders.

Conclusions: The use of OCT may increase oxidative stress levels, independent of traditional cardiovascular risk factors, in pre-menopausal women, providing new insights to the primary prevention of vascular complications in these subjects.

Key words: Contraceptives, d-ROMs test, ethinyl estradiol, norethisterone, oxidative stress marker

INTRODUCTION

Oral contraceptive therapy (OCT) is now commonly used in millions of women worldwide. Therefore, information on the risks and benefits of therapies is critically important.^[1] Estrogen can increase the bioavailability of nitric oxide (NO) in the endothelium, possibly preventing the initiation of atherosclerosis. Furthermore, intracoronary infusion of estrogen in postmenopausal women has been reported to improve endothelial-dependent vasodilatation.^[2] However, epidemiological studies have reported that the estrogens used in OCT may increase the risk of deep vein thrombosis, venous thromboembolism and stroke.^[3,4] The well-documented adverse cardiovascular effects of OCT are important issues for women's health that have not yet been resolved.^[5]

Although, various mechanisms for the adverse effects of OCT are possible, the precise mechanisms have not yet been elucidated.

An adverse effect of OCT on endothelial vascular function might be mediated by overproduction of reactive oxygen species (ROS).^[6] Although, the assessment of ROS is sometimes difficult, a clinically applicable method to measure reactive oxygen metabolites (ROMs) in blood has recently been developed (the d-ROMs test).^[7,8] Hydroperoxides, which are relatively stable reaction products in blood, are a main component of ROMs that are measured by the d-ROMs test. This test has been established as a useful method to evaluate oxidative stress.^[9] The aim of this study was to use the d-ROMs test to investigate oxidative stress in pre-menopausal women with and without OCT.

METHODS

A total of 87 consecutive Japanese pre-menopausal women (26 to 52-years old) were enrolled in this study. The subjects were recruited from women who were visiting for a routine medical examination. The institutional ethics committee approved this study, and informed consent was obtained from all subjects. Eligible subjects were healthy, free from cardiovascular disease, non-smokers and not taking any medication including antioxidant supplements. In pre-menopausal women, 24 subjects had OCT for at least six months and 63 had no OCT in the preceding 12 months. The OCT in this study was a triphasic preparation that consisted of 21 tablets of 0.035 mg of ethinyl estradiol (EE), 12 tablets of 0.5 mg norethisterone (NET) and 9 tablets of 1.0 mg NET. These subjects received the combination of EE and NET for 21 days during each 28-day interval.

All subjects were interviewed for their medical history, menstruation information, and smoking status. Measurements of body mass index, blood pressure and biochemical variables were performed in the clinic. Blood samples were obtained at the luteal phase confirmed by ultrasound, OCT users were taking 1.0 mg of NET at this time. Total cholesterol, triglyceride and high-density lipoprotein cholesterol were measured with standard enzymatic methods (Mitsubishi BCL Laboratory Co. Ltd., Tokyo, Japan) in blood samples obtained after an overnight fast. Hemoglobin A1c (HbA1c) was measured with high-performance liquid chromatography. The d-ROMs test for ROMs in blood was implemented by the Free Radical Analytical System (Diacron,

Grosseto, Italy) according to the analytical manual.^[7,8] The results of the d-ROMs test was expressed as Carr U (1 Carr U corresponds to 0.08 mg/dL of H₂O₂).^[7] A previous report showed that replicate measurements on the same serum sample had an intra-assay coefficient of variation (CV) of less than 0.5% and an inter-assay CV of less than 2.9%.^[9]

Comparisons between the groups were performed with unpaired t-tests, and were also compared using a general linear model to adjust for multiple confounders. The data on triglycerides and d-ROMs were log-transformed due to a skewed distribution. A *P* value < 0.05 was considered significant.

RESULTS

As shown in Table 1, women with OCT had significantly higher levels of d-ROMs than those without OCT. Age-adjusted analysis and age- and body mass index-adjusted analysis confirmed

Table 1: Subject characteristics in the groups with and without oral contraceptive therapy

Parameters	Non-OCT (n=63)	OCT (n=24)	<i>P</i> value level
Age, years	42.8±6.6	40.0±7.9	NS
Body mass index, kg/m ²	21.8±3.6	20.1±2.2	NS
Systolic blood pressure, mmHg	116.9±16.0	114.0±15.0	NS
Diastolic blood pressure, mmHg	75.2±11.8	75.5±11.3	NS
Mean blood pressure, mmHg	89.1±12.7	88.2±11.6	NS
Total cholesterol, mg/dL	210.8±33.6	196.2±29.0	NS
Triglyceride, mg/dL	88 (67–122)	77 (61–98)	NS
HDL cholesterol, mg/dL	70.2±14.7	71.4±15.7	NS
Hemoglobin A1c, %	4.9±0.4	4.8±0.3	NS
d-ROMs, Carr U	325 (271–369)	380 (328–502)	<0.01

NS: Not significant; OCT: Oral contraceptive therapy; HDL: High-density lipoprotein; d-ROMs: Diacron-reactive oxygen metabolites, data are presented as the mean ± standard deviation or median (interquartile range), triglyceride and d-ROMs were log-transformed because of their skewed distribution in the statistical analyses, a *P* value < 0.05 was considered significant (unpaired *t*-test between the groups)

that there was a significant difference in d-ROMs between the two groups ($P < 0.01$). Adjustment for age, body mass index, mean blood pressure, total cholesterol and HbA1c showed that the effect of OCT on d-ROMs was independent of traditional cardiovascular risk factors ($P < 0.05$).

DISCUSSION

This study demonstrates that the use of OCT could increase oxidative stress levels, as assessed by the d-ROMs test, in pre-menopausal women. It is notable that the difference in d-ROMs levels between the groups did not change when the analyses were adjusted for several well-established cardiovascular risk factors. Our findings are crucial, since oxidative stress may contribute to vascular complications.

The mechanisms responsible for the results of the present study are unclear, but there are several possible explanations. The behavior of molecules related to oxidative stress in circulating blood and at the cellular level can differ in the types and dosages of estrogen and progestin within the treatments may be important. Estrogens show various cardiovascular actions on the endothelium, increasing the bioavailability of NO via genomic and non-genomic activation of NO synthase.^[10] The most important difference between E2 and EE is that EE does not seem to protect endothelial function from oxidative stress.^[11] A previous study found that the NO production in human ECV304-endothelial cells was increased by E2 in a dose-dependent manner but not by EE.^[11] Similarly, the viability of endothelial cells after exposure to H₂O₂ was increased by E2 but not by EE, suggesting that EE does not protect endothelial cells from oxidative stress.^[11] Another investigation reported that basal NO and prostaglandin production increased in cultured aortic cells from ovariectomized rats treated with E2, whereas NO and prostaglandin production were reduced in cells from non-ovariectomized rats treated with EE.^[12] Moreover, a clinical study revealed that OCT significantly increased the plasma concentration of copper, selenium and lipid peroxides and decreased the levels of gamma-tocopherol and beta-carotene in women.^[13] Taken together, these findings indicate that OCT can increase oxidative

stress, possibly leading to vascular complications.

The administration of progesterone has anti-atherosclerotic effects with preferable lipoprotein profiles. Furthermore, progesterone may reduce ROS formation and cause vascular relaxation in a tissue-specific fashion;^[14] however, progesterone antagonizes the vasoprotective effects of estrogen on anti-oxidant enzyme expression and function, and enhances NADPH oxidase activity and the production of ROS.^[15] In OCT, a progestogen-only contraceptive implant was reported to have no negative effects on cardiovascular risk factors (e.g., C-reactive protein, total/high-density lipoprotein cholesterol ratio and NO), suggesting progesterone does not negatively impact cardiovascular risk factors in healthy young women.^[16] The risk of venous thrombosis differs depending on the type of progestogen given in combination with EE, and the appropriate dose and type of progestin may reduce the adverse effects of OCT on cardiovascular risk factors.^[17] Recently, the MEGA study indicated that the risk of venous thrombosis with OCT could be increased by different progestin up to 3 to 7 fold.^[18] Thus, progestogen may also antagonize the beneficial effects of estrogen on vasodilation in OCT.

The present study has several limitations. The sample size was relatively small. The study design was cross-sectional, and cardiovascular outcomes were not evaluated. Furthermore, there was no measurement of blood levels of antioxidants, and oxidative stress markers other than d-ROMs. A prospective evaluation in a larger population with long-term follow-up and the measurement of additional markers is necessary to confirm the results of the present study.

CONCLUSIONS

In summary, the present study showed that pre-menopausal women with OCT had increased oxidative stress levels, as assessed by the d-ROMs test, and this increase was independent of traditional cardiovascular risk factors. These findings suggest that oxidative stress due to OCT may contribute to adverse vascular effects, and can provide new insights to the primary prevention of vascular complications in women with OCT. Further research is warranted to confirm these findings.

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