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J Pharmacol Sci **120**, 89 – 97 (2012)

Journal of Pharmacological Sciences

Full Paper

Placental Extract Improves Hippocampal Neuronal Loss and Fear Memory Impairment Resulting From Chronic Restraint Stress in Ovariectomized Mice

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Received May 10, 2012; Accepted July 26, 2012

Abstract. We have recently found that combination of ovariectomy (OVX) and chronic restraint stress causes cognitive dysfunction and reduces hippocampal CA3 neurons in female rats and mice and that estrogen replacement and chronic treatment with *Ginkgo biloba* extract EGb 761 suppress the OVX/stress-induced behavioral and morphological changes. In this study, we examined the effect of placental extract on the memory impairment and neuromorphological change in OVX/stress-subjected mice. Female Slc:ICR strain mice were randomly divided into four groups: vehicle-treated OVX, porcine placental extract (120 and 2160 mg/kg)-treated OVX, and sham-operated control groups. Two weeks after surgical operation, OVX mice underwent restraint stress for 21 days (6 h/day), and all animals were then subjected to a contextual fear conditioning test followed by morphological examination by Nissl staining. Placental extract was orally administered once daily until the behavioral analysis was carried out. Chronic treatment with both doses of placental extract improved the OVX/stress-induced fear memory impairment and Nissl-positive cell loss of the hippocampal CA3 region, although it did not affect the loss of bone mineral density and increase in body weight after OVX. These results have important implications for the neuroprotective and cognition-enhancing effects of placental extract in postmenopausal women.

Keywords: postmenopausal animal model, chronic stress, ovariectomy, cognition, hippocampus

Introduction

Memory loss is the most common complaint of women going through the phases of menopause (1), and the postmenopausal memory decline might be associated with reduced ovary functions, which lead to depletion of ovarian hormones such as estrogen (2, 3). Accordingly,

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Published online in J-STAGE on September 6, 2012 (in advance)
doi: 10.1254/jphs.12115FP

estrogen or hormone replacement therapy has been shown to improve cognitive function in postmenopausal women (4 – 7). However, compliance with long-term treatment with estrogen for menopausal women is poor because of side effects. In addition, the Women's Health Initiative Memory Study reported that hormone replacement therapy increased the risk of developing memory deficits in postmenopausal women of 65 years of age or older (8, 9). Therefore, the development of safer and more effective drug therapies has been strongly anticipated by postmenopausal women with memory deficits.

Placenta contains a great variety of bioactive molecules, such as hepatocyte growth factor (10), nerve growth factor (11), epidermal growth factor (12), fibroblast growth factor (13), insulin-like growth factors (14), and transforming growth factors (15, 16), as well as estrogens (17, 18), and has growth-promoting activity. In fact, the extract of human placenta has been used as a traditional folk remedy in many Asian countries for the treatment of liver diseases and skin disorders (19). In addition, a recent randomized clinical trial demonstrated that chronic treatment of human placental extract improved some menopausal symptoms and fatigue in middle-aged women (20). However, the clinical benefits of placenta therapy for menopausal symptoms and the effects against menopausal memory decline remain unclear.

We have recently found that combination of ovariectomy (OVX) and chronic restraint stress (CS) causes memory impairment and reduces hippocampal CA3 neurons in female rodents and that estrogen treatment suppresses the CS-induced behavioral and morphological changes (21, 22). That is, we have revealed that CS-subjected OVX animals can be considered as a useful model of postmenopausal memory deficits. In this study, we examined the effects of chronic treatment of porcine placental extract on CS-induced behavioral, neurochemical, and tissue changes in OVX female mice. After chronic stress, conditioned fear performance was tested. Following the behavioral test, hippocampal cell density, bone mineral density, and uterine weight were evaluated.

Materials and Methods

Materials

Bulk powder of JPB Porcine 100 (Lot No. P-60701; Japan Bio Products Co., Ltd., Tokyo) was used for placental extract administration. According to the product information provided by the company, the bulk powder of placental extract is produced as follows: the Japanese domestic porcine (*Sus scrofa domesticus*) placentae are treated with protease and heat sterilization, and then the resulting extract is freeze-dried and ground. The resulting placental extract is assumed to contain estrogenic hormones, growth factors, and other biologically active substances. Generally, for clinical use, one ample including 112 mg of human placental extract can be used per day per person by intramuscular or subcutaneous injection. In this study, the proper oral dosage was calculated to be 120 mg/kg with reference to one ample for a 60 kg human, using the formula for dose translation based on body surface area (23). In accordance with previous animal studies (24, 25), the present study was further designed to examine the effect of a high dose of placental extract (2160 mg/kg).

Animals and treatments

Two cohorts initially totaling 47 female ICR mice (Japan SLC, Inc., Hamamatsu), age 8 – 9 weeks, weighing 26 – 33 g at the beginning of the experiments, were used. They were housed 4 – 6 per cage under standard light-dark conditions (12-h light cycle starting at 8:45 h) at a constant temperature of 23°C ± 1°C. The animals had free access to food and water and they were handled in accordance with the guidelines established by the Institutional Animal Care and Use Committee of Kanazawa University, the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society, and the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals.

One week after arrival, a quarter of the experimental animals underwent sham operation and the rest of them were bilaterally ovariectomized (OVX) under pentobarbital (40 mg/kg) anesthesia. From the next day of surgical operation, OVX animals were randomly divided into three groups (group 1 – 3), and then the animals of group 1 and group 2 were orally administered with placental extract at 120 and 2160 mg/kg, respectively, once daily to the end of behavioral analysis. The OVX mice of group 3 and sham-operated mice were orally treated with vehicle (distilled water) for 5 weeks before the behavioral analysis. Body weight of each mouse was measured before drug treatment and recorded every day.

Immobilization stress began 2 weeks after the operation and was repeated every day for 3 consecutive weeks. The stress was performed with a stainless mesh that allowed for a close fit to mice for 6 h (between 9:00 am and 3:00 pm) (21, 22, 26, 27) in their home cages. Sham-operated animals were not subjected to stress (No stress group), but were handled at 9:00 am for a few seconds. Following the repeated restraint stress for 3 weeks, behavioral analysis was performed and then mice were killed for histochemical and morphological analyses.

Contextual fear conditioning test

The day after the stress period, a mouse was placed in a plastic cage (25 × 31 × 18 cm), and the freezing response was measured for 1 min in the absence of sound (pre-tone phase). Each mouse was placed in an acrylic cage (31 × 31 × 40 cm) equipped with stainless grids (2 mm diameter, 6 mm pitch) and was allowed to explore the cage freely for 2 min (pre-conditioned phase), and then a 20-s tone (80 dB) was delivered (conditioned stimulus). During the last 5 s of the tone stimulus, a foot shock of 0.8 mA was delivered as an unconditioned stimulus through a shock generator. This procedure was repeated four to six times with 15-s intervals until the mice showed over 12-s freezing during the interval. Then 24 h after the conditioning, tone- and context-dependent tests were carried out. For the tone-dependent test, the freezing response was measured in the neutral cage for 1 min in the presence of a continuous-tone stimulus identical to the conditioned stimulus. For the context-dependent test, mice were placed in the training cage, and the freezing response was measured for 2 min in the absence of the conditioned stimulus (28).

Histochemical analysis

After completion of behavioral analyses, mice were deeply anesthetized with pentobarbital and perfused intracardially with 4% paraformaldehyde in phosphate-buffered saline (PBS). The brains were removed, post-fixed with the same fixative and cryoprotected with 30% sucrose-containing PBS. Sections (20 μm) containing hippocampus were obtained using a rotary microtome (HM505E; Microm International GmbH, Walldorf, Germany), mounted on slides and stored at −80°C until use.

Nissl staining was carried out as previously reported (21, 27). Digitized images of the Nissl-stained sections were obtained with a cooled CCD digital camera (AxioCam MRc5; Carl Zeiss GmbH, Jene, Germany) mounted on a phase-contrast microscope (Axio Imager A1, Carl Zeiss) using a 20 × magnification lens. Nissl-positive neuronal cell numbers were manually and rigidly counted within the hippocampal pyramidal cell layer (CA1 and CA3 regions) and the dentate gyrus (DG) of the scanned digital images. The total cell counts were averaged from at least three sections per animal.

Photomicrographs were taken using a microscope digital camera system (AxioCam / Axio Imager, Carl Zeiss) at 5 × magnification. To prevent variability in staining due to each experimental procedure, the brains of 4 – 6 mice, equated across experimental groups, were processed at the same slide using the same reagents and temperature conditions.

Measurement of uterine weight and bone mineral density

After the perfusion with 4% paraformaldehyde in PBS, the uterus was removed and weighed. The femurs were removed from the hind legs and stored in PBS containing 4% paraformaldehyde. The bone mineral density of femur was measured using a dual X-ray absorptiometer (DCS-600R; Aloka Corp., Tokyo) (21, 27).

Statistical analysis

Statistical analysis of the experimental data was carried out using Prism 5 for Mac OS X (GraphPad Software, San Diego, CA, USA). The significance of differences was determined by one- and two-way repeated measures ANOVA, followed by the Tukey's multiple comparison test and the Bonferroni *post hoc* test, respectively, for multigroup comparisons. The unpaired *t*-test was used for two-group comparisons. The criterion for statistical significance was $P < 0.05$.

Results

Effect of placental extract on OVX-induced gain in body weight of mice

Female mice (weight: 28.7 ± 0.3 g, n = 49) were randomly divided into four groups: sham-operated control (n = 13), vehicle-treated OVX (n = 12), low-dose placental extract (120 mg/kg)-treated OVX (n = 12) and high-dose placental extract (2160 mg/kg)-treated OVX groups (n = 12). There was no significant difference in body weight between the four groups ($P > 0.05$ by one-way ANOVA). Two weeks after surgical operation, OVX mice showed a significant increase in body weight compared with the sham-operated control (196% of sham-operated controls, $P < 0.001$ by Tukey's multiple comparison test) (Fig. 1). Chronic treatment with placental extract (120 and 2160 mg/kg) did not affect the OVX-induced gain in body weight (120 and 2160 mg/kg: 177% and 177% of sham-operated controls; $P > 0.05$ and $P > 0.05$ vs. OVX group by Tukey's multiple comparison test, respectively) (Fig. 1), and a significant difference disappeared between the groups after the stress period ($P > 0.05$ between groups by one-way ANOVA) (data not shown).

Effect of placental extract on OVX/CS-induced impairment of conditioned fear memory in mice

We have already shown that OVX mice exposed to repeated daily restraint stress (6 h/day) for 3 weeks exhibit decreases in context- and tone-dependent freezing 24 h after fear conditioning, although the OVX or CS alone does not affect the fear memory observed in sham-operated control mice (22). Similar to the previous find-

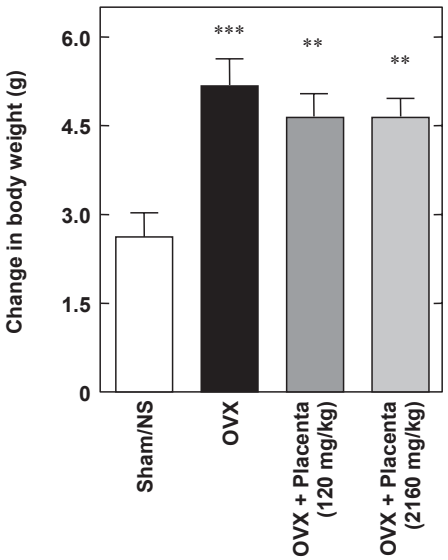

Fig. 1. Effect of placental extract treatment on OVX-induced increase in body weight of female mice. Mice were bilaterally ovariectomized or sham-operated and the body weight of each mouse was measured every day before drug treatment. Placental extract (120 or 2160 mg/kg per day) or vehicle was orally administered from the day after the operation. Results show body weight 2 weeks after the operation as means ± S.E.M. [sham-operated no stress (Sham/NS) group: n = 13; OVX group: n = 12; 120 mg/kg placental extract–treated OVX group: n = 12; 2160 mg/kg placental extract–treated OVX group: n = 12]. $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from the Sham/NS group; one-way ANOVA ($P < 0.0001$) and *post hoc* Tukey's multiple comparison test.

ing (22), the OVX/CS caused decreases in context- (Fig. 2C, left) and tone-dependent freezing (Fig. 2C, right) 24 h after fear conditioning, compared with the sham-operated–no stress (Sham/NS) group (context and tone: 32% and 45% of Sham/NS controls; $P < 0.01$ and $P < 0.001$ by Tukey's multiple comparison test, respectively). Chronic treatment with placental extract (120 and 2160 mg/kg) for 5 weeks significantly ameliorated the OVX/CS-induced decreases in context- (120 and 2160 mg/kg: 94% and 90%, $P < 0.01$ and $P < 0.05$ vs. the OVX/CS group by Tukey's multiple comparison test, respectively) (Fig. 2C, left) and tone-dependent freezing (120 and 2160 mg/kg: 87% and 91%, $P < 0.05$ and $P < 0.05$ vs. OVX/CS group by Tukey's multiple comparison test, respectively) (Fig. 2C, right) in mice without affecting the fear behaviors during the pre-conditioning (context and tone: $P > 0.05$ and $P > 0.05$ between groups by one-way ANOVA, respectively) (Fig. 2A) and conditioning sessions of the test (interval, $P < 0.0001$, group; $P > 0.05$, group × interval; $P > 0.05$ by two-way repeated measures ANOVA) (Fig. 2B).

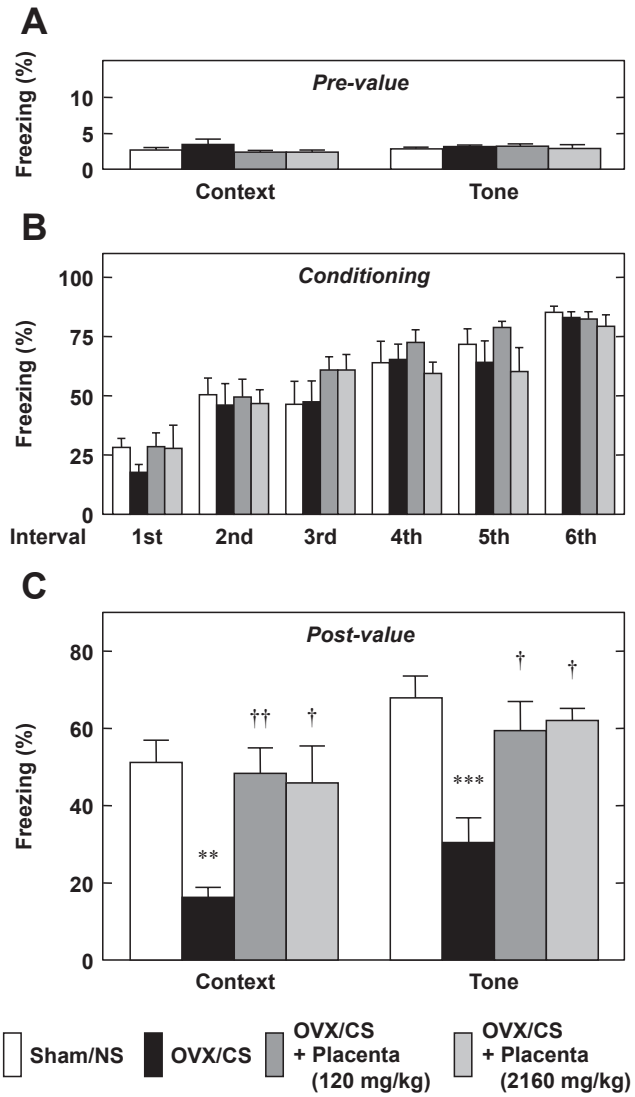

Fig. 2. Effect of placental extract on OVX/CS-induced impairment of conditioned fear memory in mice. Mice were bilaterally ovariectomized or sham-operated and then OVX animals were exposed to CS for 3 weeks. Following the CS period, all mice were trained by the pairing of an auditory conditioned stimulus and a foot-shock unconditioned stimulus and tested 24 h later for context- and tone-dependent freezing. Placental extract (120 or 2160 mg/kg per day) or vehicle was orally administered from the day after the operation to the end of behavioral analysis. Freezing time in pre-conditioning (A), conditioning (B), and 24-h later test sessions (C) was measured and is expressed as % of total time. Results represent means ± S.E.M. (Sham/NS group: n = 13, OVX/CS group: n = 9, 120 mg/kg placental extract–treated OVX/CS group: n = 11, 2160 mg/kg placental extract–treated OVX/CS group: n = 8). $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from the Sham/NS group; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, significantly different from the OVX/CS group; one-way ANOVA (A: context, $P > 0.05$; tone, $P > 0.05$. C: context, $P < 0.01$; tone, $P < 0.001$), two-way repeated measures ANOVA (B: group, $P > 0.05$; interval, $P < 0.0001$; group × interval, $P > 0.05$) and *post hoc* Tukey's multiple comparison test.

Effect of placental extract on OVX/CS-induced neuronal cell loss in the hippocampal CA3 region of mice

Our previous study also demonstrated that the combination of OVX and repeated restraint stress decreases the neuronal cell numbers in the hippocampal CA3 region, compared with those of the other three groups: stress alone, OVX alone, and sham-operated control groups (22). Figure 3 shows typical microscopic images of the Nissl-stained hippocampal CA3 region. In agreement with the previous finding (22), the OVX/CS caused approximately 20% – 25% of Nissl-positive cell loss with corresponding decrement in cell layer thickness in the hippocampal CA3 region (Fig. 3B), compared with the Sham/NS controls (Fig. 3A). Chronic treatment with placental extract (120 and 2160 mg/kg) for 5 weeks protected against the Nissl-positive cell loss in the hippocampal CA3 region of OVX/CS mice (Fig. 3: C, D). Table 1 shows the numbers of Nissl-positive cells in the CA1 and CA3 regions and DG of the hippocampus. Chronic treatment with placental extract specifically improved the OVX/CS-induced decrease in Nissl-positive cell numbers in the CA3 region (77% of Sham/NS controls, $P < 0.01$ vs. Sham/NS group by Tukey's multiple comparison test) to the control levels (120 and 2160 mg/kg: 95% and 98%, $P < 0.05$ and $P < 0.01$ vs. OVX/CS group by Tukey's multiple comparison test, respectively). In contrast, chronic treatment with placental extract did not affect the Nissl-positive cell numbers in the CA1 region and DG of the hippocampus, in which the OVX/stress did not cause neuronal cell loss.

Effects of placental extract on changes in bone mineral density and uterine weight in OVX/CS mice

After behavioral analysis, bone mineral density and uterine weight were evaluated. We preliminarily determined that OVX alone caused decreases in bone mineral density and uterine weight in normal female mice and that CS did not affect the bone mineral density and uterine weight in both sham-operated and OVX mice (unpublished observation). We found that chronic treatment with 17β-estradiol improved the loss of bone mineral density and abnormally increased uterine weight in

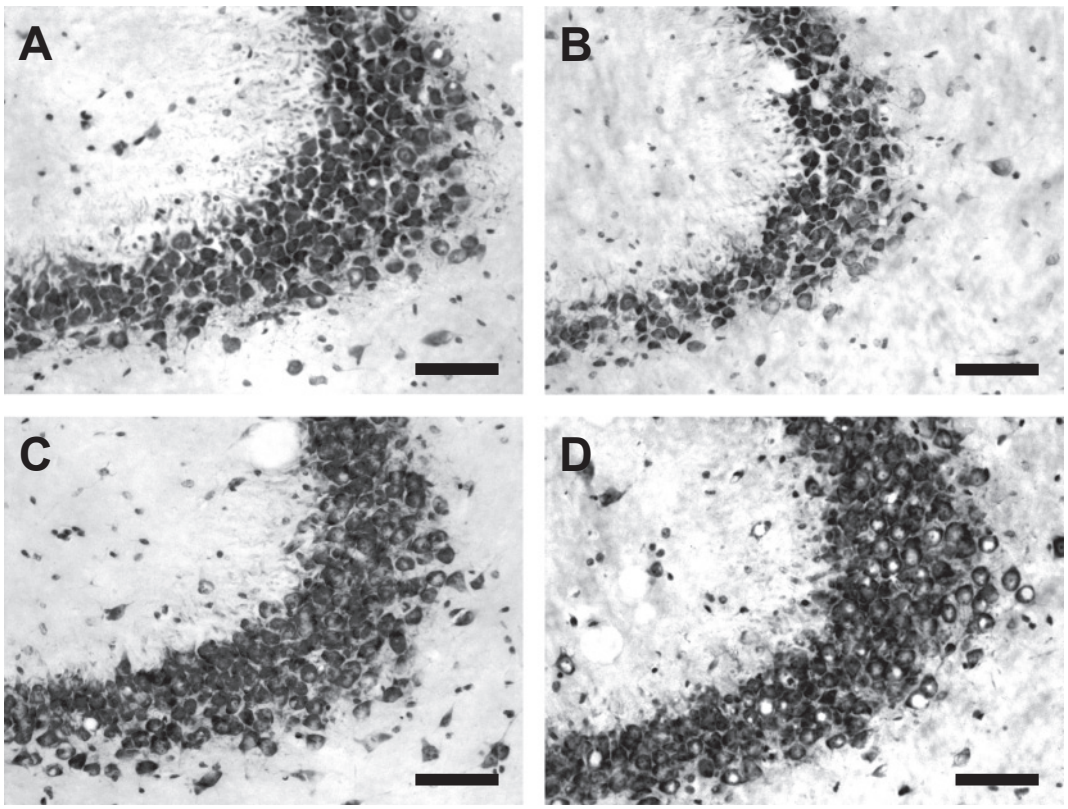

Fig. 3. Effect of placental extract on OVX/CS-induced histological changes in hippocampal CA3 region of mice. Nissl-positive cells were visualized by cresyl violet staining. Mice were ovariectomized (B, C, and D) or sham-operated (A) and then OVX animals were exposed to CS for 3 weeks. Placental extract [120 (C) or 2160 mg/kg per day (D)] or vehicle (A and B) was orally administered from the day after the operation to the end of behavioral analysis. A typical result of three independent experiments is shown. Scale bar = 100 μm.

Table 1. Effect of placental extract on OVX/CS-induced change in Nissl-positive cell number in hippocampal CA1, CA3 regions, and dentate gyrus of mice

Treatments	Nissl-positive cell number ($\times 10^3$ cells/mm^2)		
	CA1	CA3	Dentate gyrus
Sham/NS	2.59 ± 0.12	3.77 ± 0.23	7.20 ± 0.49
OVX/CS	2.57 ± 0.12	2.90 ± 0.11**	7.19 ± 0.26
OVX/CS + Placental extract (120 mg/kg)	2.79 ± 0.19	3.58 ± 0.11†	7.04 ± 0.15
OVX/CS + Placental extract (2160 mg/kg)	2.75 ± 0.11	3.70 ± 0.15††	6.44 ± 0.42

Results are shown as means ± S.E.M. with n = 5 – 6 for each group. **$P < 0.01$, significantly different from the Sham/NS group; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, significantly different from the OVX/CS group; one-way ANOVA (CA1: $P > 0.05$, CA3: $P < 0.01$, dentate gyrus: $P > 0.05$) and *post hoc* Tukey's multiple comparison test.

OVX/CS mice (22). The present study showed that the OVX/CS caused decreases in uterine weight (Fig. 4A) and bone mineral density (Fig. 4B), compared with the Sham/NS group (uterine and bone: 21% and 75% of Sham/NS controls; $P < 0.001$ and $P < 0.001$ by Tukey's multiple comparison test, respectively). This study further demonstrated that chronic treatment with placental extract (120 and 2160 mg/kg) for 5 weeks did not affect the decreases in uterine weight (120 and 2160 mg/kg: 19% and 22%, $P > 0.05$ and $P > 0.05$ vs. OVX/CS group by Tukey's multiple comparison test, respectively) (Fig. 4A) and bone mineral density (120 and 2160 mg/kg: 81% and 80%, $P > 0.05$ and $P > 0.05$ vs. OVX/CS group by Tukey's multiple comparison test, respectively) (Fig. 4B) in OVX/CS mice.

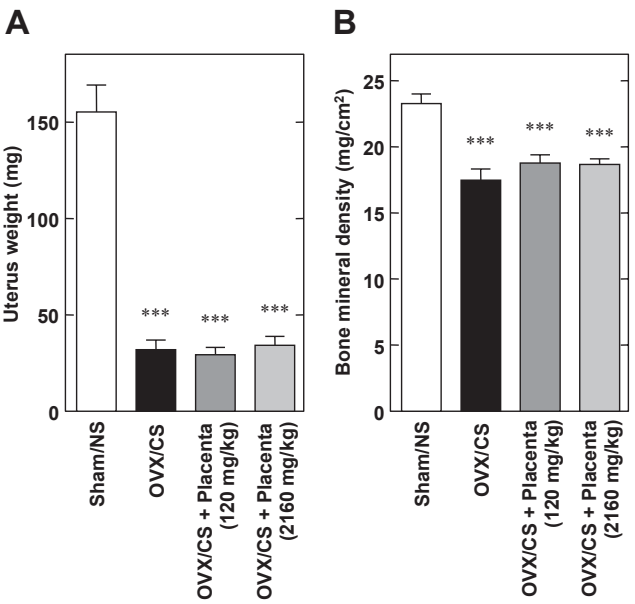

Fig. 4. Effects of placental extract on changes in uterine weight (A) and bone mineral density (B) in OVX/CS mice. Mice were ovariectomized or sham-operated and then subjected to CS for 3 weeks. Placental extract (120 or 2160 mg/kg per day) or vehicle was orally administered from the day after the operation to the end of behavioral analysis. Results are shown as means ± S.E.M. [Sham/NS group: n = 13; OVX/CS group: n = 10; 120 mg/kg placental extract–treated OVX/CS group: n = 9 (A), 11 (B); 2160 mg/kg placental extract–treated OVX/CS group: n = 10]. ***$P < 0.001$, significantly different from the Sham/NS group; one-way ANOVA (A: $P < 0.0001$, B: $P < 0.0001$) and *post hoc* Tukey's multiple comparison test.

Discussion

Estrogen replacement therapy has several beneficial effects, including a cognitive enhancing effect (4 – 7), in menopausal women. However, it is also known to have serious side effects including increased risk of breast and uterine cancers (29, 30). Further clinical study reported controversial results indicating a lack of efficacy of the therapy on cognition in women aged over 65 years (8). The present study was aimed to clarify how treatment with porcine placental extract influences the memory deficits and morphological changes in OVX/stress-subjected mice. We found that daily placental extract treatment attenuated fear memory impairment and hippocampal neuronal loss in OVX/stress-subjected mice, as did estrogen replacement (22). This is the first pharmacological evidence to demonstrate the neuroprotective effects of placental extract. Further, we found that treatment with placental extract did not affect the gain in body weight and decreases in bone mineral density and uterine weight in OVX mice, although estrogen replacement did have effects on them (22). We have recently reported the similar ameliorating effects of *Ginkgo biloba* extract EGb 761 in OVX/stress-subjected rats (27). Taken together, the present study suggests that placental extract is useful as a cognitive enhancer in postmenopausal women.

Our recent studies demonstrated that OVX over 5 weeks caused a significant increase in body weight and decreases in bone mineral density of femurs and uterine weight, compared with those of sham-operated control

rats (21, 27) and mice (22). In addition, these studies indicated that overburden of 21 days of repeated restraint stress (6 h/day) markedly decreased the numbers of hippocampal CA3 pyramidal neurons in the OVX animals, while each caused a slight decrease in cell number, and that memory impairment was only observed in the OVX/stress-subjected animals. Thus, in this study, all OVX mice received chronic stress to determine the effect of placental extract on memory deficits and neuromorphological change more simply. Moreover, in this study we examined the effects of placental extract at two doses (120 and 2160 mg/kg) on OVX/stress-induced pathophysiological changes, to assess drug efficacy and some aspects of potential toxicity. Similar to our previous finding on the effects of EGb 761, the results of this study indicated that placental extract at doses of both 120 and 2160 mg/kg did not cause adverse side effects and attenuated the OVX/stress-induced fear memory impairment in female mice without affecting gain in body weight and decreases in bone mineral density and uterine weight. The present study also revealed that treatment with placental extract attenuated the OVX/stress-induced loss of Nissl-positive cells in the hippocampal CA3 region. To date, there is no direct evidence indicating the relationship between the hippocampal CA3 atrophy and memory loss during menopause. On the other hand, evidence-based clinical research, which reviews the systematic identification of randomized clinical trials, demonstrates that surgical menopause may be accompanied by cognitive impairment that primarily affects episodic memory (31). In addition, a growing number of animal studies indicate that the hippocampal CA3 pathology is associated with the episodic-like memory dysfunction (32 – 34). Furthermore, Luques et al. (35) have demonstrated that a selective reduction of hippocampal cholinergic innervation is associated with impairment in episodic memory, as well as in spatial learning. Taken together with our previous findings showing that the cholinesterase inhibitors donepezil and galantamine ameliorated OVX/CS-induced memory impairment (22), the present study suggests that placental extract ameliorates memory impairment in OVX/stress mice by neuroprotection in the CA3 region and further that placental extract has therapeutic efficiency against memory problems in postmenopausal women.

Placental extracts contain a variety of bioactive molecules, such as proteins, peptides, glycosaminoglycans, amino acids, RNA, and DNA, and thus all of these may contribute to the regenerating effects (36). Among them, possible molecules that may be bioactive via oral administration are unknown. It appears that the large molecular size of proteins, such as hormones, enzymes, growth factors and cytokines, makes them unlikely candidates because orally administered proteins usually undergo intestinal digestion and cannot enter the general circulation. Interestingly, Alkam et al. (37) recently reported that chronic oral treatment with Leu-Ile, a hydrophobic dipeptide, prevents the impairment of recognition memory induced by amyloid β peptide in mice. Therefore, we speculate that possible bioactive molecules via oral administration are small in molecular size, such as dipeptides, which can enter the general circulation without intestinal digestion (38, 39). Further study is required to identify such bioactive molecules from placental extracts.

On the other hand, it is well documented that placenta contains estrogens (17, 18). Estrogens are known to exert various neuromodulating actions by the expression of neurotrophins such as brain-derived neurotrophic factor (40), which affects neuronal survival, differentiation, and synaptic plasticity (41 – 43). In addition, estrogens also play a greater role in uterine responsiveness and function (44), bone formation (45), and bone remodeling (46). Therefore, the present study indicates that estrogen could not be involved in the neuroprotective effects by placental extract because the extract had no effects on bone mineral density and the weight of the uterus in OVX mice.

Through a series of studies using OVX/stress animals (21, 22, 27), we have discovered that the combination of OVX and CS is necessary in induction of hippocampal neuronal loss and memory dysfunction. That is, our findings have demonstrated that stress-related molecules may act as a mediator of neuronal loss under the estrogen-depleted condition. Glucocorticoid is well known to be secreted in response to stress, and Kim et al. (47) have revealed that glucocorticoid receptor activation by dexamethasone, a synthetic glucocorticoid receptor agonist, blocks both proliferation and differentiation in hippocampal neurogenesis. Interestingly, Tongjaroenbuangam et al. (48) have recently showed that the administration of dexamethasone causes neuronal death in the CA3 layer of the hippocampus and that okra (*Abelmoschus esculentus* Linn.) extract and its derivatives, quercetin and rutin, protect the dexamethasone-induced neuronal death in mice. Taken together, these findings suggest that glucocorticoid-mediated mechanisms are responsible for hippocampal neuronal dysfunction. Thus, although further experiments are required to elucidate the precise mechanisms of neuroprotection by placental extracts, we suppose that bioactive molecules in placental extracts alleviate glucocorticoid-mediated hippocampal neuronal dysfunction.

In conclusion, the present study suggests that placental extract can attenuate neuronal loss of the hippocampal CA3 region and improve fear memory impairment in-

duced by the combination of OVX and environmental stress. Application of placental extract could thus offer an interesting approach to prevent memory problems in postmenopausal women.

Acknowledgments

We are grateful to Mr. Taiichi Kaku, MS (Japan Bio Products Co., Ltd., Tokyo) for providing porcine placental extract. This study was supported in part by grants for Scientific Research (19390062) and for the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a Grant-in-Aid for Scientific Research (20590083) from the Japan Society for the Promotion of Science.

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