The effect of HRT on cerebral haemodynamics and cerebral vasomotor reactivity in post-menopausal women

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BACKGROUND: Cerebral vasomotor reactivity (CVR) is an index of cerebrovascular dilatory capacity which can readily be assessed using trans-cranial Doppler ultrasound. Impaired CVR is associated with elevated risk of stroke. We performed a randomized, double-blind placebo-controlled trial to investigate the effect of two HRT preparations upon CVR. METHODS: We examined middle cerebral artery mean flow velocity (MFV), internal carotid artery pulsatility index (PI) and CVR to an i.v. acetazolamide bolus using ultrasound in three groups of post-menopausal women randomized to oral estradiol 1 mg + norethisterone 0.5 mg (group N), estradiol 1 mg + dydrogesterone 5 mg (group D) or placebo (group P). The MFV, PI and CVR were measured before and after 3 months treatment. RESULTS: Thirty-eight post-menopausal women were recruited (N = 12, D = 14, P = 12); mean (SE) age was 56.7 (4) years. Neither HRT preparation affected CVR [% (SE) change from baseline N +4.2 (11); D +3.8 (5.5); P +4.0 (3.8); all comparisons P = NS]. CVR was significantly reduced in recipients of dydrogesterone [% (SE) change from baseline D −5.4% (4.6); N +12.3 (6.9); P +11.6 (6.9). P = 0.025]. Middle cerebral artery velocity was significantly increased following dydrogesterone treatment compared with placebo [% (SE) change from baseline D +6.8 (3.4) N +3.9 (4.2) P −4.6% (3.4) P = 0.03 for D versus P]. CONCLUSION: HRT did not alter CVR. The reduced PI and increased MFV suggest HRT-induced intracranial vasodilatation, which is more apparent in dydrogesterone recipients. Differences may exist between progestogens with regard to changes in intracranial haemodynamics.

Introduction

Cerebrovascular disease accounts for a high proportion of health care costs throughout the developed world (Hankey, 1999). Current acute treatment strategies are limited, and stroke prevention has been recognized as a priority in the UK and elsewhere. Although stroke is a leading cause of death and long-term disability in women (Murray and Lopez, 1997; Bousser, 1999), the incidence of stroke is lower in premenopausal women than in age-matched men. After the menopause, risk of stroke increases in women and the gender discrepancy eventually disappears. These observations have generated interest in the role of female sex hormones in protection from cerebrovascular disease.

Recent large clinical trials have reported increased stroke risk in patients randomized to HRT. A post hoc analysis of the WEST study data (Viscoli et al., 2001) revealed increased risk of cerebrovascular events during the first 6 months of treatment with estradiol [relative risk of 2.3; 95% confidence interval (CI) 1.1–5.0]. Similarly, those patients in the active treatment arm of the WHI study (Writing Group for the Women’s Health Initiative Investigators, 2002) had increased stroke risk, with a hazard ratio of 1.41 (95% CI 1.07–1.85). These trials used combined HRT containing both estrogen and progestogen. These results have challenged the conclusions of older epidemiological studies which suggested significantly reduced stroke risk in HRT recipients. The mechanism through which HRT confers increased risk of stroke is elusive, although few studies have yet examined the effect of HRT on cerebral vessels directly. We performed a prospective randomized double-blind placebo-controlled study designed to examine the effect of administration of two different HRT preparations upon the cerebral vasculature.

Women over the age of 55 usually take continuous combined HRT, consisting of an estrogen combined with a progestogen. Progestogens vary in their structure according to whether they are based on testosterone (C19 progestogens) or progesterone (C21). Until recently, it was assumed that the vascular effects of HRT were largely related to the estrogen, but it has become apparent recently from preclinical studies that the progestogenic component may be of considerable importance. In animal models of both focal (Betz and Coester, 1990) and global (Cervantes et al., 2002) cerebral ischaemia, progesterone administration attenuates brain injury through an as yet undefined mechanism. In humans, progestogens affect a number of processes which may modify risk of vascular disease. For example, progestins may influence levels of
atherogenic and atheroprotective lipids (Writing Group for the PEPI Trial. 1995) and the expression of a number of circulating rheological factors (Nabulsi, 1993). Progestins can also inhibit vasorelaxation (Mercuro, 1999) and decrease proliferation of both human endothelial (Okada, 1997) and vascular smooth muscle cells (Lee et al., 1997). We chose to investigate the impact of the progestogen on the cerebral vasculature of post-menopausal women by keeping the estrogen constant while studying one C19 and one C21 progestogen.

Non-invasive ultrasonic assessment of vascular reactivity is possible through measurement of cerebral vasomotor reactivity (CVR) to acetazolamide. This agent is a carbonic anhydrase inhibitor which induces a mild extracellular acidosis, hence it provides a strong stimulus for intracranial vasodilation. The vasodilatory response is readily detectable with trans-cranial Doppler (TCD) ultrasound as an increase in velocity of blood flow through the middle cerebral artery (MCA). Impaired reactivity reflects blunted ability of the cerebral vasculature to respond to a hypoxic or ischaemic insult and has been associated with increased risk of stroke (Molina et al., 1999). Impaired reactivity is reversible and has been demonstrated in the context of hypertension, hyperlipidaemia and subcortical stroke (Settakis et al., 2003)

Methods
We performed a randomized, double-blind placebo-controlled trial to investigate the effect of two HRT preparations upon CVR. Postmenopausal women were recruited from a primary care environment. Ethical approval was obtained from the North Glasgow National Health Service Trust Ethics Committee, and patients gave written informed consent to participate. All participants in the trial were post-menopausal women aged between 50 and 65 years, who had not used HRT in the previous 3 months. Patients were deemed ineligible if they were taking any vasoactive medication or had a history of coronary, cerebral or peripheral vascular disease. Patients with contraindication to HRT use or evidence of carotid or MCA stenosis on ultrasound were similarly ineligible.

Once informed consent had been obtained and randomization completed, patients underwent baseline assessment of intracranial haemodynamics and CVR to acetazolamide. Following baseline assessment, patients received either oral estradiol 1 mg + norethisterone 0.5 mg; oral estradiol 1 mg + dydrogesterone 5 mg; or placebo to be taken daily for 3 months. At the end of the treatment interval, they returned for repeat ultrasound assessment of intracranial haemodynamics. Demographic characteristics are shown in Table I.

Haemodynamic profiles
Carotid artery. Doppler studies were undertaken by a single observer who was not involved in drug administration. Neck measurements were taken anterolaterally with an Acuson 128 with 5 MHz linear transducer (Acuson, CA). Subjects were examined reclining in a quiet room with constant lighting and temperature, having rested in a reclining position for 5 min and fasted for 2 h prior to insonation. The Doppler sample width was set to encompass the diameter of each of the common, internal and external carotid arteries, with automated velocity correction according to the ultrasound-vessel incident angle. Velocity of blood flow through the internal carotid artery (ICA) was recorded over at least five cardiac cycles, and the intensity-weighted mean velocity curve was applied to the Doppler waveforms.

Middle cerebral artery and cerebral vasomotor reactivity. As diurnal variation in CVR may occur, all examinations were performed at ~11 a.m., immediately following carotid insonation. Patients underwent TCD examination in a supine position. TCD recordings (TC 2000 with 2 MHz probe, Nicolet, UK) were obtained from the MCA at a depth of 50 mm using a temporal approach. Readings were based on 36 s recordings from each MCA. Velocity readings were based on the maximal (envelope) curve. Insonation of the right MCA was performed for a period of 2 min and the mean flow velocity (MFV) recorded. Each subject then received an i.v. infusion of 13 mg/kg acetazolamide (to a maximum of 1 g) reconstituted in sterile water for injection over 10 min. The total volume of infusate was 50 ml. Twenty minutes after cessation of acetazolamide infusion, the TCD recording was repeated for a further 2 min, and the post-acetazolamide MFV recorded. Pre- and post-acetazolamide MFVs were calculated as the average MFV of all waveforms recorded in each 2 min interval. During data acquisition, end-tidal carbon dioxide concentration was measured using a standard probe to confirm reduction of systemic carbonic anhydrase activity. CVR was calculated as MFV(post)/MFV(pre) × 100. The pulsatility indices of the ICA and MCA (a measure of vascular resistance distal to the insonated vessel) were obtained through off-line analysis of the velocity/time waveforms. All data were processed without access to treatment group information. A more detailed account of the Doppler methodology employed in our laboratory can be found elsewhere (Dyker et al., 1997).

Table I. Baseline characteristics of women

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo</th>
<th>Estradiol and norethisterone acetate</th>
<th>Estradiol and dydrogesterone</th>
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</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>56.4 ± 4.2</td>
<td>56 ± 3</td>
<td>58 ± 4.9</td>
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<tr>
<td>BMI (mean ± SD)</td>
<td>26.1 ± 2.6</td>
<td>25 ± 2.1</td>
<td>26 ± 2.8</td>
</tr>
<tr>
<td>Current smokers (n)</td>
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<td>2</td>
</tr>
<tr>
<td>Hypertensive (n)</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MABP (mean ± SD)</td>
<td>88 ± 2.7</td>
<td>89 ± 2.7</td>
<td>91 ± 3</td>
</tr>
</tbody>
</table>

Statistical measures
Statistical analysis was performed using Student’s t-test and two-way analysis of variance (StatsDirect Ltd, Cheshire, UK). The power calculation was based upon variability data acquired during earlier TCD studies. A sample size of 12 patients per group would allow a 10% difference in CVR between groups to be detected with 80% power.

Results
Forty-one women were assessed for suitability. Two women were excluded due to ineligibility (undiagnosed hypertension and carotid stenosis, respectively). One woman withdrew consent following randomization and left the study. Thirty-eight women completed the protocol. Demographics and group allocations are shown in Table I. There were no significant differences in demographics between groups. One volunteer reported painful uterine bleeding after treatment with estradiol and norethisterone for 5 weeks. The dosing interval was truncated in this case and the follow-up assessment was performed at 6 weeks. Both HRT preparations were otherwise well tolerated with no reports of other treatment-related symptoms.
Cerebral vasomotor reactivity

There were no significant between-group differences in baseline CVR. HRT administration did not affect CVR [% (SE) change from baseline, estradiol + norethisterone +4.2% (11); estradiol + dydrogesterone +3.8% (5.5); placebo +4.0% (3.8)] (Figure 1). Absolute values of CVR were consistent with those from healthy volunteers.

Middle cerebral artery mean flow velocity

MCA MFV was significantly increased following dydrogesterone treatment compared with placebo. This parameter increased by 6.8% (SE 3.4) from baseline compared with a 4.6% (SE 4.2) fall from baseline in the placebo group (P = 0.03). There was a non-significant trend towards increased MCA MFV in the estradiol + norethisterone recipients (+3.9% SE 4.2). These data are shown in Figure 2.

Discussion

Our knowledge of the effects of female sex hormones on the cerebral circulation remains limited. Studies of the effect of ageing on cerebrovascular reactivity have shown increased reactivity to acetazolamide in premenopausal women relative to age-matched men, with reduction in CVR after the menopause. A protective effect of HRT upon CVR has also been suggested on the basis of anecdotal data. Several unblinded trials (Gangar et al., 1991; Penotti et al., 1993, 1999; Cacciator et al., 1998) have reported changes in cerebral haemodynamics consistent with HRT-induced intracranial vasodilatation, an observation supported by our data. Our study is the first randomized placebo-controlled study of hormone replacement to examine the cerebral vasculature directly. We did not detect an improvement in cerebrovascular reactivity in this study of short-term intervention. We have not excluded an effect of HRT on CVR following long-term use. Similarly, as the baseline CVR of the participants in our study was similar to that of healthy volunteers, we

Internal carotid artery pulsatility index

The ICA pulsatility index (PI) fell significantly in the dydrogesterone group [−5.4% (SE 4.6%)] compared with the placebo and norethisterone groups [+11.6% (SE 6.9) and +12.3% (SE 6.9) respectively, P<0.03 for both comparisons]. These data are shown in Figure 3.

Blood pressure

There were no significant differences in blood pressure at baseline (Table I). There was a non-significant trend towards an increase in mean arterial blood pressure (MABP) in HRT recipients [+3.4% (SE 3.7) and +5% (SE 2.6)] in the norethisterone and dydrogesterone groups, respectively. MABP in the placebo group was unchanged [−1.1% (SE 2.6)] from baseline (ANOVA, P = 0.6).
have not excluded an effect of HRT upon CVR in patients with higher degrees of baseline CVR reduction.

Although no change in CVR was following HRT administration, our results suggest a modest but consistent effect of HRT on intracranial vessels, suggestive of HRT-induced intracranial vasodilatation (increased MFV and reduced carotid PI). Interestingly, significant differences between the effects of different progestins on the cerebral vasculature were seen, a phenomenon not reported before. Although this was a small study and differences observed were modest, the results support the hypothesis that not all HRT preparations exert the same effects on the cerebral vasculature, and that choice of HRT preparation may influence cerebrovascular risk.

Acetazolamide is a potent vasodilatory stimulus, acting through both endothelium-dependent and endothelium-independent mechanisms. As such, a subtle effect of HRT administration upon cerebrovascular endothelial function has not been excluded by the lack of a significant difference in reactivity to acetazolamide between groups. One plausible explanation for the changes consistent with intracranial vasodilatation observed following HRT administration is improved cerebrovascular endothelial function, similar to the estrogen-mediated improvement in endothelial function reported in other vascular beds (Gilligan et al., 1994; Liebermanhard et al., 1994).

Systemic infusion of a more specific vasoactive agent such as the nitric oxide synthase inhibitor L-NMMA would allow the effect of HRT upon cerebrovascular nitric oxide bioavailability to be examined in more detail, and further work in this area is planned.

Conclusion
In this study, HRT induced subtle but consistent and readily demonstrable changes in intracranial haemodynamics, consistent with relaxation of intracranial resistance vessels. Our results suggest a differential effect of progestins on patterns of cerebral blood flow; these may in part explain the disparity between the apparent beneficial effects of HRT upon vascular surrogate end points and the disappointing results of large clinical trials. The results of this study suggest that differences exist between the effects of different HRT preparations on the cerebral vasculature.

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References

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