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Neuroprotective effects of female sex steroids in humans: current controversies and future directions

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Abstract. Recent findings from the Women's Health Initiative (WHI) have raised considerable concern over prolonged use of opposed and unopposed oral conjugated equine estrogen (CEE), given the increased risk of serious adverse effects, including stroke and venous thromboembolic complications. Furthermore, results from the WHI Memory Study (WHIMS) indicated that over 5 years of therapy with Prempro impaired performance on global cognitive tests and nearly doubled the risk of dementia. These surprising findings were contradictory to cumulative evidence from basic science, epidemiological and some intervention studies suggesting hormone therapy was cardioprotective and could potentially reduce the risk of dementia. This review paper focuses on the neurobiology of estrogen, summarizing the clinical evidence for neuroprotective and cognition-enhancing efficacy of estrogen. Further, the paper briefly discusses variables that may account for the unexpected findings of WHIMS, and offers suggestions for future research.

Key words. Estrogen; estrogen replacement therapy; hormone replacement therapy; cognition; Alzheimer's disease; postmenopausal cognition; Women's Health Initiative; Prempro and Premarin.

Introduction

There is convincing evidence from basic neuroscience research that estrogen exerts significant neuromodulatory and neurotrophic effects on the brain. Although controversial, findings from a number of clinical studies suggest that estrogen can enhance cognitive function for both healthy older women and those with Alzheimer's disease (AD). However, recent findings from the Women's Health Initiative (WHI) and the Women's Health Initiative Memory Study (WHIMS) raised serious concerns about the safety of extended treatment and risk for cognitive decline and dementia with opposed oral conjugated equine estrogen (Prempro) for older healthy postmenopausal women. Likewise, findings from the estrogen-only arm of WHI raised concerns about the safety of Premarin. Specifically, findings from the WHI indicated that over 5 years of therapy with Prempro increased risk for coronary heart disease, stroke, venous thromboembolic complications and breast cancer [1, 2]. Likewise, extended therapy with Premarin increased risk of stroke [3]. Additionally, data from WHIMS indicated that administration of Prempro for over 5 years adversely affected performance on a global measure of cognition [4] and doubled the risk of dementia [5] for older women.

Given that Prempro and Premarin are the most commonly prescribed forms of hormone replacement therapy (HRT) [6], the WHI findings were pivotal in guiding the utility of HRT for primary prevention of chronic diseases. However, it is critical that the findings of WHI and WHIMS should not be generalized to all forms and routes of administration of estrogen and progestins. The conjugated equine estrogens (CEEs) fail to replicate premenopausal hormone profiles in several ways. For example, the major ingredient in both Prempro and Premarin is estrone and several other unidentified hormones [7, 8], while 17β-estradiol is the predominant hormone in menstruating women [9]. Further, premenopausal hormones are released cyclically during the menstrual cycle, while hormone replacement was administered continuously in WHI and WHIMS. Thus, as stressed by the authors, it would be a serious error to generalize the findings of the WHI to all forms of HRT and assume bioequivalence of Prempro and Premarin to other formulations of HRT,

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which might more accurately replicate a premenopausal hormonal milieu.

Despite the mistrust of estrogen in the general public, many questions remain unanswered concerning the therapeutic potential of estrogen, especially those related to its neurobiology [10]. Behind the scientific inquiry lies convincing evidence from both basic science and epidemiological research demonstrating that estrogen exerts multiple beneficial neuroprotective and neuromodulatory effects (see McEwen [11], Sherwin [12], Zec and Trivedi [13] and Cholerton et al. [14] for reviews) A brief summary of recent basic science and epidemiological evidence is provided here before focusing on findings from clinical research trials, examining the therapeutic potential of various forms of estrogen to address cognitive changes associated with healthy aging and disease processes, such as AD. We close with a discussion of future directions for clinical research on estrogen and cognition. Indeed, with many questions still unanswered, clinical research investigating the relationship between estrogen and cognition warrants greater attention, as opposed to halting future estrogen research.

Neurobiology of estrogen

Estrogen exerts its biological actions through estrogen receptors (ERs) distributed selectively throughout the brain, including the hippocampus, amygdala, cerebral cortex, midbrain, brainstem, pituitary gland, basal forebrain, preoptic area and hypothalamus [15–19]. Of note, several of these brain regions, including the hippocampus, are afflicted by AD pathology and mediate various cognitive functions, including memory. To date, two ER subtypes have been identified, the ERα and ERβ. Of these, the identification of ERβ [20] has led to an intense inquiry of estrogen's differential effects on brain substrata [21–23]. Both the estrogen receptor subtypes colocalize with receptors for antiapoptotic proteins, neurotrophins and neurotransmitters that are vital for learning and memory [20, 24, 25], as well as disease-modifying processes such as anti-inflammatory mechanisms [26] and apolipoprotein E expression [27, 28]. There is strong evidence to suggest that in addition to receptor-mediated effects, estrogen exerts various neuromodulatory and neuroprotective effects that are independent of its actions through the receptor binding sites (reviewed in Simpkins et al., this series).

Estrogen's cognition-enhancing effects: basic science evidence

There is well-documented and compelling evidence to support estrogen's neurobiological role in cognitive processes (see McEwen [11] for review). The recognition of estrogen's numerous genomic and nongenomic actions in the brain suggests a possible deleterious effect of chronic estrogen deprivation, both in healthy aging and disease processes. The dramatic decrease in estrogen levels occurring at menopause could theoretically result in neuronal dysregulation and loss of protective actions, and perhaps account for women's increased risk for dementia [29–31].

Estrogen's favorable neuromodulatory effects

It appears that estrogen may modulate several neurobiological processes underlying cognition. For example, estrogen appears to play a key role in the storage of explicit memories in the hippocampal formation and entorhinal cortex by augmenting the molecular changes associated with long-term potentiation (LTP) [32–35], perhaps via the ERβ in particular [21]. Estrogen also may alter cerebrovascular factors affecting cognition. In particular, estrogen may protect against ischemic injury and improve neuronal health by modulating vasodilation and improving cerebral blood flow [36–41].

Finally, there is cumulative scientific evidence portraying the facilitative role of estrogen on various neurotransmitter systems (see van Amelsvoort et al. [42] and Yaffe [43] for reviews). Among others, neurotransmitters influenced by estrogen include acetylcholine, serotonin and the catecholamines (dopamine, epinephrine and norepinephrine), all of which have been implicated in modulation of the cognitive processes. Of particular importance is the cholinergic system, a system integrally involved in several cognitive processes, including attention, learning, memory and arousal. In patients with AD, notable and early deficits are found in the cholinergic system, and the currently approved treatments for the disease are medications designed to enhance cholinergic function. Basic research suggests that estrogen facilitates the actions of the cholinergic system [44–50]. As noted earlier, estrogen appears also to influence neuronal communication through the serotoninergic (5-HT) [45, 51–53], dopaminergic [54–57], and noradrenergic and adrenergic [58–61] neurotransmitter systems. Estrogen appears to have complex and differential effects on these neurotransmitters, and there is increasing evidence that the important actions of estrogen occur in the interaction between various neurotransmitter systems [62, 63].

Estrogen's neuroprotective effects

In addition to the potential cognition-enhancing effects, there is a strong neurobiological basis supporting multiple neuroprotective and neurotrophic effects of estrogen. By facilitating the antiapoptotic actions of the Bcl proteins, estrogen may protect neurons from toxin-induced and ischemic brain injury [64–66]. Also, estrogen may interact with the regulation of the apolipoproteins, in particular apolipoprotein E (apoE) ε4 allele, which has been

strongly linked with familial and sporadic AD. While not universally supportive [67], some evidence suggests an upregulation of the apoE $\varepsilon 4$ allele in the presence of estrogen [27, 68, 69].

Estrogen also appears to act independently as an antioxidant, protecting against cell damage and death (see Chiueh et al. [70], Behl et al. [71] and Dhandapani and Brann [72] for reviews). A number of estrogenic compounds have been shown to protect cultured cells against damage induced by free radicals [73], inhibiting lipid peroxidation [74]. Furthermore, estrogen appears to play an antioxidant role with a number of cellular toxins, including β-amyloid (Aβ) Estrogen's role in the protection against Aβ is perhaps most noteworthy regarding the potential prevention and treatment of AD. A hallmark of AD pathology is the presence of abnormally high levels of insoluble and toxic Aβ, a peptide generated from amyloid precursor protein (AβPP) [75]. Physiological levels of estrogen have been implicated in the modification of deleterious actions of Aβ through several potential mechanisms, including increasing soluble, nontoxic forms of Aβ [76–78], increasing clearance of toxic forms of Aβ [79] and decreasing the inflammatory reaction to Aβ [80, 81].

In addition to protection from cell death via apoptosis, in vitro investigations support a salutary effect of estrogen on the activity of various neurotrophins, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [82–85]. Furthermore, estrogens may enhance synaptogenesis and dendritic spine density, perhaps most importantly in the hippocampal CA1 neuronal field [86–89] and the prefrontal cortex [90], areas specifically targeted in AD.

Finally, other nongenomic actions of estrogen have been proposed as a means through which the hormone can offer neuroprotection. High concentrations of the amino acid homocysteine have been associated with dementia, and cardiovascular disease, premature atherosclerosis and thrombosis [91]. There is evidence to suggest that estrogen may lower homocysteine levels [92–96], thereby reducing a person's risk for dementia. Finally, estrogen's neuroprotective actions may in part be associated with its ability to improve high and low density lipoprotein profiles and postprandial lipid metabolism [97–100].

Estrogen's cognition-enhancing effects: evidence from epidemiological and cohort investigations

Prior to the publication of the WHI data, the notion that estrogen replacement therapies may reduce a woman's risk for dementia was widely accepted. This was due in part to the large body of basic science and the generally supportive epidemiological evidence, which suggested as much as a 50% reduction in the risk for AD with postmenopausal hormone use (see Miller et al. [101], Hogervorst et al. [102] and Fillit [103] for reviews). In contrast, the surprising findings from WHIMS indicated an increased risk of dementia and cognitive decline with prolonged administration of Prempro [5, 104].

Findings from cohort studies were attributed to the inherently flawed nature of epidemiological research, such as the 'healthy-user' bias. Still, observational data continue to support the possible role of estrogen and hormone replacement therapy in the prevention of dementia (e.g. Zandi et al. [105], LeBlanc et al. [106], Maki et al. [107] and Smith et al. [108]). Bhavnani has argued that the WHI was not a true primary prevention study given the advanced age and prevalence of obesity among its participants, and the likelihood that many were beyond the opportunity to 'prevent' most of the outcome variables [7]. Still, many in the scientific community agree that while the risk of dementia associated with Prempro has been clarified, numerous questions remain unanswered regarding the use of estrogen or other forms of HRT for the primary prevention of dementia [10, 109].

Clinical trial evidence of estrogen's neurocognitive effects

While neurobiological evidence strongly supports multiple salutary effects of estrogen in the brain, clinical research involving cognitively healthy postmenopausal women and those with AD has produced inconsistent results. Several methodological problems preclude conclusive statements regarding estrogen's effect on cognition. Such problems include heterogeneity of methodology, in particular the choice of HRT, lack of rigorous methodological and statistical control, failure to assess the multiple cognitive domains generally affected by aging and AD, use of insensitive or global neuropsychological tests, and failure to assess compliance. In the following sections, we will briefly review clinical trials investigating the cognitive effects of estrogen and hormone therapies in groups of cognitively healthy and demented postmenopausal women, and then offer a discussion of the discrepant findings.

Neurocognitive effects of estrogen interventions: healthy aging

There is a vast body of research examining the neurocognitive effects of estrogen therapies on cognition in healthy aging, for both primary prevention of disease and treatment of age-associative cognitive declines. Extensive reviews of this literature are offered elsewhere (e.g. Wise et al. [110], Resnick and Maki [111], Cholerton et al. [14], Hogervorst et al. [102], Yaffe et al. [112, 113] and Sherwin [12].) Table 1 lists and briefly summarizes results of these trials.

Table 1. Trials investigating cognitive benefits of estrogen therapies for cognitively healthy postmenopausal women.

Year	Investigator(s)	Estrogen	Outcomes	
1952	Caldwell & Watson [114]	estradiol benzoate	improvement in learning and memory	benefit
1975	Rauramo et al. [115]	estradiol valerate	no improvement with treatment on tests of memory or reaction time	no benefit
1976	Vanhulle & Demol [116]	estriol	no improvement on intelligence test, tests of attention and concentration or speeded processing	no benefit
1976	Hackman & Galbraith [117]	piperazone sulphate	treatment improved score on memory test	benefit
1977	Fedor-Freyburgh [118]	17β-estradiol	improvement on reaction time, visual attention, selective attention, and memory tests	benefit
1977	Campbell & Whitehead [119]	CEEs	improved memory with treatment	benefit
1988	Sherwin [120]	estradiol valerate	treatment improved verbal memory and abstract verbal reasoning	benefit
1989	Honjo et al. [121]	CEEs	treatment group improved on screening tests for	benefit
1990	Sherwin & Phillips [122]	estradiol valerate	treatment improved verbal memory	benefit
1991	Ditkoff et al. [123]	CEEs	no improvement on treatment on tests of learning, and auditory and visual attention	no benefit
1992	Phillips & Sherwin [124]	estradiol valerate	treatment improved verbal memory	benefit
1998	Polo-Kantola et al. [125]	17β-estradiol	no differences between treatment and placebo	no benefit
1999	Hogervorst et al. [102]	17β-estradiol	trend for improved verbal memory on treatment	benefit
1999	Shaywitz et al. [126]	CEEs	no change on cognitive measures, but improved brain activation with treatment	benefit & benefit & no benefit
1999	Wolf et al. [127]	17β-estradiol	No group differences but correlation between estrogen level and cognitive performance on verbal memory measures	benefit
2000	Duka et al. [128]	17β-estradiol	treatment improved memory and nonverbal reasoning	benefit
2000	Janowsky et al. [129]	CEEs	No improvement on working memory with treatment	no benefit
2000	Rudolph et al. [130]	estradiol valerate	treatment benefited subjective well-being and to a lesser extent concentration and attention	benefit
2001	Linzmayer et al. [131]	estradiol valerate w/ or w/o dienogest	both treatments improved verbal and visual memory and information processing speed	benefit
2001	Binder et al. [132]	CEEs	no benefit with treatment on comprehensive battery of neuropsychological tests	no benefit
2002	Grady et al. [133]	CEEs	no benefit with treatment on battery of neuropsychological tests for women with coronary heart disease	no benefit
2003	Saletu [134]	estradiol valerate	treatment benefited sleep and selected cognitive abilities	benefit
2003	Woo et al. [135]	CEEs	estrogen treatment improved attention, delayed recall and score on global cognitive function	benefit
2003	Shaywitz et al. [136]	CEEs	CEEs improved reading ability and verbal memory	benefit
2003	Pan et al. [137]	CEEs	nonsignificant trend for benefit on global cognitive measures	benefit
2003	Kugaya et al. [138]	17β-estradiol	improvements in verbal fluency and divided attention, and prefrontal serotonergic binding as measured with PET	benefit
2003	WHIMS: Rapp, Espeland, Shumaker et al. [4] & Shumaker et al. [5]	CEEs	clinically important decline on Modified Mini-Mental and twice the risk for dementia for healthy postmeno pausal women on Prempro	harmful

As is evident from table 1, the majority of trials investigating the neurocognitive effects of HRT found benefits associated with estrogen therapy. Interestingly, it appears that trials utilizing estradiol therapies were more likely to demonstrate enhanced cognition, whereas investigations employing a CEE are contradictory. Only the WHIMS trial suggested a possible harmful effect of opposed CEE treatment on cognition.

As noted earlier, several reviews have been published summarizing findings from trials investigating the effects HRT on cognition in nondemented women. Given the extensive body of research, others have offered meta-analyses of the research. LeBlanc and colleagues [106] concluded from their meta-analysis of nine controlled clinical trials and eight cohort studies that only women experiencing postmenopausal symptoms benefited cog-

nitively from HRT, and only in select cognitive domains. Specifically, women symptomatic for menopause showed improvements in the areas of memory, vigilance, motor speed and reasoning. The controlled clinical trials reviewed by LeBlanc et al. tended to be short in duration (3 months or less); only one-third of these trials employed CEEs. On the other hand, most of the women included in the cohort trials were using CEEs for several years. Systematic reviews by Hogervorst et al. [102, 139] demonstrated evidence for neurocognitive benefits with estradiol for young postmenopausal women (mean age of 47), primarily in the areas of speeded accuracy, associative learning and abstract reasoning. These authors found no such benefits associated with Premarin. Finally, in a review of 42 estrogen trials, Zec and Trevedi [13] found that women on HRT outperformed women on placebo on nearly half of the memory measures; in no instance did the women on placebo surpass women on treatment. The evidence, while far from conclusive, appears to support neurocognitive enhancement with HRT, particularly with estradiol treatments.

Of note, there is a great deal of heterogeneity between investigations enrolling nondemented postmenopausal women regarding the preparation of HRT used, the dose, the duration of treatment and the concomitant use of progestins. Furthermore, there is a relative paucity of data from older postmenopausal women (>69 years of age)

Neurocognitive effects of estrogen interventions: Alzheimer's disease and dementia

The data supporting a beneficial effect on cognition for healthy older women prompted scientists to investigate the potential neurocognitive effects of estrogen for diseases. Indeed, HRT for Turner syndrome, a disease resulting from a complete or partial absence of one X chromosome in females that is characterized by short stature, impaired sexual development, infertility and neurocognitive deficits, resulted in verbal and nonverbal memory improvements for a sample of young patients [140]. Schizophrenic women with a higher endogenous estrogen level outperformed women with a lower level on neuropsychological measures [141]. Likewise, postmenopausal women with Down syndrome with higher endogenous levels of estrogen performed better on verbal memory tasks when compared to patients with lower levels of circulating estrogens [142]; furthermore, restoration of estrogen has been shown to ameliorate cognitive declines associated with AD in an animal model of Down syndrome [143]. These findings seem to support further study of the potential of estrogen to ameliorate disease-associated cognitive declines.

Table 2 lists the few human trials examining the therapeutic potential of estrogen in AD. Of the few controlled clinical trials, only three small studies [149, 153, 155] have supported cognitive benefits of estrogen in women with AD. Other larger clinical trials employing CEEs have not supported a therapeutic role of estrogen for women with AD [150–152]. With only this limited number of published studies, notably different in methodol-

Table 2. Trials investigating cognitive benefits of estrogen therapies for postmenopausal women with dementia.

Year	Investigator(s)	Estrogen preparation	Outcomes	
1986	Fillit et al. [144]	estradiol	improvement noted for 3 of 7 women on tests of orientation and attention	benefit
1994	Okura et al. [145]	CEEs	improvement on measure of global cognitive functioning with 5 months of treatment	benefit
1994	Okura et al. [146]	CEEs	improvement on global cognitive measures over 6 weeks of treatment	benefit
1995	Okura et al. [147]	CEEs	improvement in 4 out of 7 patients	benefit
1996	Schneider et al. [148]	several forms of ERT	women on ERT showed more benefit with tacrine than did women not on ERT	benefit
1999	Asthana et al. [149]	17β-estradiol	treatment group improved on measure of selective attention and verbal memory	benefit
2000	Wang et al. [150]	CEEs	no differences between groups on global measures	no benefit
2000	Henderson et al. [151]	CEEs	no differences between groups on clinician or caregiver impression of change measures	no benefit
2000	Mulnard et al. [152]	CEEs	no differences between treatment and placebo	no benefit
2001	Asthana et al. [153]	17β-estradiol	improvements on measures of verbal and visual memory and selective attention for women on estrogen	benefit
2003	Yoon et al. [154]	CEEs	opposed and unopposed CEE showed similar improvements in cognition and mood as that of Tacrine, but ERT superior to Tacrine in treating deficits in IADLs	benefit

ogy, conclusions regarding estrogen's therapeutic potential in AD would be premature. Several variables remain unexplored, in particular the differential effects of the various hormone preparations and doses, as well as the effect of concomitant progestin therapy, hysterectomy status and prior HRT use. Furthermore, estrogen may have little effect on global cognitive status, but still exhibit selective cognitive benefits when more sensitive, domain-specific cognitive outcomes are utilized.

The well-designed and important trials examining the neurocognitive effects of CEEs cannot be ignored. But, again, these findings should not be generalized to all forms of estrogen, an unfortunate consequence of which would be the failure to examine a viable treatment option for a devastating disease.

Understanding disparate findings

Psychopharmacology of estrogen replacement therapies

HRT preparation

Estimated life expectancy now predicts that women will spend as much as 40% of their lives in a postmenopausal state [156]. A postmenopausal woman's hormone profile differs from her premenopausal state in a number of ways. Furthermore, this change in hormone status occurs rapidly, as opposed to the more gradual changes noted in men. These facts may account for the increased risk of dementia for women [29–31]. Likewise, a premenopausal woman's hormonal profile is highly variable over the course of her menstrual cycle, as well as during prenatal and postpartum states. The most commonly used form of hormone therapies, oral Prempro and Premarin do not mimic premenopausal hormone profile or cycles. Rather, these therapies elevate steady-state levels of the hormone estrone and nine other estrogens [7, 8], and, in the case of Prempro, a synthetic form of progesterone, medroxyprogesterone acetate.

Prior to menopause, the predominant circulating estrogen is ovarian-produced estradiol, while after menopause the primary source of estradiol is peripheral conversion of androstenedione [9]. Estradiol has been reported to be a very potent naturally occurring estrogen, while estrone and estriol are reported to have lower binding affinities, and thus may 'fail to induce the full array of conformational changes induced by estradiol' [9]. The dramatic decrease in circulating estradiol and subsequent increase in gonadotropin hormones is theorized to account for the shift in a women's risk for cardiac disease and osteoporosis, and possibly for her increased risk of dementia [29–31]. Indeed, trials using estradiol preparations more often demonstrated cognitive benefits than did investigations utilizing a CEE (see table 1.) Thus, in order to enhance receptor-dependent cognitive actions of estrogen and to more closely mimic the premenopausal state, estradiol may be a more appropriate replacement strategy.

Cyclical variability

As noted, Prempro and Premarin do not accurately replicate premenopausal variations in hormone levels. There is some evidence from studies in rhesus monkeys to suggest cognitive benefits associated with cyclical administration of estradiol as opposed to steady-state levels [157]. However, no direct comparisons of these two forms of administration have been conducted to date.

Progestins

In order to protect against the potentially harmful effects of estrogen on uterine tissue, non-hysterectomized women must use a progestin therapy in combination with estrogen. It has been speculated that progesterone may interfere with the beneficial cognitive effects of estrogen by exerting sedative [158, 159] and detrimental mood effects [159]. On the other hand, progesterone may have important effects in the brain. Progesterone is known to regulate synaptic density in the hippocampus, and appears to work in conjunction with estrogen across the reproductive cycle [160]. Initially, progesterone augments the effects of estradiol, but then results in a more rapid decrease in synaptic spines [161]. However, it is unknown whether synthetic forms of progestins such as medroxyprogesterone act in the same manner as the endogenous hormone. While one epidemiological study found synthetic progesterone to be related to decreased performance on a gross cognitive measure [162], and another found that women using opposed estrogen actually had a greater rate of cognitive decline [163], Graham and Glasser [164] showed that levels of natural pregnanediol (a by-product of progesterone) was related to enhanced perceptual and working memory functioning.

Bioavailability

Investigations that included measurements of serum levels of estrogen suggest that levels of non-protein-bound or bioavailable forms of estrogen may be related to cognitive functioning. For example, patients with AD were found to have higher levels of sex hormone-binding globulin (SHBG), thereby a reduced level of free estrogen and testosterone, than their age-matched healthy controls [165]. While not universally supported [112, 166], high serum estradiol and bioavailable estradiol levels have been associated with improved delayed verbal memory and retrieval [167, 168]. These findings imply that it is important to verify that a hormone replacement therapy actually raises levels of the more potent and bioavailable forms of estrogen. This may be especially important given the fact that there may be individual variability in bioavailable estrogen levels, even with standard doses [169].

Route of administration

It is speculated that opposed CEE-induced cerebrovascular changes might underlie increased risk of women for dementia described by WHIMS data [170]. Preliminary findings from some recent studies suggest that, unlike oral conjugated estrogen, transdermal estradiol enhances cognitive function for postmenopausal women with AD [149, 153]. This may be related to the absence of venous thromboembolic complications [171] and plasma markers of inflammation [172] associated with transdermal estrogen administration. It may be that not only is the form of HRT influential, but the route of administration is equally critical.

History of treatment: critical period

Numerous cohort studies have depicted the beneficial cognitive effects associated with current or past history of HRT (see list above). For example, HRT use has been associated with superior verbal memory [107, 173] nonverbal memory [108], attention/executive function [108, 174, 175] and visuomotor performance [175]. On the other hand, others have found contradictory evidence, suggesting no benefit in cognitive function with HRT use (e.g. Buckwalter et al. [176]).

It may be that the timing of HRT contributes to the likelihood of benefit. In other words, there may be a critical period around menopause during which HRTs exert neuroprotective actions [111]. Therapy occurring or continuing beyond such a time frame would have little effect upon cognition and brain health. There is some neurobiological evidence to support this notion. Estrogen treatment initiated 3 months after hysterectomy was associated with an improved memory performance for aged rats; however, treatment started 10 months after surgery offered no such benefit [177]. Likewise, Verghese et al. [178] examined a small sample of women and found that HRT was associated with long-term cognitive benefits only when initiated shortly after surgical menopause. Further, there is evidence that chronic estradiol treatment does not enhance hippocampal LTP in rats [179] and that long-term estrogen treatment in women with AD may not continue to produce beneficial cognitive effects [102].

Life-time exposure to endogenous estrogens

Related to the 'critical period' hypothesis is the notion that variables associated with a woman''s reproductive cycle are associated with both risk and protection for cognitive function [107]. For example, a history of surgical menopause might confer greater risk for cognitive declines, based on the sudden and drastic changes in hormone levels resulting from surgery, as opposed to natural menopause. There is evidence to suggest that hysterectomized women may perform more poorly on neuropsychological tasks than women experiencing physiological menopause [180]. These cognitive declines were more evident if the surgery occurred at a younger age and if more time had elapsed since surgery. Considered from another perspective, this would seem to suggest that a reduced exposure to endogenous hormones could increase a women's risk for cognitive decline. Indeed, McLay et al. [181] offered evidence that age of menopause and nulliparity were positively associated with preserved cognitive function, again supporting the importance of endogenous hormones in protecting cognition.

Testosterone

Both men and women experience declines in testosterone levels with age. Current therapeutic options include a combination of estrogen and testosterone. Growing evidence suggests that testosterone also may play a role in cognitive functioning and neuroprotection (see Bates et al., this series) For example, neuronal cultures treated with testosterone were found to secrete less Aβ40 and 42 peptides [182] and that this may be mediated via changes in gonadotropin receptor expression [182a]. Barrett-Connor and Goodman-Gruen [166] found that serum levels of testosterone were related to better performance on a global test of cognitive function. Additionally, testosterone supplementation has been found to enhance cognitive performance for both men [183–186] and women [187].

Future research directions

Future research could both clarify questions concerning current HRTs as well as explore estrogen alternatives. As yet, it is unknown whether estrogen alternatives will have the same neurobiological and neurocognitive effects as estrogen. Therefore, future clinical research could compare the cognitive effects of multiple preparations of estrogen replacement therapy in healthy postmenopausal women and those with AD. Such trials should verify serum estradiol and non-protein-bound forms of estrogen, relating these variables to cognitive performance. Likewise, it is vital to further clarify the clinical effects of different forms of opposed treatment, including natural progesterones and a variety of synthetic progestagens for women with intact uteri. Clinical studies need to systematically address any differences that may occur in cognitive functioning as a result of a woman's reproductive and HRT history. Specifically, her history of HRT use, such as when therapy was initiated and the duration of treatment, her hysterectomy status, menopausal variables (age of menopause, and surgical versus physiological) and other events that influence endogenous hormone exposure, such as the number of children a woman delivered and

other events associated with drastic hormonal fluctuations.

A major drawback to HRT using CEE and medroxyprogesterone is its negative side-effect profile and the increased risk for developing breast and uterine cancers. Selective estrogen-receptor modulators (SERMs) may offer an alternative to estrogenic HRTs. One SERM, raloxifene, has been shown to prevent bone loss without negative effects associated with estrogen therapy, such as increased triglyceride levels [188], vaginal bleeding, endometrial hyperplasia, endometrial cancer [189], or breast cancer [190, 191]. The differential side-effect profile is a result of the SERMS estrogenic and anti-estogenic actions. For example, while mimicking estrogen's positive effects on bone health, SERMS have been found to increase the frequency of hot flashes in postmenopausal women by blocking estrogenic actions. Such anti-estrogenic effects may indeed negatively impact cognitive function, either directly by action in the CNS or indirectly due to decreased feelings of well-being associated with peripheral antagonist actions. Recently, Yaffe, et al. [192] found that cognitive function was not improved in postmenopausal women who took raloxifene, although they report a trend toward slower decline in both verbal memory and attention over the course of three years. In addition, they report no relationship between hot flashes and test performance. The potential for developing SERMS that act directly in the CNS and thereby possibly improving cognitive function in women with AD makes SERMS a valuable research direction. See Yaffe [43] and Dhandapani and Brann [72] for reviews). If raloxifene and potentially other SERMs have similar beneficial cognitive effects, this may offer an alternative to women who are at a high risk for developing breast or uterine cancer.

Phytoestrogens: isoflavones

With recent reports of the negative consequences of widely used forms of HRTs, many women are turning to the so-called natural or plant estrogens. Isoflavones, a class of phytoestrogens, may offer healthcare consumers an alternative to traditional HRT. Isoflavones offer several major therapeutic advantages over estrogen. Among others, these advantages include potential cardioprotective efficacy [193–195], beneficial effects on bone mineral density [196–200] and lipid profile [201–203], and an impressive safety profile with no clinical evidence for venous thromboembolic complications, cerebrovascular events or cancer-inducing properties [204, 205]. There is evidence to suggest that isoflavones exhibit SERM-like effects on the brain and likely possess both cognition-enhancing and neurotrophic properties. However, the evidence is preliminary and precludes us from drawing any major conclusions concerning the cognition-enhancing efficacy of isoflavones [206]. The few human research projects that have examined the potential beneficial effects of soy isoflavones on cognition offer intriguing support for isoflavones' efficacy to enhance cognition in healthy older adults (e.g. File et al. [207], Duffy et al. [208] and Kritz-Silverstein et al. [209]). Still, the small number of trials investigating this potential therapeutic prevents us from drawing firm conclusions.

Conclusions

The WHI and WHIMS trials provided invaluable information regarding the use of CEEs for primary prevention of several age-associated diseases, including dementia. The surprising findings that the widely used CEEs Premarin and Prempro exhibited detrimental neurocognitive effects, however, contradicted a vast body of basic science and clinical research evidence. The impact of this important and well-designed trial is enormous in terms of its influence on the use of hormonal treatment in postmenopausal women. Still, further inquiry directed toward understanding the basis of these disparate results is critically important. For example, if hormone therapies could adequately mimic a premenopausal state, women might retain the health benefits associated with premenopausal status without conferring the risks associated with CEEs. In doing so we may be able to maximize the cognitive health of older women without added risks.

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