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Antioxidant effects of hormone replacement therapy in postmenopausal women

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Summary

Questions under study: Postmenopausal women are more likely to develop coronary artery disease than premenopausal women of the same age. Postmenopausal oral oestrogen therapy is associated with increased levels of high-density lipoprotein (HDL) cholesterol and decreased levels of low-density lipoprotein (LDL) cholesterol. In this study we investigated the direct contribution of hormone replacement therapy on total antioxidant capacity rather than its effects on the serum lipid profile.

Methods: At the time of enrolment and after the drug delivery plasma total cholesterol, triglycerides (TG), HDL, LDL, uric acid, total bilirubin, albumin, oestradiol levels and total antioxidant capacity of plasma were assessed.

Results: Levels of plasma TG and total antioxidant capacity were significantly increased in the study group.

Conclusions: Our results suggest that oestrogen has an antioxidant effect following 3 months of hormone replacement therapy. Progesterone in combination with oestrogen does not have this effect. Also plasma TG levels increased in those patients receiving HRT after 3 months.

Key words: menopause; hormone replacement therapy; antioxidants; cardiovascular disease; atherosclerosis

Introduction

Menopause concerns a large population of women. It is supposed that by the year 2025, 20% of the world will consist of people above age 60. Therefore women who spend 2/3 of their life in postmenopausal term will increase in number and will be affected by the outcomes of hormonal changes.

Hormone replacement therapy (HRT) is of paramount importance in assessing adequate quality of life caused by oestrogen deficiency. Coronary heart disease, another dilemma, is one of the major causes of death in the elderly women. The reduction in Cardio Vascular Disease (CVD) during HRT is contradicted by a recent Women's Health Initiative (WHI) Study [1]. However, a large number of epidemiological studies mentioned that HRT is associated with increased levels of HDL cholesterol and decreased LDL cholesterol, which explains the protective effect on CVD [2]. On the other hand, the lipoprotein theory failed to demonstrate the reason for the decreased incidence of coronary heart disease in 50–75% of patients receiving HRT [3, 4]. Exposure of normal coronary vascular endothelium to oxidised LDL results in impaired functions of endothelium. Recent evidence suggests that oestrogens possess antioxidant activity [5]. In this study our goal was to investigate whether the cardio-protective effect of HRT was due to the antioxidant system.

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Subjects and methods

Sixty postmenopausal normocholesterolaemic (total cholesterol <200 mg/dl) women (ages 44–69, median 57.5) who had undergone a spontaneous menopause as demonstrated by amenorrhea and elevated gonadotropin levels (FSH >40 I.U./l) volunteered to participate in the study. Data were analysed prospectively. Subjects were randomised into two groups: the study group consisted of 30 patients who received HRT. The control group consisted of 30 patients who rejected or delayed HRT. Each woman in the study group was matched with one control who was closest in age and weight. Initial data in the two groups are shown in table 1. None of the patients had a history of previous HRT, and smokers, diabetics, patients with chronic inflammatory conditions, hepatic or respiratory disease, and those on antioxidants, vitamin or other medication were excluded from the study. Each subject underwent a comprehensive history and physical examination including a standard electrocardiogram.

At the time of enrolment and after the medication, venous blood samples from each subject obtained from the antecubital vein between 8–11 a.m. was drawn into ethylenediamine tetraacetic acid (EDTA, 1 mg/ml blood) containing tubes. After centrifugation of the blood for 15 minutes at 3000 g the plasma obtained was stored in several aliquots at –25 ºC until assay. Plasma total cholesterol, triglycerides (TG), uric acid albumin, and total bilirubin levels were quantified using enzymatic methods. Plasma oestradiol was measured using chemiluminesence method. HDL was measured after phosphotungstic-$MgCl_2$ precipitation of apoprotein B containing VLDL and LDL. The LDL-C was calculated according to the equation described by Friedewald et al. [6]. Plasma TAC was measured using the ABTS (Amino-diethyl-benzthiazoline sulfate) method with a total antioxidant status kit (from Randox Laboratories Ltd. UK) and the results are expressed as mmol/l [7].

The study group received 0.625 mg oestrogen and 2.5 mg medroxyprogesterone acetate orally per day, for a period of 3 months. Overall, 57 women completed the study. 1 patient from the control group and 2 women from the study group dropped out.

Two-way ANOVA was used to analyse differences between the groups in subject characteristics, all biochemical parameters and treatment induced changes. Age and body weight were used as covariates. Multivariate linear regression analysis for each biochemical parameter with treatment group, age, weight and baseline value as predictors was performed by using SPSS, version 10.0 (SPSS, Chicago, IL, USA). A cut-off of P <0.05 was used for statistical significance.

Results

Subject characteristics at baselines are listed in table 1. No significant differences were found between study and control groups with respect to age and weight at baseline. Subject body weights remained stable in each group throughout the study. The mean levels of FSH, LH, and oestradiol of the age, and weight matched two arms are also depicted in table 1.

The levels of measured parameters of both control and study groups before and after 3 months are depicted in table 2. Initial levels of total cholesterol, uric acid, bilirubin, albumin, HDL, LDL, TG, and TAC were similar between the groups at the beginning. Plasma TG levels and TAC of the patients in the study group after 3 months of HRT treatment were significantly higher than in the

Table 1

Initial subject characteristics and clinical data of the two groups of postmenopausal women. (Abbreviations; LH: Luteinizing hormone, FSH: Follicle stimulating hormone).

	Age (years)	Height (cm)	Weight (kg)	LH (IU/L)	FSH (IU/L)	Oestradiol (pg/ml)
Control group	46 ± 8	157 ± 17	74 ± 13	24.6 ± 6.1	68.3 ± 16.6	15.1 ± 4.9
Study group	49 ± 9	162 ± 19	71 ± 11	24.2 ± 5.7	84.7 ± 20.8	19.0 ± 5.3

Values are given as mean+SD.

Table 2

The levels of measured parameters of both control and study group before and after HRT treatment. (Abbreviations; HDL: High-density lipoprotein, TAC: Total antioxidant capacity, HRT: Hormone replacement therapy).

Parameter	before treatment mean [SD]		after treatment mean [SD]	
	control group	study group	control group	study group
Albumin (g/l)	45.2 [1.0]	44.5 [2.0]	44.6 [1.83]	47.3 [1.9]
Total bilirubin (mol/l)	10.3 [2.9]	11.5 [3.6]	11.3 [3.2]	11.9 [3.6]
HDL (mg/dl)	51.1 [5.9]	50.1 [8.1]	51.1 [11.7]	49.5 [10.7]
LDL-C (mg/dl)	115.5 [22.7]	121.5 [24.1]	113.1 [24.6]	125.1 [23.4]
Total cholesterol (mg/dl)	190.1 [4.1]	194.8 [17.1]	190.6 [19.0]	205.7 [19.8]
Triglyceride (mg/dl)	117.4 [49.0]	118.6 [50.2]	132.2 [50.7]	155.3 + 51.8*
Uric acid (mmol/l)	0.21 [0.01]	0.22 [0.05]	0.21 [0.03]	0.24 [0.04]
TAC (mmol/l)	1.41 [0.04]	1.39 [0.06]	1.40 [0.04]	1.53 [0.06]*

* Statistically significant p value (p <0.05). Values are given as mean [SD]

control group ($p < 0.05$). No significant difference was noted in the levels of albumin, total bilirubin, HDL, LDL, total cholesterol and uric acid in the study group after 3 months of treatment compared to the control group ($p > 0.05$).

HRT significantly increased TG levels by 17.4% (95% confidence interval, 13.4 to 27.4, $p = 0.04$) and TAC levels by 9.2% (95% confidence interval, 0.08 to 0.21, $p = 0.05$) in the study group after treatment. Plasma albumin, total bilirubin, LDL cholesterol, total cholesterol, and uric acid levels tended to increase and HDL cholesterol tended to decrease in the study group after treatment but the changes were not statistically significant ($p > 0.05$).

Discussion

Coronary atherosclerosis and cardiovascular events are less prevalent in women than in age-matched men [8]. After natural or surgical menopause, women are more likely to develop coronary heart disease (CHD) than premenopausal women of the same age [9]. The coronary vascular endothelium plays a central role in regulating arterial tone and platelet function [10, 11]. Abnormalities in endothelial control of vascular tone may be related, in part, to the oxidative modification of LDL. Oxidatively modified LDL induces monocyte recruitment, retention in the subendothelial space, and transformation into foam cells. In addition, lipid peroxidation products may be directly cytotoxic to endothelial cells, leading to intimal injury and necrosis and subsequent smooth muscle cell proliferation [12, 13]. Exposure of normal arteries to oxidized LDL results in impaired endothelium-dependent relaxation [14, 15].

Postmenopausal HRT appears to reduce the risk of developing CHD [16–21]. Large population studies have demonstrated up to a 50% reduction in the incidence of CHD in postmenopausal women receiving HRT [22–24]. Oral oestrogen therapy is associated with increased levels of HDL and decreased LDL. Clinical studies often of short duration have shown that many progestogens are able to oppose such changes, particularly in HDL [4]. But others have concluded that the use of combined therapy does not have any effects on HDL or LDL levels [25]. Medroxyprogesterone acetate (MPA) was found to have less adverse effects on plasma lipids, probably due to the absence of androgenic properties of this progestine [26]. Various studies have shown that the effect of HRT on the lipid profile depends on the dosage and duration of treatment [25–28]. Generally 0.625 mg oestrogen plus 2.5 mg or 5 mg progesterone therapy daily do not have a positive effect on the lipid profile in first 3–6 months of treatment. It has been postulated that, the beneficial effects of HRT usually occur after 6 months of therapy. In our study, after 3 months of therapy we found that the use of HRT consisting of 0.625 mg oestrogen plus 2.5 mg MPA daily had no effect on total cholesterol, LDL cholesterol, and HDL cholesterol. However the levels TG were significantly increased.

It has been postulated by a recent WHI study that HRT is associated with higher CRP (C-reactive protein), HDL and TG, and lower tPA-antigen and homocysteine. Levels of CRP were the highest in users of unopposed conjugated oestrogen. Levels of inflammatory markers like IL-6, sICAM-1, D-Dimer and total cholesterol did not differ between HRT users and non-users. They concluded that risk markers differed by HRT category, despite the large number of patients; their results remain to be proven by randomised trials [1].

In earlier observational studies, it has been reported that only 25% to 50% of the beneficial effect of oestrogen is due to effects on lipoprotein levels [19, 20, 29]. We selected the study group within the normocholesterolaemic patients because we wanted to study the direct contribution of HRT on the total antioxidant activity, rather than its effect on the plasma lipid profile. Several serum components like ascorbic acid, uric acid, vitamin E (alpha tocopherol), beta-carotene and serum proteins act as antioxidants and neutralise free radicals. Since the antioxidant system has many components, a deficiency of any component can cause a reduction in the total antioxidant status of an individual. Therefore, it is more useful to measure the total antioxidant capacity of plasma using a single assay in a clinical laboratory, rather than measuring the individual components of the total antioxidant system in plasma [30]. Total antioxidant capacity allows an assessment of the performance of the entire antioxidant system since the measurement of different antioxidant molecules separately is not practical and their antioxidant effects are additive. However there is not yet an accepted "gold standard" reference method. We preferred the colorimetric method for total antioxidant response measurement as it is the most commonly used method [31].

Our findings showed that 3 months of HRT increased the plasma total antioxidant capacity without having any effect on the plasma albumin, bilirubin and uric acid levels, which are the major components of plasma antioxidant activity. This effect is important to prevent LDL oxidation since oxidative modification of smaller LDL particles is an initial step in the atherosclerosis process [32]. It is reported that HRT decreased in vivo LDL oxi-

dation [33]. It has been also suggested that HRT with oestrogen, but not combined therapy with oestrogen and progestin, may have antioxidative effects [34]. However, antioxidative effects of HRT seem not to be enough to prevent cardiovascular diseases in postmenopausal women since some large scale recent trials showed no benefit of oral hormone replacement therapy on the overall rate of coronary events and progression of coronary stenosis [35, 36]. In addition, an increased risk of cardiovascular events was reported in healthy postmenopausal women who used HRT [37]. The increased number of early cardiovascular events might be explained by oral oestrogen induced reduction in LDL particles, which are more susceptible to oxidation [38]. Since oral oestrogen-induced increases in plasma triglyceride concentration can reduce the size of LDL particles, the antioxidant effects of oestrogen might be counterbalanced in patients showing such increases [39]. In line, it was reported that plasma triglyceride concentration and the size of LDL particles were unaffected and the oxidative susceptibility of LDL was inhibited by lower-dose oestrogen administration [40].

In conclusion, we found that oestrogen has an antioxidant feature; progesterone in combination with oestrogen does not decrease antioxidant activity. This feature was initiated in a period of 3 months following HRT during which plasma TG levels increased in those patients.

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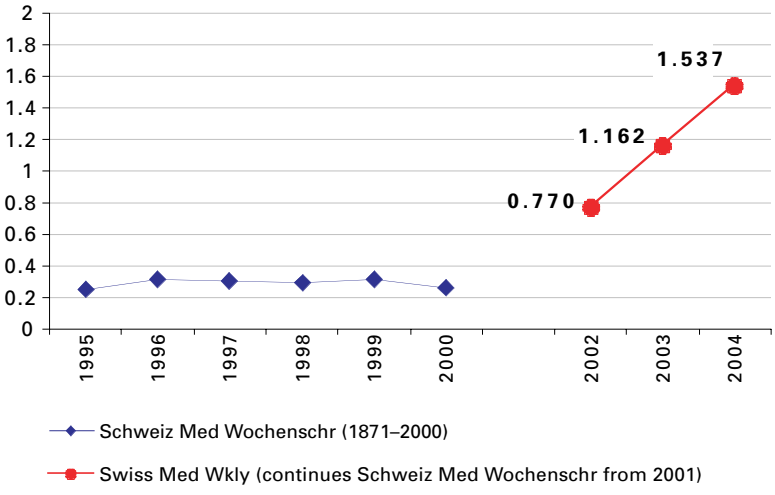

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