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COGNITIVE EFFECTS OF LONG-TERM DYDROGESTERONE TREATMENT USED ALONE OR WITH ESTROGEN ON RAT MENOPAUSAL MODELS OF DIFFERENT AGES

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Abstract—Menopause can cause cognitive decline. Hormone Replacement Therapy (HRT) is the most effective treatment for the climacteric symptoms. However, its cognitive effect has not been well clarified, especially for the progestin component. The study investigated the effects of dydrogesterone (DG) on spatial learning and memory of the ovariectomized (OVX) rat models and the impact of aging on its cognitive effects. Eighty female Sprague–Dawley rats were included in the experiment. They belonged to two cohorts, the adult (7 months) and the aged (18 months). Each cohort was divided into five groups: Sham, OVX, OVX + E2 (OVX + 17β-estrogen), OVX + E2/DG and OVX + DG. The replacement therapy lasted for 20 weeks. Two classical behavioral tests were performed: open field test (OFT) and the Morris water maze (MWM). The breast morphology and uterine weight were obtained to assess the safety and complication of dydrogesterone. In MWM, the OVX group displayed prolonged latency and less target quadrant time than the other groups. Across 5 days' testing, all the adult groups receiving hormone therapy, except the OVX + DG group, performed better than the OVX group ($P < 0.001$); but there was no significant difference among the aged groups. The uterus weight/body weight ratio of OVX + E2/DG group was lower than the sham and OVX + E2 group. The mammary glands of OVX + E2/DG group displayed normal structure or mild hyperplasia. The results suggested that DG-alone treatment had no significant benefit for the OVX rats of both adult and aged groups on the behavioral tests. DG combined with E2 could ameliorate cognition in adult rats with uterus protection and without breast harm. The cognitive-improve effects were more remarkable for the adult rats than the aged ones. The findings support the potential clinical application of dydrogesterone combined with estrogen in preventing cognitive decline, especially for the early iatrogenic menopausal women. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: estrogen, dydrogesterone, cognition, ovariectomy, Hormone Replacement Therapy, Morris water maze.

INTRODUCTION

Many cognitive abilities deteriorate across aging in humans, especially for women. Along with the menopause, the ovarian production of estrogen (E2) and progesterone (P4) decreases rapidly. The changes have the potential to influence the function of the central nervous system. It is reported that the incidence of mild cognition impairment (MCI) and Alzheimer's disease (AD) is dramatically increased in postmenopausal women (Davey, 2013). Results from multiple large-scale survey studies also show that 62–70% of the postmenopausal women report noticeable cognition and memory complaints (Betti et al., 2001; Mitchell and Woods, 2001). Thus the cognitive decline is not only an issue of aging, but also associated with gonadal steroid deficiency. Several epidemiologic observations suggest that women who experience iatrogenic menopause at a younger age exhibit higher morbidity of the cognitive decline than the aged ones (Shuster et al., 2010; Riley Bove et al., 2014; Rocca and Henderson, 2014). The duration of hormone loss, rather than the senility, appears to be the determinant factor of the cognitive status. Premature menopause or early menopause is related to a higher risk of dementia (de Villiers et al., 2013). Cognitive decline has become the hotspot of research.

Hormone Replacement Therapy (HRT) has been used for many years to alleviate climacteric complaints. Estrogen is generally regarded as effective to improve cognition and to delay the onset of Alzheimer's disease. For women with a uterus, progestin should be added to the systemic estrogen due to its endometrial protection effect (Persson et al., 1996; Wiederpass, 1999). However, the role that progestin plays in the cognition domains has not been well clarified.

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Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; CEE, conjugated equine estrogen; DG, dydrogesterone; E2, 17β-estrogen; HRT, Hormone Replacement Therapy; MPA, medroxyprogesterone acetate; MWM, Morris water maze; OFT, open field test; OVX, ovariectomized; P4, progesterone; WHI, The Women's Health Initiative.

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The Women's Health Initiative (WHI) trial is the first large-scale, rigorously designed clinical trial to exam the effects of hormone treatment on many aspects of women's health. Results from the WHI indicate that medroxyprogesterone acetate (MPA), the most commonly used synthetic progestin in HRT, combined with conjugated equine estrogen (CEE) fails to prevent the age-related cognitive decline and even increases the risk of dementia in menopausal women (Rapp et al., 2003; Shumaker et al., 2003; Coker et al., 2010). Subsequent researches compare the central effects of several progestins and show that different progestins exert different neural effects (Cagnacci et al., 2004). The progestin (MPA) investigated in WHI is less neuroprotective than the natural progesterone (Singh and Su, 2013). In addition, people enrolled in WHI are already longer than 10 years after menopause. Thus, two factors are raised which may be responsible for the inconsistent results: the type of the progestin and the age of the experimental participants.

The cognitive effects of progestins (such as MPA and natural P4) have been widely explored in numerous animal studies. It is suggested that MPA is not only detrimental to cognition, but also antagonized to estrogen's cognition-enhancing effect (Braden et al., 2010, 2011; Coker et al., 2010). Nevertheless, results are equivocal regarding the effects of natural P4 on cognition (Bimonte-Nelson et al., 2004; He et al., 2011). When combined with estrogen, few studies show their synergistic effect on enhancing cognition, while some show a neutral effect (Gibbs, 2000; Markham et al., 2002; El-Bakri et al., 2004a) and others show an antagonistic effect (Bimonte-Nelson et al., 2006; Harburger et al., 2007). These conflicting results emphasize the need to further investigate the cognitive effects of progestins.

DG, dydrogesterone (6-dehydro-retroprogesterone) is a synthetic retro-progesterone developed by Reerink et al. in the 1950s (Reerink et al., 1960). It is very close to the natural P4 in structure but with greater oral availability and receptor selectivity. After oral administration, it is excreted mainly in the urine. The mean elimination half-lives of DG and its main metabolite 20α-dihydrodydrogesterone (5–7 h and 14–17 h, respectively) are much longer than that of the natural P4, which is 15 min (Schindler, 2009b). In contrast to P4, all the metabolites maintain the 4,6-diene-3-one structure, which makes them progestationally active (Amsterdam et al., 1980). It is 10–20 times more orally potent than the natural P4. Besides, the unique retro-structure makes it different from other progestins in that it binds almost exclusively to the progesterone receptor (PR) (Schindler et al., 2003; Schindler, 2009b,a; Rizner et al., 2011). Thus, it has no estrogenic, androgenic or adrenocorticoid activity and shows limited side effects. In short, DG is suitable for clinical oral-use with less side-effects.

DG has been widely used in HRT. Clinical data show that oral E2/DG combination displays great safety and efficacy in alleviating menopausal symptoms (Mueck et al., 2009; Stevenson et al., 2013). Most importantly, DG provides good endometrial protection (Jaakkola et al., 2009) and shows advantages over the other kind of progestins in preventing breast cancer (Fournier et al., 2008a,b; Lyytinen et al., 2009; Schneider et al., 2009). Moreover, E2/DG therapy shows to be effective in relief of cardiovascular risk, osteoporosis-related fractures and neuropsychic symptoms (Lees and Stevenson, 2001; Cagnacci et al., 2004; Stevenson et al., 2010). However, not so perfect progestins role, the research on DG's role on cognition is still a blank. There are few published clinical or pre clinical studies that have investigated the effects of DG in a combined HRT regimen on cognition.

The objective of the present study is to investigate the chronic effects of DG on the cognitive functions in the menopausal rat models and also the impact of aging on DG's effect. In the present research, it is expected that DG alone therapy would improve the ovariectomized (OVX) rats' performance of spatial learning and memory; when combined with E2, DG might not antagonize the cognitive enhancing effect of E2. It is also hypothesized that the hormone effects would be more remarkable for the adult rats than the aged ones. To investigate the two hypotheses, rats at two stages of reproductive aging are included. Three kind of hormone replacement treatments are administered immediately after OVX: E2 alone, E2/DG combination and DG alone. Both the cognitive effect of DG and the role DG plays in regulating the neural actions of E2 are assessed by comparing the behavioral performance of the hormone treatment groups with the control groups. Data of the uterine weight and the breast morphology are also collected to help to assess the safety and complication of DG.

EXPERIMENT PROCEDURES

Animals

Eighty female Sprague–Dawley rats were included in this experiment. They belong to two cohorts, the adult (7 months) and the aged (18 months). They were obtained from the Experimental Animal Center of the Xuzhou Medical College (Xuzhou, China). Rats were housed 2–3 per cage in a room maintained on a 12-h:12-h light/dark schedule. The room temperature (23 ± 2 °C) and humidity (60%) were rigidly controlled. *Ad lib* access to water and food was provided.

All procedures were conducted according to the guidelines set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Special care was taken to minimize animals' suffering. This experiment was approved by the Ethics Committee on Animal Experiments of the Qilu Hospital of the Shandong University.

Ovariectomy (OVX)

After no less than one week of habituation, ovariectomy was carried out under Pentobarbital sodium anesthesia (30-mg/kg, intraperitoneal injection, i.p.). Sterile surgery was conducted according to the technique described by Robertson et al. (1984). Bilateral ovaries were removed thoroughly via the dorsal incisions. In the control groups of rats, which underwent a Sham surgery, only a small

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