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Evaluating the Role of Hormone Therapy in Postmenopausal Women with Alzheimer's Disease

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Running head: Hormone therapy and Alzheimer's disease

Abstract

Hormone therapy (HT) is prescribed during or after menopausal transition to replace the decline in estrogen and progesterone levels. While some studies indicate that estrogen and progesterone depletion in postmenopausal women might carry a significant risk for developing sporadic Alzheimer's disease (sAD), which may be reduced by HT, recent clinical trials oppose this beneficial effect. This review points to possible reasons for these mixed data by considering the issues of both preclinical and clinical trials, in particular the representativeness of animal models, timing of HT initiation, type of HT (different types of estrogen compound, estrogen monotherapy versus estrogen- progesterone combined therapy), mode of drug delivery (subcutaneous, transdermal, oral or intramuscular) and hormone dosage used, as well as the heterogeneity of the postmenopausal population in clinical trials (particularly considering their sAD stage, anti-AD therapy and hysterectomy status). Careful planning of future preclinical and clinical HT interventional studies might help to elucidate the effect of HT on cognitive status in postmenopausal women with sAD, which will eventually contribute to more effective sAD prevention and treatment.

Key points:

• The influence of hormone therapy (HT) on cognition in postmenopausal women with Alzheimer's disease (AD) is inconclusive mainly due to a translational gap based on inadequate animal models, clinical inter-/intra-group heterogeneity and often incomparable HT study design.

• Cognitive outcomes in clinical trials are mostly influenced by HT composition, its dose, timing and route of administration, as well as by ApoE carrier status, co-morbidity and concomitant therapy.

•Design of estrogen/progesterone modulators that would optimize cognitive benefits and tailored HT may lead to more successful prevention and treatment of AD in postmenopausal women.

1 Introduction

Hormone therapy (HT) is prescribed during or after menopausal transition to replace the decline in estrogen and progesterone levels to help women deal with menopausal symptoms. The post-menopausal period represents a distinctly different state of sex hormone homeostasis in which the main circulating estrogen is estrone. Estrone is less potent than 17β-estradiol (“estradiol” in further text) and due to insufficient estrogen activity such a condition consequently leads to manifestation of menopausal symptoms like flushing, mood disorders, osteoporosis, etc. [1], and is accompanied by a decrement in progesterone concentration [2-5]. This hormonal change has shown potential to additionally modulate neural processes and pathology linked to sporadic Alzheimer’s disease (sAD) [2-5]. Some studies have shown that estrogen and progesterone depletion in postmenopausal women is a significant risk factor for development of sAD and that estrogen-based HT may reduce this risk [3, 6-10]; however, more recent data argue against this beneficial effect [11-13].

Considering the data from the basic research and epidemiological trials one could hypothesize that HT has a beneficial effect on cognition [3, 6-10,14] but a large, long-term double-blind randomized clinical trial known as the “Women’s Health Initiative Memory Study” (WHIMS) showed that in cognitively unimpaired women HT can increase the risk of cognitive decline [11, 12, 15,16]. Recently, a new clinical trial has emerged, “Kronos Early Estrogen Prevention Study” (KEEPS), whose sub-study “Cognitive and Affective Study” (KEEPS-Cog) reported that there is no beneficial effect of HT on cognition [13]. The reasons for such inconsistency in results are not clear and the question of whether HT has a beneficial or detrimental effect on cognition is still open.

Bearing in mind the importance of both preclinical and clinical trials in testing the therapeutic strategy for any disease, this review aims to analyse why there is such inconsistency between the data from preclinical and clinical studies on cognitive outcome of HT in sAD women. The review discusses the issues of representativeness of animal models, as well as adequate timing of HT initiation, type of drug treatment (type of estrogen compound, treatment with estrogen alone or in combination with progesterone), mode of drug delivery (subcutaneous, transdermal, oral or intramuscular) and hormone dosage used both in animals and humans.

2 Search methodology

Data from preclinical and clinical trials were collected by searching the PubMed/MEDLINE database from 1997 to August 2016 using the terms: estrogen, Alzheimer’s disease and cognition. Data on basic research covering the mechanism of estrogen and progesterone action were collected from both original scientific papers and reviews by using the terms: ‘estrogen’, ‘progesterone’, ‘receptor’, ‘mechanism of action’, and ‘brain’. Only articles published in English were considered

Clinical data had to fulfil the following criteria: (i) double-blind, randomized controlled clinical trials investigating the effect of ≥2months of HT on cognitive function in postmenopausal women with AD (N=9 studies), (ii) ≥2.5 year randomized control trials investigating the effect of HT on cognition in cognitively unimpaired women (N=4 studies), and (iii) meta-analysis of HT therapy in perimenopausal and postmenopausal women (N=4 articles). The reviews from the reference list of meta-analysis studies were used as an additional source of data.

The preclinical data search included in vivo experiments on middle-aged and aged female animals that were modelled to mimic human menopause and AD-like cognitive decline, and cognitively tested after a sex hormone treatment. Only experiments done in the line with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were included.

3 Estrogen and progesterone mode of action and their effects on cognition

3.1 Genomic effects

Progesterone and estrogens act through the classic genomic mechanism, which includes activation of respective nuclear receptors highly expressed in brain areas associated with cognition and emotional processing such as amygdale and the limbic system [17, 18]. There are two major isoforms of estrogen and progesterone nuclear receptors: estrogen receptor-α and -β (ERα, ERβ), and progesterone receptor A and B (PR-A, PR-B) [19, 20].

Estrogen binds with similar affinity to the ERα (Kd = 0.04 nM) and ERβ (Kd 0.11 nM), demonstrating similar potency (ED50 for ERα and ERβ is 0.017 nM and 0.068 nM, respectively) [21]. Activated ERα/β can either bind directly to their target DNA sequences in the nucleus or interact with other nuclear proteins to alter gene activation, and this genomic action occurs slowly (hours–days) [19].

Basic research has showed that administration of ligands specific for ERβ, but not for ERα, has enhancing effects on hippocampal learning and memory processes similar to that of estrogen [22]. These effects are attenuated when ERβ expression is knocked down in transgenic models [16]. In line with that Zhao et al showed that oral treatment with a phyto-selective estrogen receptor modulator (phyto-β-SERM), which shows 83-fold higher binding selectivity towards ERβ over ERα, increases gene expression of apolipoprotein E (ApoE) and decreases expression of amyloid precursor protein (APP) and glycogen synthase kinase-3beta (GSK-3β) in comparison to soya extract diet (having both ERα/β acting phytoestrogens) [23]. The same experiment demonstrated increased expression of the insulin degrading enzyme (IDE) gene in both phyto-β-SERM- and soya extract-treated groups in comparison with ovariectomized (OVX) controls [23].

On the other hand, ERα-knocked down mice exhibit impaired spatial memory, which has been improved by treatment with estradiol while ERβ-knock down mice have preserved cognition and estradiol treatment does not affect their memory [24, 25]. Additionally, activation of ERα is connected to amelioration of amyloid beta (Aβ)-induced glutamate excitotoxic injury [26]. These data suggest that it is likely that both ERβ/α contribute to neuroprotection against age- and AD-related changes but possibly through activation of transcription of different genes.

Estrogen is also found to increase gene expression of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF), as well as of choline acetyltransferase (ChAT) in cholinergic neurons and to increase N-methyl-D-aspartate (NMDA) binding sites, all of which are connected to cognition [27-30]. Experiments performed in cell culture and in samples of female rat brains have shown that estrogen protects neurons from Aβ peptide-induced toxicity by increasing the expression of Aβ clearance factors including IDE, neprilysin, endothelin-converting enzyme 1 and 2, angiotensin-converting enzyme, and transthyretin (31). Although progestogens (progesterone and progestins) bind with relatively high affinity to the PR A/B, they do not bind to the ER, and their affinities differ towards the androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors [32].Regarding the neuroprotective effect of progestogens, progesterone increases the gene expression of BDNF and anti-apoptotic Bcl-2 protein. However, the most widely used progestin in HT, medroxyprogesterone acetate (MPA), has no basal effect on Bcl-2 gene expression and inhibits the one elicited by estrogen [33]. Additionally, progesterone reduces the expression of pro-inflammatory genes and lipid peroxidation, which result in reduction of cell death [34].

3.2 Non-genomic effects

In addition to regulation of gene transcription, progesterone and estrogens can also elicit their effects through rapid non-genomic mechanisms, which include the activation of non-classic membrane-bound receptors found mostly in the hippocampus, hypothalamus, cortex and substantia nigra [35, 36]. This non-genomic effect can be accomplished also through ERα/β via its interaction with metabotropic receptors [19]. The estrogen membrane receptor was defined as a G-protein-coupled estrogen receptor (GPR30 or GPER1) and the progesterone receptor as a unique G-protein-coupled receptor that acts through cyclic adenosine monophosphate (cAMP) (7TMPR) [18, 37]. Downstream of these non-genomic transduction pathways, both sex hormones can activate multiple signalling pathways, including cAMP/protein kinase A (PKA), mammalian target of rapamycin (mTOR), mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) and protein kinase B (Akt/PKB), that are involved in synaptic plasticity and neuroprotection [38, 39]. As this is a new field of research, a recent finding that estradiol-elicited mTOR activation in the hippocampus was blocked by a very specific antagonist of GRP-30 and not by the antagonists of classical ERα/β, puts a totally different light on this type of receptor in the context of cognition [39].

In neurons, progesterone is converted to allopregnanolone, a neurosteroid that binds to the discrete site in the hydrophobic domain of the gamma-aminobutyric acid A (GABA-A) receptor, resulting in the potentiation of GABA-induced chloride conductance [40]. Additionally, GABA-induced chloride conductance can also result from activation of the signal transduction pathway, which consequently phosphorylates certain subunits of the GABA-A receptor [40]. As GABA can impair memory by inhibiting the induction phase of long-term potentiation (LTP) [41], allopregnanolone could have a negative influence on the learning process [42, 43]. On the other hand, GABA has a positive effect on cell survival in models of excitotoxicity [44].

Another neurotransmitter involved in the process of learning is glutamate. Progesterone has been shown to suppress the excitatory glutamate response (in a dose-dependent manner) protecting the neurons from glutamate excitotoxicity, while estrogen has the opposite effect by facilitating glutamate transmission [45]. Therefore, the interaction between sex hormones and classic neurotransmitters is a complex one in which estrogen and progesterone can have protective as well as toxic effects.

In summary, the literature suggests that estrogen and progesterone may affect cognition at two levels (which cannot be strictly separated); fast ER/PR-independent (learning, acute response to injury) and slow ER/PR-dependent actions (neurogenesis, memory storage) in the brain areas connected to cognition.

4 Problems related to preclinical HT testing

4.1 Selection of the representative model

4.1.1 Modelling of human menopause

Before starting preclinical drug testing it has to be assured that an appropriate animal model will be used, resembling as much as possible the condition intended for treatment. Considering the HT effects on cognition, it would be important to first test the effects of sex hormone treatment in middle-aged or aged female animals, a model that more closely mimics the condition of hormone treatment during a physiological human menopause. Preclinical studies exploring this issue to date were typically performed on younger (3 month old) animals.

Additionally, all animal HT preventive studies have been generally done in a condition of surgically induced menopause (ovariectomized model) (Table 1). This is not in line with the physiological human menopausal condition since natural depletion of sex hormones in women is more gradual and not as abrupt as in the ovariectomized animal model which, therefore, might be representative only for surgically-induced menopause in women.

Our literature search revealed only one animal model with gradual sex hormone depletion, a 4-vinylcyclohexene diepoxide rodent model of ovarian follicle depletion (VCD model) [46]. When this model was used to test the effect of conjugated equine estrogen (CEE), the most common HT in menopaused women, no beneficial effect was found regarding cognition [46] (Table 1). However, when CEE was tested on ovariectomized model, it had a protective effect on cognition [47] (Table 1), emphasizing the importance of mode of animal menopause induction in HT testing.

The remaining preclinical research has been done on ovariectomized females treated with 17β-estradiol with results showing the beneficial effect of estrogen on cognition (Table 1) [48-50]. Meta-analyses of HT effect on cognition revealed that time-limited positive estrogen treatment effect was seen in women who had recently undergone surgical menopause [51]. Therefore, existing preclinical and clinical research agrees that estrogen treatment (17β-estradiol, CEE) has a beneficial effect in surgically-induced menopause. But to get to the bottom of the problem regarding HT and cognition in naturally menopaused women, experimental models that better mimic the gradual depletion of sex hormones should be used (e.g. VCD or non-human primate model) in order to improve data translation to humans.

4.1.2 Modelling of Alzheimer's disease

The modelling issue does not refer only to the modelling of hormonal depletion but also to the model of sAD used to test the beneficial effects of estrogen/progesterone therapy. There are just a few preclinical studies in which HT (estrogen and progesterone) was tested to treat experimental AD and they were performed on transgenic mouse models of AD [52, 53] (Table 2).

Transgenic AD mice models are good for testing the effect of HT on the rare, familiar early-onset AD (fAD) since they express the gene mutations known to cause fAD in humans [54]. However, the most common form of dementia (>95% cases in the World) is sAD, which is of unknown cause and not connected to the gene mutation found in fAD [55]. Therefore, the existing transgenic mice AD models are not appropriate for testing therapies for sAD, including HT.

Testing of the beneficial effects of drugs on cognitive impairment developed in non-transgenic models that more accurately represent sAD-like pathology might achieve better animal-to-human translational results. The general principle behind non-transgenic models is to inject a compound into the brain that causes changes that resemble those of sAD in humans. Existing non-transgenic models include: Aβ-based models (Aβ 1-42, 1-40), cholinergic-based models (scopolamine, ibotenic acid, choline mustard aziridium), and insulin resistance-based models (streptozotocin); all applied into the brain by various protocols [56].

Among them, streptozotocin intracerebroventriculary-treated rats (STZ-icv model) have been recognized as a model that shares major pathological similarities with the human sAD condition, in addition to cognitive decline [57]. Pathological changes found in this model are the consequence of oxidative stress and a brain insulin resistant state induced by icv administration of STZ [58], and an insulin resistant brain state has been proposed as the metabolic core in human sAD [59-61]. Neurochemical changes in insulin receptor signalling in the brain as well as cognitive decline in STZ-icv rat model demonstrate a biphasic time pattern, while structural changes and Aβ and tau pathology develop and progress slowly in a linear manner [57, 58]. Such a staging scheme suggests that late changes might correspond to the symptomatic sAD phase in humans [57]. Therefore, the STZ-icv rat model might provide a good platform for both preventive and rescue HT therapy in sAD-like conditions.

To date, only one study has tested the effect of estradiol (200 μg/day for 40 days) on learning and memory in the STZ-icv model [62]. The study found that administration of estradiol immediately after the STZ-icv treatment compensated for the decrease in energy metabolism in the brain and cognitive deficit caused by the STZ-icv treatment (Table 2).

So far none of the rodent models described in this section have led to the discovery of novel useful drug(s) for sAD [63, 64] in humans but, at the current level of knowledge of AD pathophysiology, usage of these animal models is unavoidable in preclinical drug development.

4.2 Type of memory that is tested

In general, the first and most salient symptom to emerge in patients with sAD is difficulty in acquisition of new information. Episodic or autobiographical memory is predominantly affected, with early loss of memory for everyday events. Language deficits and visuospatial deficits appear as the disease progresses [65, 66]. Cognitive domains that are frequently affected at an early stage of sAD are: episodic memory, executive functions, semantic memory and word finding [65, 66]. But, short term memory, assessed by the digit span, tends to be preserved early in sAD [65, 67].

Estradiol levels were positively associated with benefits in episodic memory, semantic memory, verbal memory, and verbal learning in premenopausal females [68-72]. In another study progesterone concentrations were significantly positively associated with verbal memory and global cognition, and estradiol was significantly positively associated with semantic memory (naming scores) among women in an early postmenopausal group only [73]. A study in which pregnant women (having high progesterone level) were compared with controls found that both pregnant groups (early and late pregnancy stages) had reduced scores on immediate and delayed verbal memory tasks, but were unimpaired on visual and procedural memory tasks [74]. These findings demonstrate a relationship between progesterone, estradiol and cognitive performance that is dependent on the type of memory and the hormone concentration. It seems that the verbal part of memory is affected the most by sex hormones. In line with that, in randomized trials, as well as in meta-analyses, verbal memory is found to be positively affected by HT (Tables 3 and 4) [51].

The prefrontal and temporal cortices in humans are the parts of the brain associated with semantic verbal memory [75]. Verbal fluency (i.e. generation of semantic category lists) was found to be impaired in sAD due to two major constraints: deterioration of semantic memory store, and variable difficulties in semantic search [75].

As semantic memory in humans represents the memory of objects and words, it is a crucial point for performance in verbal and object recognition tests [75]. Considering animal studies, it is not possible to test verbal memory or to do verbal recall tests in animals but it is possible to test object recognition. The delayed response test (DR; involving the prefrontal cortex), delayed non-matching to sample test (DPNM; involving the medial temporal lobe) or the object recognition test (involving the perirhinal cortex) are the correlates of semantic memory testing in animals. Only a few animal studies with rhesus monkeys have used these tests to explore the effect of HT on cognition and found an improvement or no effect in semantic working memory depending on the HT type and administration regime (Table 1) [76, 77].

On the other hand, episodic memory is the sum of cognitive processes involved in the acquisition, storage and recall of events that happened to the subject directly or just memories of events that happened around the subject [78, 48]. In an animal this can be translated using a what-where-when task to test spatial memory. For this purpose, spatial-delayed recognition span test (DRST), Morris-water maze test (MWM) and different radial arm tests can be used and they are the most exploited tests in preclinical research of HT and cognition [78, 48].

In clinical research, older studies used the Mini-Mental State Examination (MMSE), which is in fact only used as a screening test for dementia [79]. More recent clinical studies have used different cognitive tests that are not always comparable, so to avoid inconsistency between clinical trials, the

US National Institutes of Health have supported the development of a comprehensive assessment tool (NIH Toolbox For Assessment Of Neurological And Behavioural Function; http://www.nihtoolbox.org). The NIH Toolbox provides a specific cognition battery (NIH Toolbox Cognitive Function Battery) to test several cognitive domains (executive function, episodic memory, working memory, attention, processing speed and language) to be used in intervention studies.

The same would be useful to implement for the preclinical test battery in animal models to avoid the syndrome "lost in translation". Until then, when evaluating the effect of HT treatment in cognitive tests in animal models it would be useful to concentrate on the tests that represent semantic memory (DR, DPM or object recognition test) and that may be closest to verbal semantic memory in humans.

5 Pharmacological problems related to HT

5.1 Timing of the prevention therapy

It might be important to keep in mind the "healthy cell bias theory" proposed by Brinton and colleagues according to whom estrogen is beneficial in healthy neurons (used in vitro) but can become deleterious in diseased neurons [80]. This might explain some of the differences observed between adult and aged rats after HT treatment (Table 1) [81, 82]. It could also be the base for the "critical window theory" which suggests that if the pause between starting HT and menopause is too long, hormone treatment can have negative or no effect on cognition [78, 83].

On the other hand, a meta-analysis of clinical trials concluded that there is no connection between the time of starting the HT and its effect on cognition [51]. It could be hypothesized that health rather than absolute age per se could influence the interaction between HT and cognitive functions [84]. Randomized trials in patients with sAD have also showed that if sAD is at a later stage, the beneficial effect of HT is overwhelmed (Table 3) [85, 86]. In addition, total health status is very important for the metabolism of HT in the liver [87]. Animal studies with different hormone doses used in animals of different age have demonstrated that the concentration of estradiol is doubled in aged animals, probably due to aging-induced decrement in liver metabolism (Table 1) [81]. The same is observed in humans; Hembree et al. [87] found that the clearance of estrogen is slower in older compared to early postmenopausal women. This issue might account for some of the differences in the effect of HT seen in clinical, as well as in preclinical, studies.

5.2 HT dose and route of administration

Oral administration is the most common route of administration of HT in postmenopausal women [51, 88, 89]. However, animal studies more commonly use subcutaneous pellets and silastic capsules to deliver HT (Tables 1 and 2) [49, 83, 78, 88, 90].

The subcutaneous route of administration is associated with different drug pharmacokinetics than oral administration and avoids first pass metabolism of the hormone, which may result in different outcomes of HT [89]. Indeed, the only preclinical study in which estradiol was administered orally to middle-aged ovariectomized mice and where the effects on cognition were assessed, found that animals had improved performance in the object recognition test, but had unaffected spatial working memory and impaired reference memory following estradiol treatment [50] (Table 1).

There are few randomised double blind studies that use the transdermal route of administration in postmenopausal women with sAD (Tables 3 and 4) [51, 88, 89]. Therefore, it is important to evaluate the effects of HT in an animal model using a route of administration that is similar to the most common route used in the human population being studied.

Additionally, animal models treated with different HT doses showed that the effect on cognition is dose-dependent; lower doses had a beneficial effect, which was not the case with higher doses (Table 1) [38, 47, 83]. HT doses used in patients with AD were the same as those in healthy women experiencing menopausal symptoms and considering the animal studies, it seems that new clinical trials with different doses of HT are needed for the sAD population (Table 3).

As mentioned before, it seems that the pharmacokinetics of estradiol is changed in older animals so that the same dose administered as silastic capsules to younger and older animals yield almost double concentrations of estradiol in the older animals [81].

5.3 Type (composition) of HT

The types of hormones prescribed to postmenopausal women are different from those that are most commonly studied in animal models. Conjugated equine estrogen (CEE), the estrogen that was used in the Women's Health Initiative (WHI) [11], is the most commonly prescribed estrogen, but very few animal studies have evaluated the effects of this particular estrogen on cognition (Table 1) [88]. Basic research that found a beneficial effect of estrogen on cognition used estradiol with or without progesterone [78, 83] (Table 1). A recent study found that CEE administration to middle-aged female rats prevented overnight forgetting in the Morris water maze test only in the group with surgically-induced menopause but not in VCD group (as mentioned before this model better resembles the naturally occurring menopause in women) [46].

Furthermore, animal studies that have evaluated the effects of progestogens on cognition have used progesterone rather than MPA (the most commonly prescribed progestin in humans) (Table 1) [49,83,75,88,90]. MPA is a synthetic analogue of progesterone and acts like an agonist at progesterone receptors, but it also binds to androgen and glucocorticoid receptors, respectively [91, 92]. A recent study found that MPA administered without estrogen impaired the performance of rats in the water radial arm maze and the spatial water maze tests [93].

The impact of progestin on estrogen-induced neuroprotection depends on the type of progestin; it is synergized by progesterone and 19-norprogesterone and antagonized by MPA [33]. The WHIMS study used MPA, which could explain why HT worsened the cognition in postmenopausal women (Table 4) [11,15,16]. Formulations that contain a combination of CEE and MPA are commonly prescribed clinically for the relief of hot flashes and related symptoms [16, 51], but the effect of treatment on cognitive function in postmenopausal women remains controversial [10, 39].

So, both in preclinical and clinical trials it is important to test different combinations of hormones. One clinical study has found a beneficial effect of 19-norprogesterone, [94] which is in line with preclinical findings on cultured neurons [33]. Also, a beneficial effect of norethisterone in combination with estradiol has been found in randomized clinical trial in healthy as well as in sAD postmenopausal women [95, 96]. The KEEPS study used treatment with two types of estrogen (CEE orally and 17β-estradiol in a transdermal patch with or without micronized progesterone (MP)) and did not find any beneficial effects on cognition [13] (Table 4). However, in a group of ApoE ε4 carriers treated with low concentrations of estradiol (50μg), lower levels of Aβ deposit were found in comparison to non-carriers [97].

A meta-analysis of randomized trials that assessed the cognitive effect of HT in menopausal women showed that adding progestogens to estrogen therapy negatively affected the outcome [84]. Interestingly, 9 out of 13 clinical trials used MPA as the add-on progestin [84]. There are also studies that have used a synthetic partial agonist of the estrogen receptor, raloxifene; raloxifene administered to animals improves memory [98, 99], but clinic research has reported mixed findings (beneficial effect in some [100, 101] and no effect in others [102,103]).

From these data one could speculate that different types of estrogen and progesterone independently and interactively regulate AD-like neuropathology, suggesting that tailored and optimized HT may be more successful in reducing the risk of AD in postmenopausal women. Also future efforts should concentrate on finding new selective modulators that have the beneficial effect on cognition and no adverse effects on the periphery, and possibly to combine them with a proper progestogen compound.

6 Problems related to design of HT clinical trials

6.1 Randomised interventional clinical trials in women with unimpaired cognition

According to the four meta-analyses published during the past twenty years, the findings of previous observational clinical studies have not been consistent; three meta-analysis estimated that the risk of dementia was reduced by 29%-44% [51, 104] while the fourth one showed no risk reduction [105].

Data from large randomised clinical trials have also been mixed. On one hand there is WHIMS, and the Women's Health Initiative Memory Study of cognitive aging (WHIMSCA) reporting a negative effect of HT on cognition. [11, 15, 16] On the other hand, the Women's Health Initiative Memory Study/young (WHIMS-Y) and KEEPS-Cog showed no beneficial effectn cognition (Table 4) [13, 106] .

6.1.1. Choosing the right population

WHIMS, which was the largest multicenter, randomized, double-blind, placebo-controlled clinical trial designed to assess the effects of HT on the risk of dementia and mild cognitive impairment, found that continuous HT use (CEE with or without MPA /the latter in hysterectomised women/) was associated with an overall significant increase in dementia risk (Table 4) [11, 15, 16]. However, in spite of the valuable findings that came out of this study, there are some limitations that should be considered. First, the women enrolled were aged 65 years or older, which could have been associated with an increased risk of cardiovascular and/or cerebrovascular diseases, so it could not be excluded that the high risk of dementia was attributed to the concomitant vascular disease (Table 4). As reported in the WHI study, women on HT have an increased risk for cardiovascular disease and those with higher levels of low-density lipoprotein cholesterol at the beginning of the study were associated with an excess risk of CHD among HT users [107]. Second, there was no baseline cognitive testing and thus the results were based only on a cross-sectional analysis performed at the end of the study. Third, all types of dementia were classified in the same category without subdivision into AD, vascular dementia, Parkinson's dementia, frontotemporal dementia, etc. [11, 15, 16].

To address some of these limitations, the WHIMS-Y study was designed as a sub-trial of the WHI that enrolled only younger postmenopausal women aged 50-55 years but found no effect of HT on cognition (Table 4) [106]. However, apart from age and dementia status at enrolment, the other limitations remained the same in the WHIMS-Y study [106].

The WHIMSCA study, on the other hand, enrolled older women (mean age 74 years) and found that CEE alone had no effect on verbal and figural memory, but in combination with MPA, verbal memory declined and figural memory improved [108, 109].

In summary, it seems that if one has an increased risk for cardiovascular disease and is on HT (CEE+/- MPA) then one has increased risk for developing all-cause dementia, but any effect remains unknown in healthy women.

Additionally, a recent large randomised, double-blind study, KEEPS-Cog, showed no effect of estradiol and MP on cognition but rather beneficial effect of estradiol on amyloid deposits in ApoE ε4 carriers (Table 4) [13, 97]. This study enrolled a large sample of recently postmenopausal women, aged 42 to 58 years, without high cardiovascular risk (normal blood pressure, mean body mass index 26.3, normal lipid levels), with a mean MMSE of 29.1 (cut off <23) and naturally occurring menopause. In comparison to WHIMS, KEEPS-Cog study used different type and dose of hormones (low dose CEE, estradiol and MP vs high dose CEE and MPA in WHIMS) and two routes of hormone administration (oral and transdermal) [13]. This study also pointed to the importance of the ApoE ε4 carrier status and a need for patient stratification in this regard.

Findings from KEEP-Cog and WHIMS-Y showed that high or low dose CEE and progestogens (MPA, MP) have no protective effect on cognition in recently postmenopausal women. There may be a little spark of hope that unopposed estradiol given transdermally could have a postponed protective effect on cognition but only in ApoEε4 carriers [97]. So, it is important to consider all risks and benefits that HT could bring, depending on an individual's risk profile. Further research should be designed to clarify the effect of other types of HT (both regarding the hormone compound and the pharmaceutical formulation) on cognition in recently postmenopausal women.

6.2. Randomised interventional clinical trials in women with Alzheimer's disease

Since 1997 nine randomized, double blind, controlled trials of HT women with AD have been conducted, among which six had primarily positive results (improved visual and verbal memory [96, 110-114] while in three there was no effect on memory [86,115,116] (Table 3).

6.2.1 Trials with <50 patients

Most studies had less than 50 patients [74, 78-80, 82, 92, 93] and were of short duration (8-28 weeks) [110, 111, 113, 115, 116] or 9-15 months [96,112,114]). The two of them with the smallest number of participants (12 and 20) and with shortest duration (13 and 16 weeks) were conducted by the same investigational team [110,111]. Both included women with mild to moderate sAD, with natural or surgical menopause, who were treated with an unopposed transdermal formulation of estradiol that was reported to have beneficial effect on verbal and visual memory [110, 111]. In both studies ApoE status was not assessed and duration of sAD from diagnosis until HT was 2.2-5 years. In both of the studies no effects were found in MMSE and Blessed memory information and concentration test (BMICT) representing global cognitive function [78, 79]. A positive effect was reported on verbal and attentional memory (Table 3). In the third study done by the same team, women were treated with estrogen plus MPA, and no positive effect was found on verbal memory, but positive effects were seen on semantic and episodic visual memory [112].

***ApoE status*.** As mentioned previously, ApoE ε4 status is an important factor that can change the way that HT affects cognition in healthy perimenopausal women. The study done by Valen-Sendstad et al. [96] on women with sAD demonstrated that only women who were ApoE ε4 negative showed better performance on verbal memory task (Table 3) [96]. The other randomised studies did not stratify women regarding their ApoE status (Table 3). Future studies should pay attention to whether or not women are ApoE4 carriers.

6.2.2 Trials with >100 patients

Among nine studies in AD postmenopausal women, only two had more than 50 patients but their number hardly exceeded 100 (120 [86] and 117 [115]). However, there are certain facts that may be pointed out in these studies as possible influencing factors on cognitive outcomes.

Concomitant drug therapy. The largest was the study conducted by Rigaud et al [115] on 117 patients who were all on concomitant therapy with acetylcholinesterase (AchE) inhibitor rivastigmine. Advantage of this study is that all patients were diagnosed to have sAD for approximately 1.7 year before entering the study but no particularly comorbidity was reported [115]. The results of this study indicated that transdermal estradiol opposed with progesterone had no effect on cognition [115]. In other studies the AchE inhibitors were omitted 2-3 months prior the beginning of the study [96,110-114,116] except in The Alzheimer's Disease Cooperative Study (ADCS) [86] where 24% of women in CEE group and 13% in placebo stayed on donepezil therapy (Table 3) [86,96,110-114,116]. Bearing in mind that estradiol enhances cholinergic-mediated cognitive performance [117-119] and that combination of estrogen and cholinergic treatment may improve cognitive performance in animals as well as in humans [84-86], it becomes a problem to compare the results of these studies. Additionally, in the ADSC study 5 patients were on donepezil in placebo group in comparison to 10 and 9 patients in estrogen-treated groups (Table3) [86].

However, it cannot be excluded that possible chronic concomitant drug therapy other than anti-AD one could have influenced cognitive outcomes in these studies. This might be important particularly regarding the drugs used to treat diseases of cardiovascular system and diabetes mellitus whose influence on dementia has been investigated in AD population (e.g. calcium channel blockers [121,122], statins [123,124], metformin [125], insulin [126], rosiglitazone [122, 125, 127] etc.). Unfortunately, such concomitant therapy has been explicitly mentioned in inclusion/exclusion criteria in several studies only [96,110,111] (Table 3), while no data have been provided for comorbidity of diabetes mellitus (Table 3), a disease which itself carries a risk for AD development [128, 129].

Duration of sAD before HT treatment. It is also important to consider the issue of duration of sAD condition prior the HT (Table3). Usually the patients have mild to moderate sAD with similar scores on cognitive tests (MMSE as inclusion criteria) but if one considers healthy bias theory and appropriate time to start HT, then it would be useful to know the duration of disease before starting the therapy and, if possible, which part of the brain has been affected at that stage. This seems to be important considering the preclinical results showing that a choline acetyltransferase (ChAT) protein level is altered in a site-specific manner after the treatment with estradiol [120]. This research demonstrated that estradiol treatment initiated immediately after ovariectomy significantly increased ChAT levels in the middle-aged rat hippocampus but not in the prefrontal cortex while the vice-verse effect was induced by estradiol treatment initiated 5 months after ovariectomy; it increased ChAT levels in the prefrontal cortex, but not in the hippocampus [120]. Having this in mind and looking back into the ADSC study, there is a higher percentage of patients with mild sAD (74%) in placebo group in comparison to similar sAD-staged patients in the estrogen-treated groups (55%), while other patient in both groups had moderate sAD (26% in placebo and 45% in estrogen-treated group) [86]. This may indicate that at the beginning of the study the participants in estrogen group were in disadvantage regarding the timing of HT in comparison to placebo group.

Baseline serum estradiol level. The ADCS study (120 patients enrolled) found no effect on cognition in surgically-induced postmenopausal women but the mean baseline serum estradiol levels were 5.4 pg/ml in placebo group (N=35), 48.0 pg/ml in the low-dosed estrogen group (N=42, concomitant treatment with 0.625 mg of CEE per day), and 58.4 pg/ml in the high-dosed estrogen group (n=39, concomitant treatment with 1.25 mg of CEE per day) [86] (Table 3). It can only be speculated that the reason for such a huge difference in the mean baseline serum estradiol levels between the groups was due to previous estrogen-based treatment or because a large inter-group age range (55-91 years).

Pharmacokinetics and pharmacodynamics of HT. Another methodological inconsistency between the clinical studies can be looked for in the pharmacological aspects; the type of HT (estrogen compound), route of hormone administration (pharmaceutical formulation), opposed or unopposed with progestogens, and inter-individual differences in hormone levels achieved following HT (not assessed in any of the studies) (Table 3). Some trials tested oral CEE while in others transdermal estradiol was used (Table 3) which might be a source of biotransformation-related differences; as women become older the pharmacokinetics of sex hormones is changed and estradiol is metabolized more rapidly in the early versus late stage of menopause [87]. Timing of cognitive testing following the HT initiation seems to be very important as well. The largest randomized study conducted by Kantor et al that lasted for 38 months has reported that beneficial effect of CEE treatment in sAD patients begins between 6 and 9 months after the HT initiation and lasts until 12 months of the therapy while after that, the test scores decline [65]. This observation suggests that CEE therapy may improve cognition at its beginning but eventually disease progression overwhelms the beneficial effect of HT.

To summarize, considering the design of clinical trials, in particular intra- and inter-group heterogeneity in sAD stage, concomitant AchE inhibitor or other drug therapy, comorbidity, patient age and hysterectomy status seem to have a large impact on inconsistency in the results of HT clinical trials in postmenopausal women with sAD which might be further complicated by different sensitivity of cognitive tests used to measure the primary outcome.

Although this review may have some limitations related to a single data base used as a source of information and particular inclusion criteria used in search (time frame, article type, etc), preclinical and clinical trials that have been critically evaluated as the results of such search seem to provide a rather representative sample of a larger number of similar studies dealing with the issue of HT influence on cognition.

7 Conclusion

While there are studies showing that estrogen and progesterone depletion in postmenopausal women carries a significant risk for developing AD which may be reduced by estrogen-based HT, data from recent clinical trials oppose this beneficial effect. Possible reasons for such inconsistency might be found both in preclinical and clinical trials as well as in the HT itself:

1. Inappropriate animal model (incorrect translation of animal-to-human or human-to-animal condition of sex hormones depletion; abrupt/surgical or gonadal/physiological, widely exploited models represent rare familiar but not the prevailing sporadic AD)

2. Heterogeneous postmenopausal women groups in clinical trials (particularly regarding the sAD stage, anti-AD therapy, hysterectomy status, possible co-morbidity and concomitant drug therapy)

3. Incomparable HT treatment design (different estrogen/progestogen composition as well as dose androute of administration, pharmaceutical formulation, timing of treatment initiation as well as of cognitive testing)

In line with that further research is needed both in humans and animals that will be focused on other types of estrogen and progestogen compounds including other progestins besides MPA (e.g. norprogesterons, levonogestrel and others) and selective modulators of estrogens receptors. It is essential to have randomized, controlled double-blind studies in population with uniformed early stage of AD and patient groups that are more homogeneous regarding the AchE inhibitor therapy (or other anti-AD therapy), age and hysterectomy status as well as co-morbidity condition and ApoE carrier status, to elucidate and define the therapeutic role of different HT for postmenopausal women with AD. As we go deeper in understanding of mechanisms underlying estradiol and progesterone effect and their pharmacokinetics, we are getting closer to the design of estrogen/progesterone modulators that would optimize cognitive benefits for possible prevention and treatment of AD and minimize associated side effects.

8 Compliance with ethical standards

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Jelena Osmanovic Barilar and Melita Salković-Petrišić both have no conflicts of interest to declare.

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Table 1: Experiments in cognitively unimpaired animals subjected to sex hormone therapy.

Reference	Animal/ Age	HT type/dose	HT administration	HT duration	Blood c (E2)/ pg/ml	Length of hormone deprivation after ovariectomy	Results of cognitive testing: Type of test / Outcome	Results of cognitive testing: Factors that affected/might have affected or had no influence on the results
COGNITION IMPROVED								
Daniel et al 2006 [78]	Rat/12m and 17m	E2 (25%)	sc sil.cap.	5m	15 -25	0m 5m	**RAM** Only rats with 0m of hormone deprivation showed enhanced working memory	-Dependent on post-OVX time -Single test used
Frick et al 2002 [48]	Mouse/ 27–28m	E2-3-benzoate /1 or 5μg	sc injection (cyclic)	11 d	ND	Intact gonades	**MWM** Only 5 μg of E2 benzoate significantly improved spatial learning and memory	-Dependent on E2 dose -Single test used
Talboom et al 2008 [81]	Rat/ 2m 14m 21m	E2/0.25 or 0.50 mg	sc pellets	60d	14m/0.25=57 14m/0.50=44 2m/0.25=77 2m/0.50=49. 21m/0.25=104	1m	**MWM** only 21m-Ovx +E2 had better platform acquisition. 14m-Ovx +E2 exhibited faster learning	-Dependent on animal age -Single test used
NO EFFECT ON COGNITION								
Baxter et al 2013 [77]	Rhesus monkey/ 17.7–25.7 y	E2/150 pg/ml +/- P4/ 100mg estradiol cypionate/ 100 μg+ P4/100mg	sil. implant oral d/cyclic im injection (cyclic)	16m	E(150 pg/ml): 99.45–337.55 80.17–449.28 91.08–248.96 P oral: 2.84–3.40 P4cyclic: 1.45–7.00 E (100 μg): 50.46–155.82 P: 2.61–5.08	6-12w	**DP** No effect **DPNM** No effect **OR** No effect	Independent of: -cognitive test - E2 formulation and dose - E2+/-P4 composition
COGNITIVE TEST - DEPENDENT EFFECT ON COGNITION								
Fernandez and Frick,	Mouse/16-17m	E2/ 70, 80,110μg	oral	6w	10-40	1w	**WRAM** No effect	

2004 [50]		/kg					**OR** improved cognition	Independent of E2 dose and test
Rapp PR et al., 2003 [76]	Rhesus monkey/ 22y+/-7m 5.2y+/-7m	cyclic estradiol cypionate /100 µg /1 ml	Im injection	6w	≈290(1st day of injection ≈150(2nd day) ≈90 (3rd day)	30+/-1.7 w	**DP** Improved spatial working memory in 22y old monkeys only**.** **DPNM** No effect	-Dependent on age -Independent of age
Engler-Chiurazzi et al 2012 [47]	Rat/13m	CEE(Premarin) 12,24,36 µg	osmotic pump	44d	Estron: ≈7. ≈20 ≈ 17 E2: ≈3.5 ≈6.5 ≈ 8.0	0d	**WRAM** Only Ovx+CEE (36 µg) group exhibited better performance compared to the control one. **MWM** No effect except in low-dose CEE group which showed impaired learning **DPM plus maze** No effect except low-dose CEE group which showed impaired learning	Dependent on estrogen dose regardless the test
ESTROGEN+/-PROGESTOGENE -DEPENDENT EFFECT ON COGNITION								
Bimonte-Nelson et al 2006 [49]	Rat/12m	E2 +/-P4/ (25%E2) E2 10 µg+/- P4/	sc sil.cap. sc injection (cyclic)	3m 3m	20 40 c(P4) 12ng/ml	3w	**MWM** Low-dose (20 pg/ml) and cyclic estradiol (dose of 10 µg) treatments improved spatial reference memory -addition of progesterone significantly reversed these benefits	-P4-dependent worsening -Single test used
Lowry et al 2010 [90]	Rat/10-12m	E2/47µg/ kg+/-P4 +/-MPA/ 41.7 µg/kg	sc sil.cap. sc sil.cap.	6m	ND	0m	**MWM** No effect E2+ MPA resulted in worse performance in comparison to other groups receiving HT but not to the control one.	-MPA-dependent worsening -Single test used
Gibbs et al 2000 [83]	Rat/13m	E2/.(25% E2) E2+P4	sc sil.cap. sc injection	1.5m 7m 7m	15 -25 50	3m 10m	**DPM-** Only E2+P4 sc treatment (3m of hormone deprivation) significantly enhanced acquisition of the DMP task	-P4-dependent improvement -Dependent on post-OVX time -Single test used
NATURAL / SURGICAL HORMONE DEPLETION -								

DEPENDENT EFFECT ON COGNITION								
Acosta et al 2009 [46]	Rat/7m OVX VCD	CEE/ 30 µg/	sc njection (cyclic)	2m	ND	18+/-1d	**WRAM** CEE treatment impaired spatial working and reference memory in VCD+CEE group but enhanced it in OVX+CEE animals **DPM** enhanced performance in OVX animals but not in VCD group. **MWM** CEE enhanced performance in OVX animals ($p = 0.05$), but not in VCD (3rd test day only)	OVX-dependent improvement regardless the test

All experiments had a control group with sham surgery.

m-month, **w**-week, **y**-year, **d**-day, **c-** concentration, **sil cap-**- silastic capsulae, **HT**-hormone therapy**, E2**-17-β estradiol, **P4**-progesterone, **CEE-** conjugated equine estrogen, **MPA**- medroxyprogesterone acetate, **sc**- subcutaneous, **im**.-intramuscular, **RAM-** radial arm maze, **OR-** object recognition**, DP-**delayed response (prefrontal cortex), **DPM**-delayed matching to sample test, **DPNM-**delayed nonmatching to sample test (medial temporal lobes), **MWM-** Morris water maze test (spatial reference memory and working memory), **WRAM**-8-arm radial arm maze (spatial working and reference memory), **OVX**- ovariectomized, **VCD**- 4-vinylcyclohexene diepoxide rodent model of ovarian follicle depletion **ND**-no data

Table 2: Experiments in cognitively impaired and ovariectomized animal models for Alzheimer's disease subjected to sex hormone therapy.

AD model	Reference	Animals / Age of HT treatment initiation	HT			Blood c(E2)/pg/ml	Length of hormone deprivation	Results of cognitive testing	
			dose/type	administration	duration			Type of test/ Outcome	Factors that affected/might have affected or has no influence on the results
TRANSGENIC MICE MODELS	**PROGESTOGENE ADD-ON-DEPENDENT EFFECT ON COGNITION**								
	Carroll JC et al 2007 [131]	3xTg AD mouse/ 3m WT mouse/6m	E2/0.025 mg +/-P4/25 mg	sil.cap oral	3m	104±28	0m	**Y-maze**-E2+/-P4 improved performance only in 3xTg AD mice WT mice-No effect	-Dependent on age (AD<WT) -Familiar AD model used -Single test used -Independent of P4 add-on
	Carroll JC et al 2010 [52]	3xTg AD mouse/3m WT mouse/4-6m	E2/ 0.025 mg +/- P4 /25 mg cyclic or continuous	sil.cap oral	3m in 3xTg 1m in WT	ND	0m	**Y-maze** E2 +/-cyclic P4 improved performance in 3xTg AD mice WT mice –No effect	-Cyclic P4-dependent improvement -Dependent on treatment duration (WT<AD) - Familiar AD model used -Single test used
	COGNITIVE TEST -DEPENDENT EFFECT ON COGNITION								
	Heikkinen T et al 2003	APPsw/PS1 mouse /3-	E2 /0.18mg	sc pellet	3m	ND	0m	**MWM-**No effect **T maze-** improved performance only in 9-	-Dependent on age -Dependent on test

	[53]	12m						12m old mice **RAM-**No effect on working memory and improvement in reference memory only in 6 m old animals	- Familiar AD model used
	NO EFFECT ON COGNITION								
	Palm R et al 2014 [132]	3xTgAD mouse/ 18m	E2 1.1 ng/day	sc pump	3m	12-58	0m	**MWM** –No effect	- Familiar AD model used -Single test used
NON –TRANSGENIC MODEL	**POSITIVE EFFECT ON COGNITION**								
	Lannert H et al 1998 [62]	STZ-icv rat/12m	E2 200µg/d	sc injection	40d	ND	0m	**Holeboard test-** STZ+E2 group performed better than STZ **PA test-**improvement in STZ +E2 group	- Sporadic AD model used
	Savonenko et al 2003 [130]	Rat/12-13m and 20 m + /- scopalamin	E2(25%)	sil.cap.		ND	0m	**T-maze active avoidance-** improvement only in scopalamin 12-13 m +E2 group	-Dementia model used -Single test used -Dependent on age

m-month, **d**-day, **HT**-Hormone therapy, **E2**-17 beta estradiol, **P4**-progesterone, **sc-** subcutaneous, **sil.cap.**- silastic capsulae, , **AD**-Alzheimer disease, **STZ-icv**-streptozotocin intracerebroventriculary treated rats, **APPsw -**Tg2576 mice which express human APP with the Swedish double mutation, **WT**-wiled type, **RAM**-radial arm maze, MWM-Morris water maze, **PA**-passive avoidance test **ND**-no data

Table 3 Randomized double-blind, placebo-controlled trials in postmenopausal women with sporadic Alzheimer's disease subjected to hormone therapy

Reference	n	Age (years)	HT			Type of menopause	Cognitive outcome/Test	Factors that might have influenced cognitive outcome	
			dose/type	administration	duration			inclusion/exclusion criteria specification	miscellaneous
Asthana et al., 1999 [110]	12	66-89	0.05 mg E2	TD	8w+5w follow up	Natural	**No effect BS**: MMSE, BMICT, BNT, VR, PR, TMT, Verbal fluency, Token test **Positive effect BS:** verbal memory (BSRT) and attention (SCWIT) Positive effect declined after E2 discontinuation (E2 concentration correlated with cognitive decline)	-Comorbidity: ND -DM-ND -Concomitant drug therapy: antihypertensive (except β blockers) -Exclusion criteria: depression, Hachinski score >4, neurogical diseases, psychiatric disease	-Untreated dementia or AD therapy withdrawn 3m before E2 -Only 6 patients per group -Mixed mild + moderate sAD (MMSE 17-25, BMICT 18-30) - ApoE status: ND -Time between AD diagnosis and HT initiation unspecified -Gradual menopause
Wang et. al., 2000 [116]	50	72.6+/-9.1	1.25 mg CEE	po	12w	Natural	**No effect BS**: MMES, CDR, CASI, BEHAVE-AD	-Comorbidity: ND -DM-ND -Exclusion criteria: Hachinski score>4, uncontrolled DM or hypertension, endometrial/ breast Ca	-Mixed mild + moderate sAD (MMES 10-26; CDR 1-2) -AD therapy withdrawn during CEE - ApoE status: ND -Time between AD diagnosis and HT initiation unspecified -Gradual menopause

Valen-Sendstad et al., 2010 [96]	55	65-89	1mg E2+ 0.5 mg norethisterone (most usual HT in Europe)	po+ po	16 m	Natural	**No effect BS** DRS,CERAD-MMSE,WLM,CERAD-BNT,CP, TMT, WAIS DSC **Positive effect BS:** Only APOE4 negative group showed better performance in WLM (verbal memory) Regardless of ApoE, HT reduced the cognitive decline (GDS) in women with a level of education≥9.	-Comorbidity: ND -Concomitant drug therapy: antihypertensive, statins, aspirin, sedatives, vitaminB12,antidepressants -Exclusion criteria-AF, IHD, thromboembolic events, neurological disease, MCI, HDT, uncontrolled DM or hypertension, major depression	-Untreated dementia -BS analysis only -Mixed mild + moderate sAD (mean MMES 22+/-4) - ApoE status used as: stratification factor -Gradual menopause
Mulnard et al., 2000 [86]	120	56-91	0.625 and 1.25 mg CEE	po	15 m	Surgical	**No effect BS (0.625+1.25 vs placebo):** ADAS-CGIC, ADAS-Cog, MMSE, NDT, TMT, CF, LF, EFRT **Worsening BS (0.625+1.25 vs placebo)**: CDR, CF, FTT **Worsening BS (0,625 vs 1.25 vs placebo)**: CDR in both E groups and FTT only in low dose CEE	-Comorbidity: ND -DM-ND -Concomitant drug therapy: neuroleptics, anxiolytics, sedatives, hypnotics, stable use of donepezil or tacrin -Exclusion criteria: MI, thromboembolic disease, hyperlipidemia, major depressive disorder	-Mild sAD only 55% in E group vs 74% in placebo -Moderate sAD 45% in E group vs only 26% in placebo -Donepezil 24% in E group vs only 13% in placebo - ApoE status: ND -Basal E value 48 pg/ml in E group vs only 22.7 in placebo -Time between AD diagnosis and HT initiation unspecified -Abrupt menopause
Henderson et al., 2000 [113]	40	78	1.25mg CEE +/-10 mg MPA for 14 days	Po+ po	16w	Surgical+ Natural	**No effect BS**: ADAS-Cog, ADAS- CGIC WMS, BNT Token test, VR, LMS **Positive effect BS:** in TMT 4w after treatment	-Comorbidity: ND -DM-ND -Concomitant drug therapy: ND	-Mixed mild + moderate sAD (MMS 19-20 +/-1) - ApoE status: ND -MPA cyclic only in 9/20 subjects

									-BMI ≥35 -4 years from AD diagnosis -Mixed gradual + abrupt menopause
Asthana et al., 2001 [111]	20	61-90	0.10 mg E2	TD	8w+8 w of follow up	Surgical+ Natural	**No effect BS:** MMSE, BMICT, BNT, TMT, Story recall, TVS, SCWIT, VP,OMDR **Positive effect BS:** recent verbal memory in BSRT(p=0.049) when one good preforming subject was omitted from E group (p=0.07) and recent visual memory (p=0.03) in Figure Copy /memory test **No effect WS**	-Comorbidity: ND -DM-ND -Concomitant drug therapy: antihypertensive(except βblockers), Gingko, Vitamine E, -Exclusion criteria: depression, Hachinski score >4, neurogical disease	-AD therapy withdrawn 2m before E2 -Mixed mild + moderate sAD (MMSE 10-29) - ApoE status: ND -5/10 patient in E2 and 3/10 in placebo group were on HT before entering the study -Only 10 patients per group -2-5.5 years from AD diagnose -Mixed gradual + abrupt menopause
Rigaud et al., 2003 [115]	117 (-33) **84**	75.8 (SD 6.5)	0.025 mg E2+ 100 mg P	TD+ po	28w	Surgical+ Natural	**No effect BS:** ADAS-Cog, MMES, GDS	-Comorbidity: ND -Concomitant drug therapy: rivastigmin -Exclusion criteria ND	-BS analysis only -0.7(SD1) years from AD diagnosis -MMES 10-26 - ApoE status: ND -Mixed gradual + abrupt menopause

Wharton et al., 2011 [112]	43 (-20) **23**	55-85	50 or 100 μg E2 +/- 2.5 mg MPA	TD+ po	15m	Surgical+ Natural	Results only after 3m **No effect BS:** MMSE, BMICT, CFT, VPA, PR TMT, SCWIT **Positive effect BS:** on semantic memory (BNT) in E2+/-MPA, positive effect on episodic visual memory (FMT) was more pronounced in E2+MPA.	-Comorbidity: ND -Concomitant drug therapy: ND -Exclusion criteria: Hachinski score >4, Hamilton depression scale >14, Ca of endometria or breast	Mixed mild + moderate sAD (MMSE placebo:21.8 SD6.4, E:23.5 SD 3.9) -BS analysis only -Mixed gradual + abrupt menopause -ApoE/+ status in 70% E2+/-MPA vs 71% in placebo group
Birge et al., 1997 [114]	20	≥70	0.625 mg CEE (daily)+ 5mg MPA (13d every 3m)	po+ po	9m	ND	**Positive effect WS:** CIBIC (8/10 sub), also improvement in orientation and concentration memory, TMT, paired associate learning. Controls: 5/10 declined on CIBC, none improved	-Comorbidity: ND -Concomitant drug: ND -Exclusion criteria: other forms of dementia, depression	-Only mild sAD (CDR <2) - ApoE status: ND -Cycled MPA -Type of menopause unspecified

m-months, **w**-weeks, **sub**-subjects, **n**-number, **E2**-17 β estradiol, **CEE**- conjugated equinon estron, **P**-progesterone, **MPA**- medroxyprogesterone acetate, **po**-oral, **TD**-transdermal, **WS**- within subjects (versus baseline), **BS** –between subjects, **HT**-hormone therapy, **MI**-Miocardial infraction, **CIBIC-** Clinical Interview-Based Impression of Change, **TMT**-trial making test, **APOE4**- apolipoprotein E, **BMICT**-Blessed Memory Information and Concentration Test, **BNT**-Boston Naming Test, **BSRT**-Buschke selective reminding test, **MMSE**-Mini-Mental State Examination, **ADAS-Cog**- Alzheimer disease assessment scale, **ADAS-CGIC-** Alzheimer's disease cooperative study version of the clinical global impression of change scale, **WLM**- Word list memory, **CP**- Constructional praxis, **WMS-** Wechsler Memory scale, **WAIS DSC**-Wechsler Adult Intelligence Scale–Digit Symbol-Coding, **DRS**-Dementia Rating Scale, **CDR**- Clinical Dementia Rating Scale **SCWIT**- Stroop color word interference test, **VR**- Visual reproduction, **TVS**- Treisman visual search, **AF**-atrial fibrillation, **IHD**-ischemic heart disease, **MCI**-mild cognitive impairment, **HDT**-hormone dependant tumors, **LMS**-logical memory subtest, **NDT**-New dot test, **CF**-category fluency, **LT**-Letter fluency, **EFRT**-Emotional face recognition est, **FTT**-Finger taping test, **CASI**-Cognitive abilities screening instrument, **BEHAVE-AD**- Behavioral pathology in Alzheimer's disease, **PR**-paragraph recall, **VPA**-visual paired associates, **CFT**-Complex figure test, **FMT**- figural memory test, **GDS**- global deterioration scale, **OMDR**-Oculomotor delayed response, **CERAD**- consortium to establish a registry for Alzheimer's disease, **DM**- Diabetes mellitus, **ND**-no data

Table 4. Large long-lasting, double-blind, placebo-controlled, randomized clinical trials in cognitively unimpaired postmenopausal women subjected to hormone therapy

Study/ Reference	n	Age (y)	HT			Type of menopause	Cognitive outcome		Factors that might have influenced or had no influence on cognitive outcome	
			dose/type	administration	duration		test/ all- cause dementia	MCI	inclusion/exclusion criteria specification	miscellaneous
WHIMS/ Shumaker et.al.2003 [15]; Rapp et. al.2003[16]; Espeland et.al., 2004[11];	4532	65-79	-0.625 mg CEE/d -0.625 mg CEE+2.5 mg MPA/d	-oral -oral	5 y	Mixed natural or surgical	MMSE, ADAS-Cog -Higher risk for developing dementia (CEE +/- MPA)	No effect (CEE+/- MPA)	-Co-morbidity: ND -DM: ND -Concomitant drug therapy: ND -Baseline cognitive status: ND -Postmenopausal period: ND	-No dementia subtype differentiation -Advanced age -BMI>35 -ApoE status: ND
WHISCA/ Resnick et al., 2006 [108] Resnick et al.2009 [109]	1416 88	Mean 74	0.625 mg CEE+2.5 mg MPA/d 0.625 mg CEE/d	oral oral	2.7y	Mixed natural or surgica Surgicall	CVLT, VF, BVRT, DF,DB, -Verbal learning decline, figural memory improved (CEE +MPA) -No effect on verbal and figural memory (CEE)	ND	-Co-morbidity: Hypertension (55%), DM, (5%) -Concomitant drug therapy: ND -Baseline cognitive status on word list and geometric figures -Postmenopausal period: ND	-Advanced age -ApoE status: ND

WHIMS-Y/ Espeland MA et al., 2013 [106]	1326	50-55	-0.625 mg CEE/d -0.625 mg CEE+2.5 mg MPA/d	-oral -oral	5 y	Mixed natural or surgical	EBMT, OTMT, VF, DS -No effect (CEE +/- MPA vs placebo) -VF-A (semantic memory) improved in MPA+CEE vs CEE	No effect (CEE +/- MPA)	-Co-morbidity: hypertension 21%,cardiovascular risk 78%, DM-ND -Concomitant drug therapy: ND -Baseline cognitive status. ND -Postmenopausal period: 1-12y	-No dementia subtype differentiation -ApoE status: ND -BMI 20-35
KEEPS-Cog/ Gleason CE et al., 2015 [13]	693	42-58	-0.45 mg/d CEE+200 mg/d MP -50μg E2+200 mg/d MP	-oral -TD	4y	Natural	MMSE-modified, CVLT, WMS, SCWI, TMT,CF,WAIS-3,BVRT, PF,DS, PR -No effect	ND	-Co-morbidity: mild mood disorders allowed, -DM-ND -Inclusion criteria: normal BP and lipid profile, BMI 20-34, -Concomitant drug therapy: antidepresives -Baseline cognitive status: inclusion criteria MMSE 24-30 -Postmenopausal period: 1.4y	High degree of inter/intra-group homogeneity (high education, perimenopausal age, health and ApoE status, 21.5% past use of HT)

n-number, **y-**year, **d-**day**, HT-** Hormone therapy **, MP**-micronized progesterone, **MPA**- medroxyprogesterone acetat **E2-**estradiol, **CEE-** conjugated equine estrogen, **TD**-transdermal, **KEEPS** –Kronos early estrogen prevention study, **WHISCA**- The Women's Health Initiative study of cognitive aging, **WHIMS-**The Women's Health Initiative Memory Study, **WHIMS-Y**-The Women's Health Initiative Memory Study/young, **MCI**-mild cognitive deficit, **ADAS-Cog-** Alzheimer disease assessment scale, **MMSE**-Mini Mental State exam, **CVLT-** California verbal learning test, **DF-**digits forward, **DB-**digits backward, **FTD**-finger tapping dom, **FTN**-finger tapping nondom, **WMS**- Wechsler Memory scale, **WAIS**-3. Wechsler adult intelligence scale 3 rd edition, **SCWI**-Stroop color word interference test, **TMT**- trial making test, **CF**-category fluency, **BVRT**-Benton visual retention test, **VF**-verbal fluency, **PF**- Phonemoc fluency, **PR**-Paragraph recall, **OTMT**-Oral trail making test, **DS**-Digiti span, **EBMT**-East Boston memory test, **BP**-blood pressure, **DM**- Diabetes mellitus, **BMI**-body mass index, **ApoE**- Apolipoproteine E, **ND**-no data.