Estrogen Levels Do Not Correlate With Improvement in Cognition

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Objective: To investigate whether an association exists between estradiol and estrone levels and measures of cognitive functioning in women with Alzheimer disease (AD) treated with conjugated equine estrogen (Premarin; Wyeth-Ayerst, Philadelphia, Pa).

Methods: We studied 120 postmenopausal women who underwent hysterectomy and who had AD treated with Premarin for 1 year. Plasma estradiol and estrone levels were determined at multiple points during the 1-year treatment trial. The change from baseline level at 2 and 12 months was associated with the change score on 7 different assessments of cognitive functioning.

Results: At baseline, estradiol levels were low and there were no associations between the estradiol level and the 7 neuropsychological measures. A similar pattern was observed for estrone treatment. During treatment with 0.625 mg/d of Premarin, estradiol levels increased about 4-fold; while receiving 1.25 mg/d of Premarin, estradiol levels increased about 8-fold. A similar pattern was seen with estrone treatment. For both estradiol and estrone levels, there were no significant associations between the change in plasma level and the change in neuropsychological test scores at either 2 or 12 months.

Conclusion: Although Premarin elevated estradiol and estrone levels, there was no association between hormone levels and cognitive functioning after either 2 or 12 months of treatment.

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Alzheimer disease (AD) is the most common cause of dementia in the United States affecting approximately 4 million individuals. It occurs with higher frequency in women. Considerable evidence has emerged from epidemiological and preclinical studies suggesting that orally administered estrogens may be beneficial in improving cognition and mood in AD. This evidence includes up-regulation of choline acetyltransferase activity by estrogens, colocalization of estrogen and nerve growth factor receptors on cholinergic neurons, and improvement in neuropsychological functioning in postmenopausal women taking estrogens. However, 3 recent randomized, controlled clinical trials of conjugated equine estrogens (Premarin; Wyeth-Ayerst, Philadelphia, Pa) have failed to substantiate this claim. In contrast, 2 small controlled clinical trials using an estrogen patch reported improvement in attention and verbal memory on selected tasks although improvement was not seen on global cognitive or functional scales. In addition, plasma estradiol levels in a very small subset of patients in studies reported by Ashtana et al correlated with delayed recall, a measure of memory.

We previously reported on the rate of decline over a 1-year period in a relatively large multicenter trial examining the effects of Premarin in postmenopausal women who had AD. Although the overall results of this trial show no significant cognitive or global effects of conjugated equine estrogens over placebo treatment, women treated with Premarin did demonstrate increased plasma estradiol levels. It is possible that women with higher levels of circulating estrogens might have responded and that this response was masked in the overall group analyses. We report herein on a further analysis correlating the change in plasma estradiol levels with the change in a variety of global measures of cognition as well as with specific tests of memory, attention, and language to determine whether increasing circulating estradiol levels predicted response. These analyses were then repeated using estrone, the major estrogenic compound in Premarin. Results of the plasma estra-
diol and estrone levels from this multicenter study have not been previously reported.

**METHODS**

The clinical trial from which plasma estradiol levels were assayed was a 1-year study in women with AD treated with conjugated equine estrogens and no progestin. One hundred twenty women who underwent hysterectomy (to allow for exposure to estrogen therapy without the need for a progestin) were recruited into a multicenter clinical trial comparing the dose effects of placebo (n = 39), 0.625 mg/d of Premarin (n = 42), and 1.25 mg/d of Premarin (n = 39). Informed consent was obtained from the patient or the patient’s caregiver acting as proxy for the patient, according to local internal review board guidelines. The study participants had baseline Mini-Mental State Examination (MMSE) scores between 12 and 28 and, therefore, represented subjects with mild to moderate AD. The study medication was randomized and double-blinded with 12 months of estrogen exposure followed by a 3-month, single-blind washout period. The primary outcome measure was the Alzheimer’s Disease Cooperative Study Clinical Global Impression of Change. In addition, a range of secondary outcome measures were obtained including the MMSE, Alzheimer’s Disease Assessment Scale—Cognitive (ADAS-Cog), Clinical Dementia Rating (CDR) Scale, Hamilton Depression Rating Scale, Emotional Face Recognition Test (Elizabeth Koss, PhD, written communication, June 3, 1998), New Dot Test, Letter Cancellation test, Trail Making Test, Digit Symbol Test, Category Fluency test, Letter Fluency test, Grooved Pegboard Test, Finger Tapping Test, Blessed Dementia Rating Scale (activities of daily living), and the dependent scale. The relationship between a change in hormone levels and the change in 7 selected neuropsychological measures was explored in this analysis.

A plasma sample was collected at the screening; baseline; and months 2, 6, 9, 12, and 15 visits; they were assayed for estradiol and estrone levels. The association between estradiol and estrone levels and neuropsychological measures was first examined at baseline. Using multiple linear regression models, we then examined the association between the change in estradiol level at 12 months and age, weight, and apolipoprotein E status with the change in the MMSE and ADAS-Cog scores, 2 global measures of cognition, with change in word recall and delayed word recall, from the ADAS-Cog to assess learning and memory, and with change in Letter Cancellation, Trails A, and Digit Symbol tests scores to evaluate parameters of attention and language. The analyses were then repeated for estrone.

**RESULTS**

At baseline, estradiol levels were low and equivalent in all 3 groups reflecting the postmenopausal status and the absence of estrogen supplementation. There were no significant associations between baseline estradiol levels and any of the 7 neuropsychological measures. An identical pattern was observed for estrone.

During treatment, patients receiving placebo maintained uniformly low levels of estradiol averaging approximately 5 pg/mL (18 pmol/L) (Figure). Individuals receiving 0.625 mg/d of Premarin had mean estradiol levels of approximately 20 pg/mL (73 pmol/L) and those receiving 1.25 mg/d of Premarin had levels averaging 35 to 40 pg/mL (128-147 pmol/L). All estradiol levels returned to pretreatment values by 3 months after discontinuing Premarin treatment. A similar pattern was seen with estrone with levels averaging approximately 175 pg/mL (648 pmol/L) while receiving 0.625 mg/d of Premarin and 350 pg/mL (1295 pmol/L) while receiving 1.25 mg/d of Premarin (data not shown).

Across all treated patients, levels of estradiol ranged from 0 to more than 100 pg/mL (0 to >367 pmol/L). This spread in estradiol levels allowed us to examine the change scores between cognitive measures across a wide range of plasma estradiol levels. There were no significant associations between the MMSE or ADAS-Cog change score and plasma estradiol change at either 2 or 12 months after initiating therapy. There was a significant negative association between change in delayed recall and change in estradiol level at 12 months but not at 2 months (Table). Further exploration of this relationship revealed that this association was largely dependent on 3 outliers in which large increases in plasma estradiol levels (55, 62, and 73 pg/mL [202, 227, and 268 pmol/L, respectively]) were associated with decreases in delayed word recall at 12 months (−4, −4, and −3 words, respectively). After removal of the 3 outliers, the association between estradiol level and delayed recall disappeared (P = .75). There were no significant associations between the change scores in estradiol level and word recall, Letter Cancellation, Trails A, or Letter Fluency test results at 2 or 12 months (Table). Repeating the analyses with estrone revealed an identical pattern with a significant negative association existing at 12 months only for the change in delayed recall and the change in estrone level (P = .05) that disappeared when the 3 outliers were removed (P = .88). In addition, there was a separate and independent main effect of apolipoprotein E on change in the delayed recall test results at 12 months for both estradiol and estrone that also disappeared when the 3 outliers were removed.

**COMMENT**

In this large, multicenter, randomized, controlled clinical trial, commonly used doses of Premarin elevated plasma estradiol levels approximately 4-fold at the low dose and approximately 8-fold at the higher dose, resulting in mean levels of approximately 20 pg/mL (73 pmol/L) while receiving 0.625 mg/d of Premarin and 40 pg/mL (147 pmol/L) while receiving 1.25 mg/d of Premarin.
Thus, a dose-dependent increase in estradiol levels was achieved. These levels are comparable to the levels reported by Fillit et al.\textsuperscript{26} in women with AD treated with estradiol but lower than the levels achieved by Asthana et al.\textsuperscript{9,10} using a transdermal estradiol patch where mean levels of 75 to 130 pg/mL (275–477 pmol/L) were obtained. Levels achieved in our study were also substantially higher than those reported in cognitively healthy older women residing in the community where levels of supplementation averaged 6 pg/mL (22 pmol/L).\textsuperscript{27}

The spread in estradiol levels across this group of 80 individuals exposed to Premarin therapy allowed us to assess the association between cognitive function and estradiol levels. The highest estradiol levels obtained were over 140 pg/mL (>514 pmol/L), providing a wide spread to examine the associations between various cognitive measures and estradiol levels. Unfortunately, change in estradiol levels did not predict change in global cognitive scores on the MMSE or ADAS-Cog nor did it predict change on other neuropsychological measures except for a weak negative association between change in estradiol and estrone levels and change in delayed recall test results that disappeared when 3 outliers were removed. An identical pattern was noted for estrone. Thus, these weak associations seem to be best explained by a few outliers that showed a large change on the delayed recall test results.

Five studies have recently been published reporting on results of randomized, controlled clinical trials of estrogens for patients with AD. The 3 largest trials all used Premarin, the most commonly used estrogenic preparation in the United States and the preparation reported to be associated with protection against the development of AD in virtually all epidemiological studies (see Hogervorst et al.\textsuperscript{28} for review). The largest trial randomized 120 women and it is from that trial that these estradiol and estrone levels are derived. Two additional trials enrolled 40 subjects\textsuperscript{8} and 50 subjects\textsuperscript{9} and randomized their subjects to 1.25 mg/d of Premarin for 3 to 4 months. Unlike our study,\textsuperscript{7} these subjects had not undergone hysterectomy. As in the present study, studies by Henderson et al.\textsuperscript{9} and Wang et al.\textsuperscript{8} failed to detect cognitive improvement. In contrast, 2 very small studies by Asthana et al.\textsuperscript{9,10} reported improvement on tests of attention and memory in 12 and 20 women, respectively, randomized to treatment with an estradiol patch delivering 0.05 mg/d or 0.1 mg/d of estradiol-17β. The lower-dose patch produced plasma estradiol levels reaching about 70 pg/mL (257 pmol/L) while the higher-dose patch resulted in estradiol levels of approximately 120 pg/mL (440 pmol/L). A correlation was found between the level of plasma estradiol and the test results of delayed cued recall in 6 of 12 subjects receiving 0.05 mg/d of Premarin.\textsuperscript{9} In the second study, using the higher-dose patch, improvements were noted in tasks of attention, total recall, and figure copying but not in global status, mood, or functional assessments.\textsuperscript{10} In our study, we enrolled many more subjects and attained plasma estradiol levels using Premarin that were as high as the levels reported by Asthana et al.\textsuperscript{9,10} We failed to observe a significant positive association between change in plasma estradiol levels and performance on a variety of cognitive measures. Comparability across trials is apparent in that 2 of the 3 larger studies\textsuperscript{6,8} included patients who did not undergo hysterectomy and our study\textsuperscript{7} attained plasma estradiol levels over 140 pg/mL (>514 pmol/L), consistent with the findings of Asthana et al.\textsuperscript{9,10} Thus, we conclude that the results obtained by Asthana et al are most likely secondary to the use of small sample sizes and the presence of a few outliers.

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\textbf{Abbreviations: ADAS-Cog, Alzheimer’s Disease Assessment Scale—Cognitive; MMSE, Mini-Mental State Examination.}
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REFERENCES