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Conjugated Estrogen Administration Improves Common Carotid Artery Elastic Properties in Normotensive Postmenopausal Women

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Summary

Background: Various vascular effects of estrogens have been proposed to explain further the beneficial effect of replacement therapy in cardiovascular events.

Hypothesis: The study was undertaken to assess the effect of conjugated estrogen on the elastic properties of the large arteries in normotensive, healthy, postmenopausal women.

Methods: Toward this end, we investigated the acute effect of conjugated estrogen on the elastic properties of the common carotid artery (CCA) in 20 normotensive, healthy, postmenopausal women (age 54 ± 3 years) at baseline and 20 min after the intravenous administration of 1.25 mg conjugated estrogens. The CCA distensibility was derived by a combination of surface ultrasonographic data and simultaneous blood pressure measurements at the brachial artery. The carotid pulsatility index, a measure of brain impedance, was determined electronically by tracing the CCA Doppler waveform.

Results: At baseline, CCA distensibility had a negative correlation with both patients' age and time since menopause ($r = -0.57$ and $r = -0.48$, $p < 0.05$ for both cases). After estrogen administration, estradiol and estrone plasma levels were restored to the range of usual premenopausal values. Estrogen induced a significant increase in CCA distensibility by 0.92 ± 0.005 dyne$^{-1} \cdot$cm$^2 \cdot 10^{-6}$ (from 2.03 to 2.95 dyne$^{-1} \cdot$cm$^2 \cdot 10^{-6}$) and a significant reduction in CCA pulsatility index by 0.24 ± 0.06, (from 2.17 to 1.93) ($p < 0.001$ for both cases). The improvement in CCA distensibility had a negative correlation with both patients' age and time since menopause ($r = -0.46$ and $r = -0.44$, respectively, $p < 0.05$ for both cases).

Conclusions: Acute conjugated estrogen administration induced an improvement in CCA elasticity and a reduction in brain impedance in normotensive, postmenopausal women. As the age of women and the time since menopause increased, the improvement in carotid distensibility decreased in such selected subjects.

Key words: conjugated estrogen, carotid artery distensibility, postmenopausal women

Introduction

Menopause is associated with structural and functional cardiovascular adaptations regardless of the presence or absence of high blood pressure.[1,2] The reduced arterial elasticity which accompanies menopause contributes to increased pulse pressure which has been shown to be an independent predictor of future cardiovascular events.[2–5] Although stroke is an important cause of morbidity and mortality in women, there is little information about the risk of stroke in current postmenopausal hormone users, and these results are still conflicting.[6,7] Furthermore, since only 25–50% of the replacement therapy beneficial effect in cardiovascular events is attributable to lipid lowering, a variety of vascular effects of estrogens is suggested.[8–10] Estrogen may inhibit atherosclerosis and also play an integral role in the maintenance of arterial hemodynamics that prevent ischemia.[11,12] In this study, we report the acute effect of conjugated estrogens on the elastic properties of common carotid artery (CCA) in normotensive, postmenopausal women in an attempt to clarify the possible mechanisms by which hormone replacement therapy exerts its favorable effect on the risk of stroke.

Subjects and Methods

Study Population

Women were eligible if menopause had occurred at least 1 year previously, without their undergoing any hormone replacement therapy, and whose plasma estradiol concentration was within the appropriate range of postmenopausal values for our laboratory. All participants were free of vasomotor

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symptoms associated with the effect of menopause. Women with a history or clinical evidence of coronary artery disease, cerebrovascular disease, valvular disease, myocardiopathy, congestive heart failure, hypertension, diabetes mellitus, liver or renal or any systemic disease were excluded, as were women with a history of thromboembolism, smoking, familiar hypercholestorelemia, and alcohol or drug abuse. We looked carefully for evidence of plaques (defined as a thickening of the wall ≥ 50% of the surrounding wall) bilateral on the common, internal, and external carotid artery in order to exclude such women with presence of plaques. Finally, 20 healthy, unmedicated postmenopausal women, aged 54 ± 3 years with 4.8 ± 2.5 years since menopause fulfilling the above criteria were selected.

All subjects underwent physical examination, 12-lead electrocardiogram, chest x-ray, and standard laboratory examinations. The study protocol included duplex ultrasound scanning of carotid arteries and assay of plasma estradiol (E2) and estrone (E1) concentrations before as well as 20 min after intravenous administration of 1.25 mg conjugated estrogens (Premarin®, Wyeth-Ayerst). The study protocol was approved by the ethics committee of the Institutional Review Board of our hospital. After description of the procedure, written informed consent was obtained from all women participating in the study.

Carotid Artery Duplex Ultrasonography

Carotid arteries duplex ultrasound studies were performed in the echocardiography department of our clinic by an experienced investigator, using a Hewlett Packard Sonos 2500 (Hewlett Packard [Philips Medical Systems/Agilent Technologies, Andover, Mass., USA]), equipped with a 7.5/5.5 MHz phased linear-array transducer. The ultrasonographic studies were scheduled to start at 11.00 A.M. under a controlled room temperature of 22˚C, with constant noise and light intensity, during a day's hospitalization. All subjects had had a similar meal at least 3 h prior to the study and rested in a supine position for 15–20 min. Patients were studied in the supine position with slight neck hyperextension. Long axis B-mode images of the right CCA were obtained in three projections (anterior, posterior, and lateral) in order to obtain simultaneously a fine image of the near and far wall.[13–17] The CCA systolic and diastolic diameter was determined in frames corresponding to the maximal and minimal diameters, respectively. Both CCA systolic and diastolic diameters were measured as the distance between the intima-lumen interface of the near and far walls.[13–16] Values were measured over five consecutive cardiac cycles and averaged.

For the calculation of CCA distensibility, pulse rate and blood pressure were measured at the brachial artery simultaneously with the performance of CCA ultrasonographic studies, with the use of an automatic oscillometric device (Dinamap XL, Johnson & Johnson, Inc.). Blood pressure (BP) values were obtained for measurements after the achievement of a steady hemodynamic state, manifested by a pulse rate variation of less than five beats/min and systolic and diastolic BP variation of < 5 mmHg over two consecutive measurements. Pulse pressure was calculated as the difference between systolic and diastolic BP values. The CCA measurements were combined with the simultaneous oscillometric BP readings to calculate the carotid artery distensibility by means of the formula: Carotid artery distensibility = 2× [CCA systolic diameter −CCA diastolic diameter]/[CCA diastolic diameter × pulse pressure] [$dyne^{-1} \cdot cm^2 \cdot 10^{-6}$]).[13–16]

When the CCA diameter measurements were completed, the pulsed Doppler range gate was placed across the right carotid artery 1.5 cm proximal to the carotid bifurcation, which was the measurement site, to obtain flow velocity waveforms. We made certain that the sample volume was placed in exactly the same position for all repeated measurements and that at least five consecutive Doppler waveforms of adequate quality (representing 5 cardiac cycles) were recorded. Common carotid artery pulsatility index was determined electronically by tracing the CCA Doppler waveform and defined as the difference between the peak systolic shifted frequency and end-diastolic shifted frequency divided by the mean frequency shift over the cardiac cycle given.[13, 17, 18]

After the above baseline CCA measurements, conjugated estrogens were administered intravenously through a peripheral vein in a bolus dose of 1.25 mg over a 2 min period.[19] Electrocardiogram, BP, and carotid artery duplex scanning parameters were continuously monitored and recorded repeatedly at 20 min after the end of conjugated estrogen administration. At the same time, blood was drawn for determination of E_1 and E_2 concentrations. All images were recorded on super VHS videotapes for subsequent off-line analysis. Five consecutive cycles were measured blindly by two observers and the mean value was used in the analysis.

Repeatability of Arterial Measurements

Repeatability of our measurements was investigated in five subjects through calculation of the repeatability coefficient (RC) as defined by the British Standard Institution, according to the formula $RC^2 = (\Sigma Di^2)/N$, where N is the sample size and Di the relative difference between each pair of measurements.[20] Repeatability coefficient values for the intraobserver repeatability (comparison of two determinations obtained at 24 h intervals by the same observer) concerning CCA systolic and diastolic diameter and pulsatility index at baseline were 0.031 and 0.035 mm, and 0.09, respectively. Also, RC values for the interobserver repeatability (comparison of two determinations obtained at the same time by two observers) concerning CCA systolic and diastolic diameter and pulsatility index at baseline were 0.041 and 0.039 mm, and 0.08, respectively. These values were small compared with the mean values of CCA systolic and diastolic diameter and its pulsatility index in the sample.

Plasma Estradiol and Estrone Assay

Blood samples were drawn and centrifuged immediately and plasma was frozen at −20˚C for estimation of E_1 and E_2 concentration by radioimmunoassay.

TABLE I Clinical characteristics of the study subjects

Age (years)	54.2 ± 3
Time since menopause (years)	4.8 ± 2.5
Body surface area (kg/m^2)	1.72 ± 0.8
Total cholesterol (mg/dl)	215 ± 45
Plasma estradiol (pg/ml)	45 ± 23
Plasma estrone (pg/ml)	32 ± 16
Clinic blood pressure (mmHg)	124/82

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). A value of $p \leq 0.05$ was accepted as statistically significant. The variables compared before and after conjugated estrogen administration intervals included BP, heart rate, E_1 and E_2 plasma levels, CCA systolic and diastolic diameter, CCA distensibility, and CCA pulsatility index. Significant differences of the parameters studied at baseline and after estrogen administration were determined by use of the paired *t*-test. Multiple linear regression analysis, with inclusion criteria at the 0.01 level and exclusion criteria at the 0.05 level, was used to evaluate the relation of clinical, demographic, and laboratory parameters with CCA elasticity indices. Pearson correlation coefficient was employed to detect possible significant correlation between CCA elasticity indices and demographic and clinical parameters.

Results

All women underwent the study without reporting any adverse symptoms after conjugated estrogen administration. The clinical and demographic characteristics are presented in Table I. After estrogen administration, E_1 and E_2 concentration increased substantially, peaking to 308 ± 52 pg/ml and 341 ± 133 pg/ml, respectively, at 15 min ($p < 0.001$) and remaining at typical premenopausal levels throughout the study. Heart rate and systolic and diastolic BP as well as pulse pressure did not change significantly after estrogen administration. The CCA ultrasonographic measurements and the oscillometric blood pressure data, at baseline as well as after the acute administration of conjugated estrogen, are presented in Table II.

The CCA systolic and diastolic diameter was significantly increased by 0.035 and 0.02 cm, respectively ($p < 0.001$ for both cases), after estrogen administration. Similarly, the pulsatile changes of CCA diameter increased significantly by 0.015 cm ($p < 0.001$). Estrogen induced a significant increase in CCA distensibility by 0.92 ± 0.005 $dyne^{-1} \cdot cm^2 \cdot 10^{-6}$ (from 2.03 to 2.95 $dyne^{-1} \cdot cm^2 \cdot 10^{-6}$) ($p < 0.001$) and a significant reduction in CCA pulsatility index by 0.24 ± 0.06 (from 2.17 to 1.93) ($p < 0.001$).

Correlations

Before estrogen administration, pulsatility index had a positive correlation with age ($r = 0.90$, $p < 0.0001$) and with time since menopause ($r = 0.94$, $p < 0.001$), while CCA distensibility had a negative correlation with age ($r = -0.57$, $p < 0.001$) and with time after menopause ($r = -0.48$, $p < 0.05$).

After estrogen administration, the reduction of pulsatility index had no significant correlation with age or the time since menopause, while the improvement of CCA distensibility had a negative correlation with age and with time since menopause ($r = -0.46$ and $r = -0.44$, respectively, $p < 0.05$ for both cases) (Figs. 1 and 2, respectively).

Furthermore, CCA distensibility at baseline and the change of CCA distensibility after estrogen administration was entered in multiple regression models to identify significant relations with age, time since menopause, body mass index, arterial pressure, total serum cholesterol levels, and E_1 and E_2 levels. It was revealed that both CCA distensibility at baseline and the change of CCA distensibility after estrogen administration were significantly related with age ($p = 0.004$ and $p = 0.002$, respectively) and time since menopause ($p = 0.002$ and $p = 0.02$, respectively).

Discussion

In the present study, we investigated the acute effect of conjugated estrogen on CCA mechanics in normotensive, postmen-

TABLE II Carotid artery and oscillometric blood pressure measurements

CCA parameters	Baseline	20 min	p Value
Systolic diameter (cm)	0.690 ± 0.03	0.725 ± 0.02	0.001
Diastolic diameter (cm)	0.660 ± 0.02	0.680 ± 0.02	0.001
Pulsatile change of diameter (cm)	0.03 ± 0.01	0.045 ± 0.01	0.001
Heart rate (beats/min)	76 ± 5	77 ± 5	NS
Systolic blood pressure (mmHg)	128.7 ± 6	128.4 ± 6	NS
Diastolic blood pressure (mmHg)	84.1 ± 3	83.7 ± 3	NS
Pulse pressure (mmHg)	44.6 ± 4	44.7 ± 4	NS
Distensibility ($dyne^{-1} \cdot cm^2 \cdot 10^{-6}$)	2.03 ± 0.4	2.95 ± 0.7	0.001
Pulsatility index	2.17 ± 0.13	1.93 ± 1.15	0.001

Abbreviations: CCA = common carotid artery, NS = not significant.

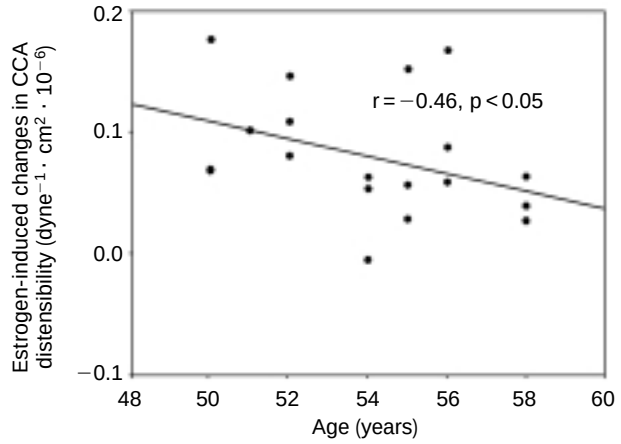

FIG. 1 Relation between changes in common carotid artery (CCA) distensibility induced by estrogen and age of postmenopausal women.

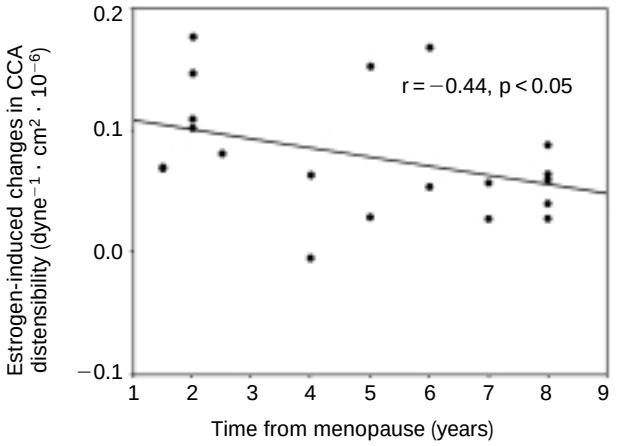

FIG. 2 Relation between changes in common carotid artery (CCA) distensibility induced by estrogen and time since menopause in postmenopausal women.

opausal women. We found that estrogens induce an improvement in CCA distensibility apart from the reduction of brain impedance in this setting. Furthermore, we found that the older the women and the longer the time since menopause, the less improvement in CCA elasticity after estrogen administration.

Large arteries, such as aorta and carotid, function both as a conduit delivering blood to the tissues and as an important modulator of the cardiovascular system, buffering the intermittent pulsatile output from the heart to provide steady flow to capillary beds.[3–5] Aging, along with or without hypertension, exerts further ill effects through structural and functional modifications of the arterial wall.[5] Recently, it has been reported that CCA stiffness is an independent predictor of all-cause mortality in end-stage renal disease.[21] Furthermore, menopause is associated with early structural and functional manifestations of heart disease in both hypertensive and normotensive women.[1] Similarly, it is supported that the effect of menopause on the elastic properties of the aortic root is abrupt and devastating in hypertensive women.[2] In agreement with this study, we found that CCA distensibility decreased as patients age, and as the time since menopause increased in normotensive postmenopausal women.

Pulsatility index represents the impedance to blood flow downstream from the point of measurement; the higher the value the greater the impedance.[17, 18] In our postmenopausal women, the pulsatility index was found to have a positive correlation with age and time since menopause, and these findings are in agreement with a previous study by Gangar *et al.*[17]

Epidemiologic studies have shown that the rates of cardiovascular and cerebrovascular mortality in women who receive estrogen replacement therapy are a third to a half of those in untreated women;[3, 4] however, in the Nurses' Health Study, there was no significant association between stroke and use of combined hormones or estrogen alone.[22] Graby *et al.*[23] using meta-analytic statistical methods, concluded that there is no convincing evidence that estrogens decrease stroke risk significantly. Even nowadays, the data on stroke are unclear, and this issue will be addressed more directly in the next decade, when the results of clinical trials, such as the Women's Health Initiative, will be known.[24] The apparent benefit of estrogen replacement therapy on cardiovascular events cannot be accounted for only by its favorable effect on lipids profile. Our findings that conjugated estrogen improves CCA elasticity and decreases brain impedance are in agreement with previous studies suggesting a variety of vascular effects of estrogen on both peripheral and coronary vessels and may contribute to the better understanding of the rationale for the estrogen-induced protection from stroke.[5–8, 13, 19, 25, 26]

Although the present study was not designed to assess the mechanism of estrogen vasomotor effects, we may suggest that conjugated estrogen actions on the CCA function could be interpreted, at least partly, by an endothelium-independent mechanism. We may support this, since we excluded women with atherosclerotic plaques in the carotid arteries, as well as women with other conditions which are associated with endothelium dysfunction such as hypertension, smoking, or hypercholesterolemia. Thus, it is possible that conjugated estrogen improves the distensibility through a calcium-channel antagonist effect. Toward this direction, Stefanadis *et al.* have proved that estrogen improves aortic distensibility by an active mechanism, suggesting a calcium-antagonist effect.[27] In addition, Collins *et al.* have supported the view that if estrogens have a calcium-channel antagonist effect on human beings, then this mechanism may contribute significantly to its protective effect on the development and progression or regression of atherosclerosis in coronary and other arteries in women.[28] It is obvious that we cannot rule out the possibility that conjugated estrogen may enhance endothelium-dependent vasodilatation by facilitating nitric oxide release. Our finding that the estrogen-induced improvement on CCA elasticity is inversely related with patients' age and the time since menopause may suggest that replacement therapy should be started early after menopause. However, it is unclear to what extent the benefits and risks of replacement therapy apply to older women. Only

randomized trials with clinical outcomes that are of sufficient size and duration can answer this question.

Limitations

In the present study, the distensibility of CCA was determined by pulse pressure obtained at the site of brachial artery. Given that amplification of pulse pressure from central arteries to a more distal one is a well known phenomenon attributable to pulse-wave reflection, the difference between CCA BP and brachial artery BP may affect the accuracy of CCA distensibility.[5] However, the pulse pressure, which was estimated noninvasively at the brachial artery, correlates well with that measured directly from the aorta and has been extensively used for determination of the aortic and CCA elastic properties.[15, 16, 29] Furthermore, we used a video imaging analysis for estimation of CCA systolic and diastolic diameter. Although the video imaging analysis has much less reproducibility than does the echo-tracking method,[30] our method has been used previously[15, 16, 30] and is preferred in clinical practice due to our good repeatability in arterial measurements.

We chose to administer conjugated equine estrogens since they are the most commonly prescribed medications for the amelioration of menopausal symptoms, and their effect on the function of peripheral circulation has not been well investigated. However, it is known that estrogen may have class-specific and dose-dependent vasomotor properties. In our case, a dose of 1.25 mg conjugated equine estrogen given intravenously increased plasma E_1 levels to approximately 300 pg/ml, typical mid-cycle premenopausal values providing precise physiologic replacement without producing any potential harmful side effect. In addition, the conjugated estrogen used in this study contains several vasoactive estrogenic compounds, which may account for some of the effects observed.

Another limitation of our study may be the rather small study population. However, this small sample may be balanced against the strict inclusion criteria, and, consequently, our women are part of a highly selected population.

Conclusions

Acute conjugated estrogen administration induced an improvement in CCA elasticity and a reduction in brain impedance in selected normotensive, postmenopausal women. As the women's age and the time since menopause increased, the improvement in CCA distensibility decreased. These data provide evidence that conjugated estrogen may have a direct effect on carotid artery function and may explain some of the protective effects of estrogen replacement therapy in postmenopausal women. However, further investigation is required to determine whether its apparently favorable effects on this intermediate biological outcome can be translated into the reduction in cerebrovascular events in postmenopausal women who receive replacement therapy.

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