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Effect of miroestrol on ovariectomy-induced cognitive impairment and lipid peroxidation in mouse brain

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ABSTRACT

Miroestrol (MR) is a phytoestrogen isolated from *Pueraria candollei* var. *mirifica* (KwaoKrueaKhao), a Thai medicinal plant used for rejuvenation. We examined the effects of MR on cognitive function, oxidative brain damage, and the expression of genes encoding brain-derived neurotrophic factor (BDNF) and cyclic AMP-responsive element-binding protein (CREB), factors implicated in neurogenesis and synaptic plasticity, in ovariectomized (OVX) mice. OVX decreased serum 17β-estradiol level and uterine weight. OVX also impaired object recognition performance in the novel object recognition test and spatial cognitive performance in the Y-maze test and the water maze test. Daily treatment of MR dose-dependently attenuated OVX-induced cognitive dysfunction. Moreover, OVX mice had a significantly increased level of thiobarbituric acid-reactive substances, and down-regulated expression levels of BDNF and CREB mRNAs in the hippocampus and frontal cortex. MR treatment as well as hormone replacement therapy with 17β-estradiol significantly reversed these neurochemical alterations caused by OVX. These results suggest that MR ameliorates cognitive deficits in OVX animals via attenuation of OVX-induced oxidative stress and down-regulation of BDNF and CREB mRNA transcription in the brain. Our findings raise the possibility that MR and *Pueraria candollei* var. *mirifica*, the plant of origin of MR, may have a beneficial effect on cognitive deficits like AD in which menopause/ovariectomy are implicated as risk factors.

Introduction

Ovarian hormone 17β-estradiol has a wide variety of functions in the central nervous system, especially in cognition, learning, and memory, and also exerts a protective effect against oxidative stress-mediated degenerative conditions. Therefore, the decrease in the 17β-estradiol level after menopause or ovariectomy is known to enhance the incidence of inflammatory pathology involving oxidative stress (Pozzi et al. 2006) and can be a risk factor for neurodegenerative diseases such as Alzheimer's disease as well as cardiovascular dysfunction. Recent studies have suggested the preventive effects of hormone replacement therapy (HRT) or phytoestrogen supplement therapy on oxidative stress-mediated neurodegenerative disorders (Xu et al. 2007). However, it has been demonstrated that HRT in postmenopausal women is likely to be relevant to the development of breast, cervix, and endometrial cancer (Zucchetto et al. 2009). Thus, an alternative phytoestrogen might be of benefit compared with conventional HRT with a poor safety profile or side effects.

Miroestrol (Fig. 1), a potent phytoestrogenic compound, is a chromene derivative with a chemical structure similar to estradiol. However, unlike 17β-estradiol, miroestrol is not a steroidal compound. Miroestrol was isolated from the tuberous root of *Pueraria candollei* var. *mirifica* (KwaoKrueaKhao), which belongs to the family Leguminosae. This plant has long been used in Thai traditional medicine for rejuvenation in the elderly. The other active compounds from the tuberous roots of *P. candollei* are isoflavonoids, such as peurarin, daidzin, genistin, daidzein, and genistein. Recent evidence showed that miroestrol binds with higher affinity to the estrogen receptor α than other isoflavonoids isolated from this plant (Sugiyama et al. 2009). Moreover, it increased the levels of glutathione and the activity of antioxidant enzymes in the liver and uterus of β-naphthoflavone-treated mice (Jearapong et al. 2013) and exhibited a preventive effect against

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bone loss in ovariectomized mice (Udomsuk et al. 2012). However, the effects of miroestrol on cognitive function and oxidative stress in the brain in ovariectomized mice have not been investigated.

The main objectives of this study were to investigate the effect of miroestrol on ovariectomy-induced cognitive dysfunction and oxidative brain damage in mice and to compare the effects of miroestrol with those of 17β-estradiol. This study also aimed to examine the possible involvement of the expression of genes encoding brain-derived neurotrophic factor (BDNF) and cAMP-response element-binding protein (CREB) in the effect of miroestrol, since the translated forms of these genes play important roles in learning and memory via neurogenesis and synaptic plasticity.

Materials and methods

Plant materials and isolation of miroestrol

Tuberous root bark of *P. candollei* var. *mirifica* was collected in Ubon Ratchathani, Thailand, in March 2010 and was identified by Dr. Thaweesak Juengwatanatrakul, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand. The reference specimen (NI-PSKKU 007–010) was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Ten kilograms of dried tuberous root bark of *P. candollei* var.*mirifica* was powdered and extracted by maceration method with hexane 3 × 20 l, and the product of maceration was extracted with ethyl acetate 3 × 20 l. The ethyl acetate crude extracts were combined, evaporated, and fractionated by column chromatography (Silica gel 60 with hexane:ethyl acetate 3:1, 3:2, 1:1, and 0:1 v/v). The fractionated samples were combined and evaporated at 60 °C. Purification of the miroestrol-rich fraction continued using high-performance liquid chromatography (HPLC). The HPLC separation was performed using a TSK gel C18 reverse phase column (5 μm, 2 × 60 × 2 cm) and a mobile phase consisting of 16% acetonitrile with a flow rate at 45.0 ml/min. The UV detection wavelength was set at 205 nm for obtaining chromatograms. Miroestrol (Fig. 1) was identified using ^{1}H NMR and ^{13}C NMR spectra and compared to the previous report (Chansakaow et al. 2000). The purity of miroestrol applied in this study was >99%, according to the quantitative analysis of our previous report (Chatuphonprasert et al. 2013).

Animals

Fifty-two 5-week-old female mice were obtained from the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand). The animals were housed in a light-controlled room with a 12-h light/dark cycle and were allowed free access to food and water in the Laboratory Animal Unit of the Faculty of Pharmaceutical Sciences (Khon Kaen University, Khon Kaen, Thailand). All animal research procedures used in the present study were in accordance with the Guiding Principles for the Care and Use of Animals (NIH Publications No. 80-23, revised in 1996). The present study was also performed in accordance with the Animal Ethics Committee for Use and Care, Khon Kaen University, Khon Kaen, Thailand (Approval No. AEKKU 01/2555).

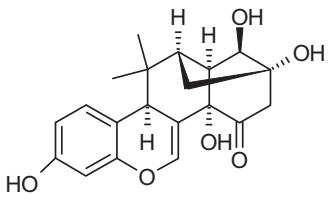

Fig. 1. Chemical structure of miroestrol.

Surgical procedure

To mimic an estrogen-deprived state in animals, OVX mice were used in this study. The animals underwent bilateral ovariectomy via dorsolateral incision under pentobarbital anesthesia (Nembutal: 60 mg/kg; Ceva Sante Animale, France). The exposed ovary and associated oviduct were removed and then, the skin incisions were closed. The sham-operated group underwent the same procedure without removal of the ovaries. After a 3-day recovery period, the animals were divided into five groups: (1) sham, (2) ovariectomy (OVX), (3) ovariectomy + 1 μg/kg 17β-estradiol, (OVX + E2), (4) ovariectomy + 0.1 mg/kg miroestrol (OVX + MR 0.1), and (5) ovariectomy + 1 mg/kg miroestrol (OVX + MR 1). The sham and OVX control groups were intraperitoneally administered corn oil 0.2 ml per mouse once daily for 8 weeks. 17β-estradiol and miroestrol were dissolved in corn oil and intraperitoneally administered 0.2 ml per mouse once daily for 8 weeks. In order to determine the period of drug treatment after OVX surgery, we evaluated the effect of ovariectomy on learning and memory activities in different periods (4 and 8 weeks) after surgery using Y-maze test at first, in preliminary experiments. We found that 8-weeks treatment period showed significant improvement in cognitive function (data not shown). To assess the effects of 17β-estradiol and miroestrol in OVX mice, the drugs were administered 1 h before the behavioral test.

Y-maze test

The Y-maze was used to elucidate the hippocampus-dependent spatial working memory of the animals. The Y-maze consisted of three arms 40 cm long, 18 cm high, 3 cm wide at the bottom, and 12 cm wide at the top, which were positioned at equal angles. The maze floor and walls were constructed from dark opaque polyvinyl plastic. One hour after the drug administration, the Y-maze test was conducted. The animals were individually placed on one arm, and the sequence of arm entries was recorded manually over an 8-min period. An actual alternation was defined as entries into all three arms on consecutive choices (i.e. 123, 312, or 231, but not 212). When all fours limbs were within an arm, an animal was judged to have entered it. The percentage of alternation was calculated according to the following equation:

$$\%\,\text{Alternation} = \frac{\text{Number of alternations}}{\text{Total arm entries} - 2} \times 100.$$

Novel object recognition test (ORT)

The novel object recognition test was carried out as described previously (Le et al. 2013; Zhao et al. 2012). The apparatus consisted of a square arena (50 cm × 50 cm × 40 cm high). The height of the objects was sufficient to prevent the mice from climbing on them. The ORT consisted of three different sessions: habituation, sample phase trial, and test phase trial. About 24 h before the test, each mouse was individually habituated to the test box, with 10 min exploration in the absence of objects. In the sample phase trial, each mouse was placed into the observation box where two identical objects (objects O1 and O2) were placed in two adjacent corners and was allowed to explore for 5 min. The animals were considered to be exploring the object when the head of the animal was facing the object or when the animal was touching or sniffing the object. The time spent exploring each object was recorded. In the test phase trials performed 30 min after the sample phase trials, one of the two objects was replaced by a novel object. The total time spent