**Estrogen and Non-Feminizing Estrogen for Alzheimer’s Disease**

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**Introduction**

ESTROGEN use by postmenopausal women has many health benefits, such as prevention of osteoporosis and arteriosclerosis. In addition to these benefits, recent studies have suggested that estrogen may be useful for the treatment and prevention or delay of the onset of Alzheimer’s disease (AD). The preventive effect of estrogen on AD has become clearer with many epidemiological reports. However, the therapeutic effects of estrogen have been controversial until now.

Excessive precipitation of amyloid and loss of neurons are histological features of AD. Production of amyloid precursor protein (APP) is increased by injury to or inflammation of the brain. In AD patients, astrocytes have poor ability to remove the precipitation of amyloid. Amyloid β (Aβ), which is broken up from APP by β-secretase, is the main component of the precipitation. Apolipoprotein E (apo E) is a well-known factor for coagulating soluble Aβ and accelerates the precipitation of amyloid. The mechanism by which Aβ induces neurons to cell death is considered to be as follows. Aβ increases the number of calcium pores on the cell membrane and accelerates calcium inflow to the cell. Activation of nitro-oxygen synthetase follows the increase of intracellular calcium and induces intracellular peroxidation [1]. The serum apo E level is markedly suppressed with estrogen therapy [2]. Apo E has been reported to induce precipitation of amyloid dose-dependently [3]. Thus, the decrease in apo E level may reduce the precipitation of amyloid. However, it is still unclear how estrogen affects the production of apo E in the human brain. Estrogen regulates the metabolism of APP and decreases Aβ broken up from APP. Estrogen probably activates α-secretase preferentially to β-secretase [4]. Also estrogen has been reported to have antioxidant effects [5]. Estrogen is useful for prevention of AD due to both decrease of amyloid precipitation and antioxidant effects.

Many basic studies have shown several beneficial effects of estrogen to maintain, protect and repair neuronal functions. Estrogen is considered to 1) improve the depressive status [6], 2) increase the brain blood flow [7], 3) stimulate the cholinergic neurons [8–10], and 4) increase the number of developed glial cells [11]. The prevention of arteriosclerosis is considered to be one of the important effects of estrogen to protect the brain, because AD and vascular dementia of the small vessel type frequently show overlap both in clinical symptoms and histological findings [12]. An apo E isoform apo E4 is also considered to be an important risk factor of both AD and arteriosclerosis [13].

In this report, we summarize the reports about AD and estrogen and add our new basic data of non-feminizing estrogen, J 861.

1) Preventive effect of estrogen

The preventive effect of estrogen on Alzheimer’s disease (AD) has been established by epidemiological data [14–19]. Table 1 shows the epidemiological studies reported until now. In all the case-control studies, except that of Brenner et al. [15], it was denied that estrogen had a preventive effect. Paganini-Hill et al. [16] reported that the risk decreased significantly with both increasing dose and duration of estrogen therapy. In cohort studies, the relative risk was 0.40 by Tang et al. [18], 0.46 by Kawas et al. [19]. Tang et al.
[18] reported that the age at onset of AD was significantly later in women who had taken estrogen than in those who did not.

2) Therapeutic effect of estrogen

Many clinical studies have supported the therapeutic effect of estrogen on AD, since Fillit et al. [20] first reported an open trial of estradiol-17β (E₂) therapy for AD (Table 2) [20–32]. However, the therapeutic effect was not established. The number of patients was small, the design was not a case-control study, and the method of assessment was too simple in most of these studies. Recently the results of randomized, double-blind, placebo-control trials including a large number of women with mild to moderate AD, have been reported. The therapeutic effect of estrogen was denied in these studies. However a few questions can be

### Table 1. Preventive effect of estrogen on AD

<table>
<thead>
<tr>
<th>Reference</th>
<th>AD patients (estrogen users)</th>
<th>Control (estrogen users)</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paganin-Hill, 1994 Am J Epidemiol[14]</td>
<td>136 (51)</td>
<td>545 (252)</td>
<td>0.69</td>
</tr>
<tr>
<td>Paganin-Hill, 1996 Arch Intern Med[16]</td>
<td>246 (96)</td>
<td>1139 (578)</td>
<td></td>
</tr>
<tr>
<td>Duration of HRT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–14 years (25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;15 years (17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waring, 1999 Neurology[17]</td>
<td>222 (8)</td>
<td>222 (19)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

### Table 2. Therapeutic effects of estrogen on AD

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>AD patients</th>
<th>administration (mg/day)</th>
<th>duration</th>
<th>estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fillit et al.[20]</td>
<td>open trial</td>
<td>7</td>
<td>Estradiol 2 mg</td>
<td>6 weeks</td>
<td>improved</td>
</tr>
<tr>
<td>Honjo et al.[21]</td>
<td>open trial</td>
<td>7</td>
<td>CEE 1.25 mg</td>
<td>6 weeks</td>
<td>improved</td>
</tr>
<tr>
<td>Honjo et al.[22]</td>
<td>double-blind</td>
<td>7</td>
<td>CEE 1.25 mg</td>
<td>3 weeks</td>
<td>improved</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohkura et al.[23]</td>
<td>open trial</td>
<td>15</td>
<td>CEE 1.25 mg</td>
<td>6 weeks</td>
<td>improved</td>
</tr>
<tr>
<td>Ohkura et al.[24]</td>
<td>open trial</td>
<td>10</td>
<td>CEE 0.625 mg</td>
<td>5 months</td>
<td>improved</td>
</tr>
<tr>
<td>Fillit[25]</td>
<td>double-blind</td>
<td>4</td>
<td>Estradiol transdermal</td>
<td>3 months</td>
<td>not changed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohkura et al.[26]</td>
<td>open trial</td>
<td>7</td>
<td>CEE 0.625 mg</td>
<td>Over 5 months</td>
<td>improved</td>
</tr>
<tr>
<td>Birge[27]</td>
<td>double-blind</td>
<td>10</td>
<td>CEE 0.625 mg</td>
<td>9 months</td>
<td>improved</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Henderson et al.[28]</td>
<td>observational</td>
<td>9</td>
<td>CEE 0.625 or 1.25 mg</td>
<td>variable</td>
<td>improved</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>untreated</td>
<td></td>
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<tr>
<td>Asthana et al.[29]</td>
<td>double-blind</td>
<td>6</td>
<td>Estradiol transdermal</td>
<td>8 weeks</td>
<td>improved</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mulnard et al.[30]</td>
<td>double-blind</td>
<td>42</td>
<td>CEE 0.625 mg</td>
<td>12 months</td>
<td>not changed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CEE 1.25 mg</td>
<td></td>
<td>not changed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>placebo</td>
<td></td>
<td>not changed</td>
</tr>
<tr>
<td>Wang et al.[31]</td>
<td>double-blind</td>
<td>25</td>
<td>CEE 1.25 mg</td>
<td>12 weeks</td>
<td>not changed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>placebo</td>
<td></td>
<td>not changed</td>
</tr>
<tr>
<td>Henderson et al.[32]</td>
<td>double-blind</td>
<td>18</td>
<td>CEE 1.25 mg</td>
<td>16 weeks</td>
<td>not changed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>placebo</td>
<td></td>
<td>not changed</td>
</tr>
</tbody>
</table>
pointed out in these studies [30–32]. The estrogen treatment (ERT) groups already had a high serum E\textsubscript{2} level as baseline, the average of which was 50 pg/ml [30]. Estrogen was given continuously in these studies. Hagino [33] pointed out that in aged animals, cessation of cyclic changes in circulating estrogen suppressed the activity of the hippocampus, and this might lead to the initiation of AD. In the clinic, cyclic estrogen treatment might be better. More patients in the ERT groups took donepezil during the course of the trial compared with the placebo group. Donepezil is a specific non-competitive reversible inhibitor of acetylcholinesterase (AchE). Donepezil and estrogen treatment may interfere with each other because estrogen promotes the development of AchE-positive neurons [22].

Our first trial showed that administration of high-dosage estrogen (1.25 mg/day of conjugated equine estrogen [CEE]) for 6 weeks improved cognitive functions in women with mild to moderate AD [21]. Furthermore a double-blind study was conducted in women with mild to moderate AD and the therapeutic effect of high-dosage estrogen was confirmed [22]. We administered low-dosage estrogen (0.625 mg/day of CEE) to women with mild to moderate AD. One cycle of ERT consisted of CEE 0.625 mg/day for 3 weeks and no drugs for 1 week. We observed the therapeutic effect over 6 cycles. Improvement of cognitive function was recognized during the third week from the beginning of the trial and maintained as long as ERT was continued (Fig. 1). Ohkura et al. [24] also reported the long-term beneficial effects of cyclic ERT in women with AD. In our studies patients with ERT showed a better response to registration and recall of a few easy words, questions of common knowledge, and orientation in space. Spontaneity, emotion, and social interaction were improved. In detail, there was marked improvement in willingness to talk, expressionless look, bad mood, rebellious attitude toward guardians, and defect of attention to guardians. It became easier for their guardians and nurses to care for them [34].

3) Non-feminizing estrogen J 861

CEE often used for HRT mainly consists of estrone sulfate and equilin sulfate, but also includes other estrogen sulfates. One of them 8, (9)-dehydroestrone comprises 10% of CEE, and has a neurotrophic effect. Estradiol-17\textalpha{} and E\textsubscript{2} have an antioxidant effect. J 861 is a derivative of estradiol-17\textalpha{} and is one of the 8,(9)-dehydro estrogens. The chemical structure of J 861 is 14\textalpha{}, 15\textalpha{}-Methylenenestra-1,3,5,(10),8-tetraene-3, 17\textbeta{}-diol as in shown Fig. 2 [35]. From its structure, J 861 was expected to have both antioxidant and neurotrophic effects. The neurotrophic effect of J 861 was stronger than that of 8,(9)-dehydroestriol and the antioxidant effect of J 861 was stronger than that of E\textsubscript{2} [36, 37]. J 861 had considerably less effect on the uterus, breasts, seminal glands and prostate than E\textsubscript{2}.

4) Neuro-protective effect of E\textsubscript{2} and J 861

i) Effects of E\textsubscript{2} and J 861 on intracellular calcium, peroxidation and apoptosis induced by amyloid β

Rat pheochromocytoma PC12 cells were induced and differentiated to the neurotype by incubation for 6 days in RPMI 1640 medium containing 100 ng/ml nerve growth factor (NGF). Differentiated PC12 cells were treated with various concentrations (10^{-12}, 10^{-10}, 10^{-8} M) of E\textsubscript{2} or J 861 for 3 days before the exposure to Aβ which consisted of a cytotoxic sequence between amino acid residues 25 to 35. To examine the interaction with estrogen receptor (ER), ICI 182780 (blockade of estrogen receptor) was added to each culture. After preincubation, the supernatant was re-
newed with the medium containing Aβ at the concentration of 10 μM. The intracellular calcium concentrations in each culture were measured by flow cytometry with 5 μM of Fluo-3-AM. We determined the quantity of the peroxidation by measuring the fluorescence intensity with 100 μM of 2',7'-dichlorofluorescein diacetate (DCF-DA). Statistical analysis was performed by one-way ANOVA and post hoc multiple comparison by Fisher's PLSD test.

A flow cytometric analysis using Fluo-3-AM revealed that exposure to Aβ increased the intracellular calcium concentration. Preincubation with E2 or J 861 for 3 days prevented the increase of intracellular calcium concentration dose-dependently. The inhibitory effect of J 861 was stronger than that of E2. Those inhibitory effects were attenuated by the administration of ICI 182780.

The quantity of peroxidation also increased after exposure to Aβ. These increases were prevented in the cultures preincubated with E2 or J 861 for 3 days. The inhibitory effect of J 861 was also stronger than that of E2. Referring to the change of ER antagonist, pure anti-estrogen ICI 182780 significantly attenuated the effect of E2 and J 861.

E2 and J 861 has been suggested to prevent both the intracellular calcium increase and peroxidation induced by Aβ by directly and via an ER-mediated system. The physiological level of E2 is considered to have a sufficient antioxidant effect. The anti-oxidant effect of J 861 is probably stronger than that of E2.

**ii) Effects of E2 and J 861 were compared on cholinergic neurons in substantia innominata (SI).**

Wistar female rats were divided into three groups: ovariectomized, E2-treated and J 861 treated groups. After oophorectomy, rats in the E2-treated group were given 5 μg of E2 daily for 2 weeks by subcutaneous injection and the rats in the J 861-treated group were given 5 μg/day of J 861 just like E2. The ABC (avitin-biotin complex) method was used to stain choline acetyltransferase (ChAT). The number of positive ChAT-immunoreactive cells and morphological changes were compared among the three groups. The ovariectomized group had fewer positive ChAT-immunoreactive cells, fewer dendrites and shorter axons than either the E2 or J 861 group. J 861 showed neuroprotective and neurotrophic effects similar to E2.

**5) Effects of E2 and J 861 on vascular factors**

**Effects of E2 and J 861 on adhesion of monocyte to endothelium under physiological flow conditions in vitro**

Human umbilical venous endothelial (HUVE) cells were cultured on gelatin and fibronectin-coated glass slides. Various reagents such as E2, J 861, α-tocopherol were added at 10^{-7} M–10^{-5} M. HUVE cells were incubated for 24 hours, then stimulated with 20 U/ml of interleukin-1 (IL-1) for 4 hours. U937 cells derived from human myeloma in HBSS (10^5 cells/ml) were passed through the flow chamber at 1.0 dyne/cm^2 of shear stress for 7 min. The number of adherent U937 cells was counted in 20 microscopic fields by analyzing the recorded videotape. The number of adherent U937 cells under each condition was compared with that under the condition without any reagents, stimulated with IL-1 alone for 4 hours. E2 (10^{-7} M ) did not inhibit adhesion of monocytes. About a 25% decrease was recognized by addition of 10^{-5} M of E2.

J 861 and α-tocopherol suppressed adhesion of monocytes dose-dependently. About a 40% decrease was recognized in the presence of 10^{-5} M of J 861 and 10^{-5} M of α-tocopherol. The concentration of 20 U/ml is the same serum level of IL-1 as that detected endogenously under shock status. The shear stress of 1.0 dyne/cm^2 is the same stress exerted on the venous wall in the slowest blood flow. This model was similar to the physiological blood flow conditions. E2 probably did not suppress adhesion of monocytes at a physiological serum level. α-Tocopherol is considered to suppress adhesion of monocytes sufficiently in a physiological serum level. J 861 suppressed adhesion of monocytes just like α-tocopherol.

E2, J 861 and α-tocopherol may inhibit adhesion of...
monocytes by suppressing the expression of adhesive factors (E-selectin and intercellular cell adhesion molecule-1 [ICAM-1]) on endothelium of vessels. In our study, these reagents suppressed the expression of adhesive factors induced by stimulation of IL-1. Like α-tocopherol. J 861 suppressed the expression of adhesive factors induced by stimulation of IL-1. Suppression was stronger by J 861 and α-tocopherol than that by $E_2$. Blockade of ER did not change the suppression by α-tocopherol, but reduced the suppression by J 861. J 861 is considered to suppress the expression of these adhesive factors directly and partially with the help of ER. ERβ has been recognized in HUVE cells. These reagents were suggested to suppress arteriosclerosis in the first process by inhibiting adhesion of monocytes to the vascular endothelium.

**Conclusion**

The effects of HRT on the health of women are dependent both on the dose and duration of the treatment. J 861, a non-feminizing estrogen derivative, showed neuroprotective effects and may be promising for the prevention and treatment of AD not only in women but also in men.

**Acknowledgements**

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**Summary**

The preventive effect of estrogen on Alzheimer’s disease (AD) has become clearer with many epidemiological reports. However, the therapeutic effects of estrogen have been controversial until now. In our trials, estrogen treatment showed a beneficial therapeutic effect for women with mild to moderate AD. Improvement of cognitive function was recognized during the third week from the beginning of administration and maintained as long as estrogen treatment continued. The longer the duration of HRT, the more HRT is useful for the prevention and therapy of AD. However, in most cases, administration of estrogen is discontinued because of the adverse effects on the uterus and breast. J 861 is a derivative of estradiol-17α, which has little effect on the sexual organs. The effects of estradiol-17β ($E_2$) and J 861 on neuronal function and vascular factors were investigated. J 861 was suggested to prevent both the intracellular calcium increase and peroxidation induced by amyloid β (Aβ), more effectively than $E_2$. The effect of J 861 may be related with both the direct non-genomic and the ER-mediated systems. J 861 showed neurotrophic effects like $E_2$. J 861 inhibited the adhesion of monocytes to vascular endothelium, more effectively than $E_2$. Also, J 861 suppressed the expression of adhesive factors, such as E-selectin and intercellular cell adhesion molecule-1 (ICAM-1), more effectively than $E_2$.

**References**


