Prospective Randomized Study of the Effect of “Add-Back” Hormone Replacement on Vascular Function During Treatment With Gonadotropin-Releasing Hormone Agonists

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Background—Gonadotropin-releasing hormone agonists (GnRHAs) are a group of drugs that with long-term use induce a pseudomenopausal state in which estrogen production is suppressed. They are commonly used in the treatment of sex steroid–dependent conditions. “Add-back” hormone replacement therapy is used to prevent menopause-like symptoms and bone loss during GnRHa treatment, but it is also recognized that hypoestrogenism adversely affects vascular function. The aim of this study was to examine the effect of GnRHa and add-back therapy on vascular reactivity. This model serves as a paradigm for the effect of hormone replacement therapy in postmenopausal women.

Methods and Results—Measurements of endothelium-dependent and endothelium-independent vascular reactivity were compared in 2 groups of women treated with a GnRHa for 6 months. One group received estrogen/progestogen add-back therapy during the second 3 months of GnRHa treatment. Vascular reactivity was examined by use of ultrasound measurements of changes in brachial artery diameter. Endothelium-dependent changes were assessed during reactive hyperemia, whereas endothelium-independent changes were measured after the administration of glyceryl trinitrate sublingual spray. Treatment with the GnRHa alone had an inhibitory effect on endothelium-dependent relaxation. However, endothelium-dependent relaxation significantly improved in the group receiving add-back therapy (14.6%) compared with the group treated with GnRHa alone (8.6%) \( P<0.01 \). There were no significant endothelium-independent changes in either group.

Conclusions—These results suggest that the administration of add-back therapy has a protective effect on vascular function in GnRHa-induced hypoestrogenism. As a model for the menopause, these results also suggest that the long-term administration of hormone replacement therapy would result in endothelium-dependent arterial relaxation, an observation previously attributed only to the acute administration of estrogen. (Circulation. 1998;98:1631-1635.)

Key Words: hormones ▪ vasculature

The gonadotropin-releasing hormone agonists (GnRHAs) are analogues of GnRH that after prolonged exposure cause inhibition of gonadotropin secretion and hypoestrogenism. This effect has been used to advantage in the treatment of sex steroid–dependent conditions such as endometriosis and uterine fibroids. However, a number of disadvantages are associated with the long-term use of a GnRHa. These are related to hypoestrogenism and include bone loss, the development of vasomotor symptoms, and memory complaints. The problem of bone loss is especially important, because this has not always been shown to be reversible on withdrawal of treatment. To overcome these problems, low-dose “add-back” hormone replacement therapy (HRT) has been used during GnRHa treatment, and this has in most cases been found to prevent the unwanted side effects of treatment without interfering with its primary action. The side effects of GnRHa treatment are similar to those experienced by postmenopausal women. Estrogen deficiency is recognized to be a cause of vasomotor symptoms and bone loss, but postmenopausal women are also at increased risk of cardiovascular disease. This is partly due to the development of a more atherogenic lipid profile, but more recently, direct effects on the cardiovascular system have been identified as being more important contributors to the increase in risk.

Despite convincing evidence to show that the menopause increases the risk of cardiovascular disease, few studies have been undertaken on the effects of GnRHAs on the cardiovascular system. It remains unclear whether the administration of a GnRHa has a detrimental effect on vascular function, and if so, whether this effect is reversed with add-back HRT. The aim of this study was to compare differences in vascular reactivity between women using a GnRHa alone and women using a GnRHa in combination with add-back therapy. This
study design can be used as an in vivo model of the effect of HRT on vascular reactivity in postmenopausal women.

Methods

Patients

A prospective, randomized, controlled study was performed in the Prince of Wales Hospital in Hong Kong between January 1996 and July 1997. Fifty women with a laparoscopic diagnosis of endometriosis that had been made within 3 months of their entry into the study were recruited. The decision regarding their need for treatment with a GnRHa had been made by medical staff who were not involved in the study. All subjects were Hong Kong Chinese women in the reproductive age range. All had been shown to be ovulatory before the laparoscopy on the basis of biphasic basal body temperature charts or luteal-phase progesterone assays. The study period was of 9 months' duration. A schematic diagram of the study design is shown in Figure 1. Subjects were randomly allocated to 1 of 2 treatment groups. Randomization was performed with block randomization and a random table, with 25 subjects being randomized to each group. Subjects in group 1 received a GnRHa alone for 6 continuous months. Subjects in group 2 were also given a GnRHa for 6 continuous months, but in addition, they received add-back therapy during the second 3 months of GnRHa treatment. The GnRHa was administered by monthly injection of 3.75 mg leuprolerin acetate IM (Enanotide SR, Takeda Chemical Industries Ltd). The add-back was in the form of 2 mg/d of estradiol and 1 mg/d of norethisterone acetate (Kliogest, Novo Nordisk). The progestogenic component of the types of preparations is necessary to protect the endometrium of the uterus against estrogen-induced hyperplasia. This combination of estradiol and norethisterone acetate has previously been shown to be an effective method of providing add-back for women using GnRHa, but the same combination is more commonly prescribed as HRT for postmenopausal women. Compliance with add-back therapy was assessed by direct questioning and by inspection of used packages of the drug.

To determine the effect of add-back therapy on vascular function, blood flow studies were performed before, during, and after the completion of treatment with a GnRHa. This study was approved by the Ethics Committee of the Chinese University of Hong Kong. Vascular reactivity was examined by use of measurements of endothelium-dependent and endothelium-independent relaxation of the brachial artery as described by Celermajer et al.11

Measurements of Vascular Response

The peripheral vascular response was studied with a color duplex Doppler ultrasound (Aloka S-680 with a steered 7.5-MHz peripheral vascular ultrasound probe (Aloka UST-5518-7.5). All vascular response studies were performed by the same investigator, who had no knowledge regarding the treatment of individual subjects. To provide a stable environment in which to conduct these studies, all subjects fasted for at least 2 hours before each examination. These were performed in a quiet room with constant light and a room temperature between 21°C and 22°C. Subjects rested in a supine position for 15 minutes before measurements were made.

Endothelium-dependent and -independent changes in vascular reactivity were assessed by measurement of changes in the diameter of the brachial artery. The right side was chosen for the convenience of the operator, with a previous study of a similar nature having established that there were no significant differences between the right and left sides.12 All measurements were made over the brachial artery, where a mark was made on the skin with a marker pencil at each examination to minimize error related to movement during the examinations. The artery was scanned in the longitudinal plane, and the depth of focus was set at the center of the artery. To provide more accurate measurements of changes in arterial diameter than would have been possible with the calipers on the ultrasound monitor, all of the examinations were recorded with scan magnification onto s-VHS tapes, as shown in Figure 2, and studied retrospectively. A video frame grabber was used to capture individual frames into digitized images, each with a resolution of 768 x 640 pixels. A software digital micrometer was designed to measure between fixed reference points on these images. The number of pixels between a 10-mm vertical gradation scale on a single captured image was determined and subsequently used to transform pixel distance measurements into real distances. The brachial artery diameter was assessed during each phase of the experiment by determining the perpendicular distance (in millimeters) between the inner and outer edges of the arterial wall on each of the digitized images. The precision of measurement was determined by taking 5 repeated measurements in 5 subjects over a period of 10 minutes. ANOVA found the between-measurement variance to be 0.02 mm, with confidence limits of ±0.24 mm. These measurements also showed that repeated ultrasound examinations did not affect the diameter of the artery.

Endothelium-dependent relaxation was assessed by measurement of the change in diameter of the artery occurring as a result of reactive hyperemia. A resting measurement was taken, and then a pneumatic cuff was inflated to a pressure of 200 mm Hg for 4 minutes. The diameter of the artery was recorded again 45 to 60 seconds after cuff deflation. Fifteen minutes was then allowed for recovery before testing for endothelium-independent relaxation. A repeat baseline measurement of the diameter of the artery was made before a 400-μg dose of sublingual glyceryl trinitrate spray (GTN) was administered (Nitrolingual spray, Pohl-Boskamp GmbH). The brachial artery diameter was then measured 3 to 4 minutes after the GTN had been given.

Statistical Considerations

The results were analyzed with the Statistical Package for Social Sciences. The Wilcoxon matched-pairs signed-rank test was used to compare the means of changes in brachial artery diameter within the study groups. The Mann-Whitney U test was used for comparison between groups. Because many external factors affect the resting measurement of the diameter of the artery, statistical analysis between groups was performed by comparing changes in the diameter of the artery rather than by comparing measurements of the diameter at the resting stage and after intervention. Nonparametric
therapy. In group 2, there was a 14.6% increase in the diameter of the artery in response to the release of the cuff, compared with an 8.6% increase in group 1 ($P<0.01$). Three months after all treatment was completed, the measurements did not differ significantly from those taken before the study commenced. Within group 1, there was a significant reduction in the mean endothelium-dependent change in arterial diameter during GnRHa treatment ($P<0.01$ at both 3 and 6 months), but after completion of treatment, the mean change did not differ significantly from the value taken before the study commenced ($P=0.40$). Within group 2, there was a significant reduction in the mean endothelium-dependent change in diameter after 3 months on the GnRHa alone ($P<0.05$), but with add-back therapy, the change was no different from the value taken before the commencement of treatment ($P=0.26$).

Table 3 shows the endothelium-independent changes in brachial artery diameter during the study period. There were no significant differences in the mean endothelium-independent changes in arterial diameter between the 2 groups. In addition, there were also no significant differences in the mean endothelium-independent change in arterial diameter within each group.

**Discussion**

Hypoestrogenism resulting from the menopause predisposes women to an increased risk of osteoporosis and cardiovascular disease.9,14 The prolonged use of a GnRHa induces a reversible menopausal state, and women using GnRHAs have been shown to experience symptoms similar to those of postmenopausal women and also to suffer bone loss. In a situation analogous to that of postmenopausal women using HRT, the administration of add-back therapy to women using a GnRHa has been shown to reduce symptoms of hypoestrogenism and to protect against bone loss. This appears to occur

**TABLE 1. Mean Baseline Brachial Artery Diameters Before, During, and After Treatment**

<table>
<thead>
<tr>
<th>Months After GnRHa</th>
<th>Group 1 (n=21)</th>
<th>Group 2 (n=20)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.21 (3.07–3.37)*</td>
<td>3.49 (3.00–3.87)</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>3.60 (3.12–4.04)†</td>
<td>3.54 (3.12–3.90)</td>
<td>0.07</td>
</tr>
<tr>
<td>6</td>
<td>3.43 (3.26–3.90)‡</td>
<td>3.44 (3.14–3.60)</td>
<td>0.49</td>
</tr>
<tr>
<td>9</td>
<td>3.34 (2.89–3.79)</td>
<td>3.54 (3.18–3.85)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Values are median (IQR) (mm). IQR indicates interquartile ratio.

Group 1 received GnRHa alone from 0 to 6 months.

Group 2 received GnRHa from 0 to 6 months and also add-back HRT from 4 to 6 months.

* vs †, * vs ‡; $P<0.01$.

**TABLE 2. Mean Endothelium-Dependent Changes in Arterial Diameter**

<table>
<thead>
<tr>
<th>Months After GnRHa</th>
<th>Group 1 (n=21)</th>
<th>Group 2 (n=20)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.55 (0.37–0.74)*</td>
<td>0.45 (0.36–0.60)$§$</td>
<td>0.34</td>
</tr>
<tr>
<td>3</td>
<td>0.26 (0.21–0.43)†</td>
<td>0.30 (0.22–0.37)$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.30 (0.19–0.44)‡</td>
<td>0.39 (0.37–0.99)</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>9</td>
<td>0.56 (0.12–0.92)</td>
<td>0.48 (0.22–0.65)</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Values and groups as in Table 1.

* vs †, * vs ‡; $P<0.01$; § vs ||, $P<0.05$.

**TABLE 3. Mean Endothelium-Independent Changes in Brachial Artery Diameter**

<table>
<thead>
<tr>
<th>Months After GnRHa</th>
<th>Group 1 (n=21)</th>
<th>Group 2 (n=20)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.53 (0.22–0.72)</td>
<td>0.49 (0.32–0.89)</td>
<td>0.39</td>
</tr>
<tr>
<td>3</td>
<td>0.49 (0.24–0.93)</td>
<td>0.54 (0.30–0.86)</td>
<td>0.86</td>
</tr>
<tr>
<td>6</td>
<td>0.44 (0.22–0.80)</td>
<td>0.49 (0.27–0.77)</td>
<td>0.66</td>
</tr>
<tr>
<td>9</td>
<td>0.39 (0.16–0.57)</td>
<td>0.43 (0.28–0.66)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Values and groups as in Table 1.

No significant differences in values within groups.

The sample size was calculated on the basis of a previous study by Anderson et al13 that showed a 13.8% increase in brachial artery diameter in normal subjects in response to reactive hyperemia compared with a 7.2% increase in those with endothelial dysfunction. With a minimum of 5 patients in each arm of our study, such a sample size should be able to detect a difference between 2 groups with $\alpha$ of 0.05 and power of 80%. However, to provide greater statistical power and to allow for study dropouts, 50 women were recruited.

**Results**

The mean age of the subjects in group 1 was 34.2±5.8 years, whereas in group 2, the mean age was 35.1±6.7 years ($P=0.70$). During the study period, 9 patients defaulted from treatment, 4 from group 1 and 5 from group 2. Two of these moved away from Hong Kong, and 2 others wished to stop treatment early to attempt to conceive. The remainder were lost to follow-up and could not be contacted. The results of these subjects were excluded from the final data analysis. All subjects who completed the study remained amenorrheic during treatment except for 5 subjects who received add-back therapy, who reported at least 1 episode of vaginal spotting. The spotting did not result in dropout from the study or failure to comply with treatment.

Table 1 compares the mean baseline brachial artery diameters before, during, and after treatment between the 2 groups. There were no significant differences in the baseline diameters between the groups throughout the study. Within group 1, there was a significant increase in the resting diameter after treatment ($P<0.01$ at both time intervals), but the value 3 months after the completion of treatment did not differ significantly from the pretreatment value ($P=0.25$). In group 2, there were no significant changes in baseline arterial diameter throughout the study period.

Table 2 compares the mean endothelium-dependent changes in arterial diameter between the 2 groups. The mean change in diameter after the reactive hyperemia was consistently found to be greatest 1 minute after the release of the tourniquet. There was no significant difference between the means of the change in diameter between the 2 groups after 3 months of GnRHa treatment, but after the addition of the add-back therapy, the increase in diameter in the artery was significantly greater in the group that received add-back therapy. In group 2, there was a 14.6% increase in the diameter of the artery in response to the release of the cuff, compared with an 8.6% increase in group 1 ($P<0.01$). Three months after all treatment was completed, the measurements did not differ significantly from those taken before the study commenced. Within group 1, there was a significant reduction in the mean endothelium-dependent change in arterial diameter during GnRHa treatment ($P<0.01$ at both 3 and 6 months), but after completion of treatment, the mean change did not differ significantly from the value taken before the study commenced ($P=0.40$). Within group 2, there was a significant reduction in the mean endothelium-dependent change in diameter after 3 months on the GnRHa alone ($P<0.05$), but with add-back therapy, the change was no different from the value taken before the commencement of treatment ($P=0.26$).

Table 3 shows the endothelium-independent changes in brachial artery diameter during the study period. There were no significant differences in the mean endothelium-independent changes in arterial diameter between the 2 groups. In addition, there were also no significant differences in the mean endothelium-independent change in arterial diameter within each group.
within a regimen of add-back as was prescribed in our study. None of these demonstrated a difference in pain scoring during add-back therapy, and for this reason, a pain score was not used in our study.

Few studies have investigated the effects of GnRHAs on cardiovascular risk. Most of these have examined changes in the lipid profile, but these changes have been of small magnitude and did not appear to persist after withdrawal of the treatment.

There is accumulating evidence to show that the direct effect of estrogen on blood flow may play a far more important role than changes in the lipid profile. Estradiol has been shown to have an acute effect on vascular resistance, resulting in an increase in blood flow through both endothelium-dependent (via nitric oxide) and -independent (smooth muscle) mechanisms. Arterial relaxation through an endothelium-dependent mechanism has been demonstrated with the acute administration of estrogen, but long-term treatment is thought to alter vascular function through other actions, like those of a calcium antagonist, or by the inhibition of atheroma formation.

To the best of our knowledge, there has been only 1 study on the effect of GnRHa administration on cardiac function in young women. In this study, 15 women underwent Doppler echocardiography during GnRHa treatment, and significant reductions in peak flow velocity and cardiac index were demonstrated. There was also a nonsignificant reduction in flow velocity and a significant decrease in mean acceleration. It was concluded that the hypoestrogenism associated with this treatment had a direct effect on cardiovascular performance. One other study examined changes in the resistance index of the uterine arteries of women with uterine fibroids treated with a GnRHa. After >4 months of this treatment, there was a significant increase in vascular resistance, and this increase correlated closely with the reduction in uterine size during treatment. The only study in which the use of a GnRHa was found to have no effect on blood flow was that reported by Penotti et al. In that study, a 6-month course of treatment was found to produce no significant changes in the pulsatility index in the internal carotid and middle cerebral arteries, and it was concluded that because the resistance of these arteries was unaltered by treatment, they must be under extraestrogenic control.

Our study investigated whether a 6-month course of treatment was associated with changes in vascular function in an endothelium-dependent or -independent fashion. Of note, over the duration of the study period, the resting diameter of the brachial artery tended to change in both groups, as shown in Table 1. Many external factors can affect the resting diameter of the artery, and we believe that these may have contributed to the observed differences. This emphasized the importance of studying changes in diameter of the artery rather than comparing measurements of the diameter at the resting stage and after intervention.

Within the group that received no add-back therapy (group 1), there was a significant reduction in endothelium-dependent arterial relaxation during treatment with the GnRHa, but 3 months after discontinuation of treatment, values returned to baseline. In those who received add-back therapy, however, the inhibition of relaxation observed with the GnRHa alone was removed, suggesting a positive effect of add-back therapy on endothelium-dependent relaxation. In this group, values also returned to baseline after the discontinuation of treatment.

Significant changes in endothelium-independent vascular function were not found in this study, although endothelium-independent mechanisms are thought to account for most of the cardioprotective effect of estrogen in the chronic situation. There were no significant differences in brachial artery diameter within the groups, nor were there significant differences between groups. These results suggest that the effects of GnRHAs and add-back therapy are dependent on the endothelium and are presumably mediated via nitric oxide in a chronic situation.

Although it might be expected that the progestogenic component of the add-back therapy could interfere with the favorable effect of estrogen on vascular function, this aspect of therapy was not investigated in our study. Some studies in humans have found that the addition of a progestogen to estrogen therapy attenuates the improvement in vascular reactivity attributable to estrogen. This has been demonstrated in studies using the progestogens norgestrel and norethindrone. However, 1 report on the effect of medroxy-progesterone acetate on the pulsatility index suggested that this progestogen did not interfere with the beneficial effect of estrogen. To have investigated the contribution of the progestogen in our study, it would have been necessary to compare standard add-back therapy (in which both estrogen and a progestogen are used) with estrogen alone. In our study, however, the aim was to examine the effect of standard add-back therapy on vascular reactivity.

The results of our study are of clinical significance not only to women using GnRHAs but also for postmenopausal women using HRT. As far as GnRHas are concerned, treatment for a 6-month period appears to interfere with endothelium-dependent relaxation, but the administration of hormonal add-back therapy negates this adverse effect of treatment. As a clinical model for the effect of HRT on vascular function, these results more importantly suggest that the same advantages would be offered to postmenopausal women using this or other similar treatments. These are important findings, as previous studies have shown an endothelium-dependent effect of estrogen only during acute rather than chronic administration. The results of this study provide an additional explanation for the cardioprotective effect of HRT in postmenopausal women.

References


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