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Tonic Premarin dose-dependently enhances memory, affects neurotrophin protein levels and alters gene expression in middle-aged rats

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Abstract

Premarin™ is the most commonly prescribed estrogenic component of hormone therapy, given since 1942. The current study is the first examining cognitive effects of tonic Premarin treatment in an animal model. Middle-aged ovariectomized (Ovx) rats received vehicle or one of three doses of Premarin (12, 24 or 36 μg daily). Rats were tested on a spatial working and reference memory maze battery. Both medium- and high-dose Premarin enhanced memory retention, while low-dose Premarin impaired learning and memory retention. Correlations with serum hormone levels showed that as the ratio of estrone:17β-estradiol increased, animals tended to show better working memory performance. Taken together with the dissociation of dose-specific estrogenic profiles, results suggest that higher levels of estrone, in the presence of 17β-estradiol concentrations higher than that of Ovx levels, may be beneficial for memory. Moreover, Premarin exerted dose and brain-region specific effects on BDNF and NGF protein levels, with most marked changes in cingulate and perirhinal cortices. Hippocampal gene expression profiling demonstrated significant Premarin-induced transcriptional changes in genes linked to plasticity and cognition. These findings indicate that Premarin can impact memory and the brain, and that dosing should be recognized as a clinically relevant factor possibly affecting the direction and efficacy of cognitive outcome.
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Keywords: Premarin; Estrogen; Hormone replacement; Working memory; Spatial memory; Neurotrophins; Gene expression

1. Introduction

Conjugated equine estrogen, trade name Premarin (Wyeth Pharmaceuticals, Philadelphia, PA), has been administered since 1942 and is the most widely used estrogenic component of hormone therapy in North America (Segal, 1997; Sitruk-Ware, 2002). Premarin is given unopposed to women who have undergone surgical menopause including uterus removal (Farquhar et al., 2009; The North American Menopause Society, 2003). As well, Premarin is the estrogenic component of Prempro, the most prescribed combination hormone therapy for women with a uterus (Segal, 1997; Sitruk-Ware, 2002). Clinical findings assessing cognitive effects of Premarin-containing therapies have been inconclusive. Premarin-containing therapy has been reported to improve memory in case studies (Ohkura et al., 1995), non-randomized small quasi-experimental designs (Carlson and Sherwin, 1998) and small double-blind placebo-controlled studies (Campbell and Whitehead, 1977; Kantor et al., 1973). Also, a randomized, double-blind placebo con-

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trolled crossover trial showed that Premarin treatment altered brain activation patterns in women during memory task performance (Shaywitz et al., 1999). Yet, findings from the large placebo-controlled WHI Memory Study (WHIMS), conducted by the National Institutes of Health, showed that Premarin treatment yielded a non-significant increased incidence of probable dementia and mild cognitive impairment in women 65 and over (Espeland et al., 2004; Shumaker et al., 2004). Further, there was an elevated probable dementia risk, and no effect on mild cognitive impairment, in women taking Premarin+medroxyprogesterone acetate (Shumaker et al., 2003). This combination therapy also had a negative effect on verbal memory, but a trend for positive effects on figural memory, in women 65 and over that were free of probable dementia (WHI Study of Cognitive Aging, WHISCA, Resnick et al., 2006). Together, the clinical studies indicate that Premarin-containing therapy can result in both beneficial and detrimental actions on cognition in women.

Cognitive effects of estrogen replacement have been evaluated in animal models. In young and middle-aged ovariectomized (Ovx) rodents, 17β-estradiol enhances spatial working memory (Bimonte and Denenberg, 1999; Daniel et al., 1997, 2005; Fader et al., 1999; Gibbs, 1999; Hruska and Dohanich, 2007; Luine and Rodriguez, 1994) and spatial reference memory (Bimonte-Nelson et al., 2006; El-Bakri et al., 2004; Feng et al., 2004; Frick et al., 2002; Markham et al., 2002). Like the clinical findings testing Premarin, not all animal studies testing 17β-estradiol have shown positive effects (Chesler and Juraska, 2000; Fernandez and Frick, 2004; Galea et al., 2001; Galea et al., 2002; Holmes et al., 2002; Singh et al., 1994). To date, 17β-estradiol has been the primary type of estrogen used to test cognitive effects of hormone therapy in the animal model. 17β-estradiol is the most potent naturally circulating estrogen, followed by estrone and estriol, in order of receptor affinity (Kuhl, 2005; Sitruk-Ware, 2002). Premarin is derived from the urine of pregnant mares, and is comprised of a complex mixture of estrogen sulfates that have been conjugated by the horse's liver before excretion in urine; many of the estrogens present in Premarin are unique to horses (Bhavnani, 1998). Premarin contains the sulfates of at least ten estrogens, is over 50% estrone sulfate, 20–25% equilin sulfate, and has only trace amounts of 17β-estradiol; after metabolism, the resulting biologically active circulating hormones are primarily estrone and, after estrone's conversion, 17β-estradiol, as well as equilin (Bhavnani, 2003; Sitruk-Ware, 2002). It is hypothesized that these three metabolites are primarily responsible for the estrogenic effects of Premarin (Sitruk-Ware, 2002). It is noted that there are other estrogens and related metabolites present in Premarin that could alter efficacy of 17β-estradiol effects, and may initiate effects on their own; these hormones include, but are not limited to, delta 8,9 dehydroestrone, dihydroequilin-17β and equilenin (Kuhl, 2005). Therefore, the animal studies done thus far testing the cognitive effects of 17β-estradiol cannot be directly compared to potential effects of Premarin.

Like the cognitive enhancements seen after 17β-estradiol treatment given via subcutaneous injection (Bimonte-Nelson et al., 2006; Chesler and Juraska, 2000; Daniel and Dohanich, 2001; Luine et al., 2003; Sandstrom and Williams, 2001), we recently showed cognitive enhancements after Premarin treatment given via cyclic, intermittent subcutaneous injections in middle-aged Ovx rats (Acosta et al., 2009). Specifically, with this regimen Premarin improved spatial working memory delayed-match-to-sample (DMS) plus-maze performance and attenuated overnight forgetting on the spatial reference memory Morris water maze (MWM). However, cyclic intermittent versus tonic estrogen administration may influence realization of memory benefits. With tonic estrogen treatment, estrogen receptors become down-regulated, while with cyclic intermittent estrogen treatment, estrogen receptor recycling and other physiological changes occur that may enhance ultimate responsiveness for many parameters, including learning and memory (Blaustein, 1993; Brown et al., 1996; Kassis and Gorski, 1981; Rosser et al., 1993). Women, including those enrolled in the WHI study, typically take hormone therapy as a daily oral tonic regimen, not intermittent in nature. The cognitive effects of tonic Premarin treatment have not been evaluated in an animal model.

In vitro studies provide evidence that Premarin, or components thereof, has positive effects on the brain. Premarin enhances neuronal growth and increases neuronal survival after experimentally induced insult in in vitro preparations, including in cognitive brain regions (Brinton et al., 2000a,b; Zhao and Brinton, 2006). While these in vitro experiments provide compelling evidence that Premarin could result in brain changes ultimately leading to enhancement in brain functions such as learning and memory, direct evaluations testing tonic Premarin's effects on cognition have not been done in an animal model.

Discerning the mechanism of the potentially cognitive enhancing effects of Premarin could have wide implications for future research and treatments for optimizing hormone therapy. Neurotrophins may be one mechanism of estrogen-induced neuroprotection or mnemonic changes. Survival and maintenance of cholinergic neurons are dependent upon neurotrophins, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF); age-related neurotrophin changes have been reported in animal models, and NGF and BDNF have been associated with cognitive function (Bimonte et al., 2003; Bimonte-Nelson et al., 2008; Granholm, 2000; Hall et al., 2000; Kesslak et al., 1998; Seigal and Chauhan, 2000). 17β-estradiol treatment significantly impacts neurotrophin systems in young and aged Ovx rats, increasing neurotrophin and its receptor mRNA levels in basal forebrain, frontal cortex and hippocampus (McMillan et al., 1996; Pan et al., 1999) and elevating NGF and BDNF protein levels in cognitive brain regions (Bimonte-Nelson et al., 2004). Whether Premarin induces cognitive change, and whether such changes are related to neurotrophin alterations, has not yet been evaluated.