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Polycystic ovary syndrome (PCOS)-like phenotypes in the D-galactose-induced aging mouse model

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ABSTRACT

The D-galactose (D-gal)-induced animal model, which is established by consecutive subcutaneous D-gal injections for approximately 6 weeks, has been frequently used for aging research. This animal model has been shown to accelerate aging of the brain, kidneys, liver, and blood cells. However, aging of the female reproductive organs in this animal model has not been reported. The aim of this study was to investigate changes in the ovary in the D-gal-induced aging mouse model. First, we evaluated anti-Müllerian hormone (AMH) as a marker of ovarian aging in blood plasma. We speculated there would be lower AMH levels in D-gal-treated mice because ovarian aging would be induced by D-gal, as reported for other tissues. However, the results showed that AMH levels in D-gal-treated mice were approximately four-fold higher than control mice. Abnormally high AMH levels are detected in ovarian cancer and polycystic ovary syndrome (PCOS) patients. Therefore, we examined PCOS-related markers in this mouse model. Total testosterone levels were high and abnormal estrous cycles were induced in D-gal-treated mice. These changes, including AMH levels, in D-gal-treated mice were inhibited by aminoguanidine treatment, an advanced glycation end product reducer. In addition, ovarian cysts were observed in some D-gal-treated mice. These results indicate that with respect to female reproduction, D-gal-treated mice are suitable for PCOS studies, rather than aging studies.

1. Introduction

The D-galactose (D-gal)-induced aging mouse model is established by consecutive subcutaneous D-gal injections for approximately 6 weeks [1]. This animal model shows accelerated aging in the brain, ear, kidney, liver, and blood cells [2–5]. The aged phenotype in this model may be caused by excessive formation of reactive oxygen species (ROS) and accumulation of advanced glycation end products (AGEs) due to the administered D-gal [1,6,7]. ROS damage and AGEs accumulation are well known among the many causes of aging [8]. Therefore, this animal model has been frequently used for aging studies and anti-aging screenings [4–6,9]. However, aging of the female reproductive organs in this animal model has not been reported.

Many studies of reproductive aging in females have focused on age-related changes in hormone production around menopause. Anti-Müllerian hormone (AMH) is produced by follicular granulosa cells and AMH levels reflect the size of the ovarian follicle pool [10]. Because decreases in AMH are detected long before menopause (at approximately 35 years of age in humans [11]), serum AMH is a very useful early marker for ovarian aging [12,13]. Therefore, we first investigated serum AMH levels in D-gal-treated mice to confirm ovarian aging. However, the results were contrary to our expectations. Plasma AMH levels in D-gal-treated mice were significantly higher than controls. It is known that elevated AMH levels are related to polycystic ovary syndrome (PCOS) [14,15] and ovarian cancer [16]. Because we could not find any tumor-like lesions in the ovarian tissue sections of D-gal treated mice, we investigated the predictive markers of PCOS.

In the present study, to confirm the PCOS-like phenotypes in D-gal-treated mice, we measured the levels of AMH and testosterone in blood plasma, monitored estrous cycles, and examined ovarian morphology.

2. Materials and methods

2.1. Animals and treatment

ICR female mice, aged 7–8 weeks, were used. Mice were given free access to water and a normal diet, and were housed on a

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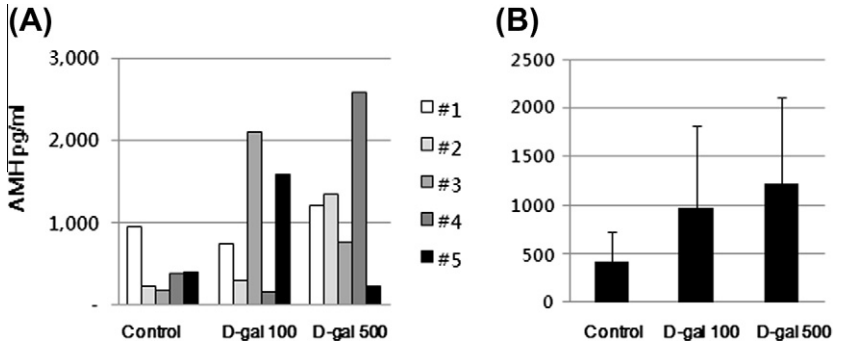

Fig. 1. Plasma levels of AMH. (A) Five mice (#1–5) in each group were examined. The three groups were treated with 100 mg/kg or 500 mg/kg D-gal or vehicle only (control). (B) The average of each group. Values shown are the mean ± S.E.M. from five mice per group.

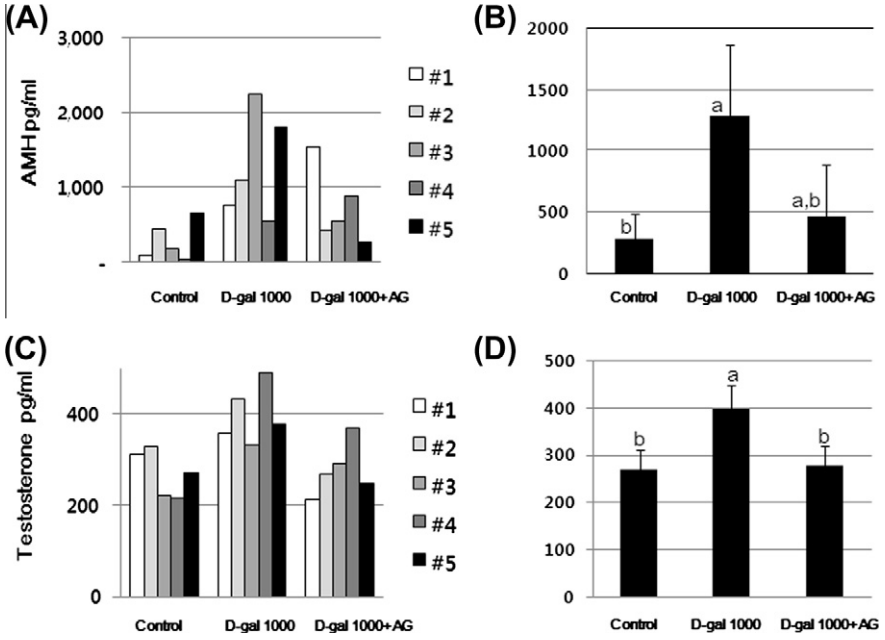

Fig. 2. Plasma levels of AMH (A, B) and testosterone (C, D) in the three groups. Five mice (#1–5) in each group were examined. Panels (B) and (D) show the average of each group (from Panels A and B). Data represent the mean ± S.E.M. from five mice per group. Values with different superscripts are significantly different ($P < 0.05$). Aminoguanidine (AG), D-galactose 1000 mg/kg (D-gal 1000).

12 h light–dark cycle at 24 ± 1 °C and 50% humidity. Mice were randomly divided into three groups, containing five mice each. After a 1-week adaptation period, mice from each group were injected subcutaneously daily with 100, 500, or 1000 mg/kg D-gal or vehicle (0.9% saline) as a control for approximately 6 or 7 weeks. One D-gal treated group of mice was constantly given 0.1% aminoguanidine (AG), an AGE blocking agent, in their drinking water. All animal studies were conducted in compliance with the Guidelines for the Care and Use of Research Animals established by Dankook University's Animal studies Committee.

For blood plasma preparation, whole blood was obtained from incising the tail vein with a sharp surgical blade and was centrifuged at 1500*g* for 15 min at room temperature. The plasma was aliquoted and frozen at −80 °C until use.

2.2. AMH and testosterone assays

AMH measurements in blood plasma were performed using an enzyme-linked immunosorbent assay (ELISA) kit (USCN Life Science, China) according to the manufacturer's instructions. Testosterone was assayed using a total testosterone assay kit (Demeditec Diagnostics GmbH, Germany) per the manufacturer's instructions.

2.3. Vaginal smears

Estrous cycles were monitored by vaginal smear at 6 weeks after D-gal treatment for 10 consecutive days. Vaginal cells were collected between 9:00 AM and 11:00 AM daily from vaginal lavage. The different phases of the estrous cycle were determined according to the predominant cell type present in the vaginal smears as detected by microscopic examination [17].

2.4. Ovarian morphology

All mice were sacrificed by cervical dislocation after 6–7 weeks of D-gal treatment. Ovaries were dissected and placed in Bouin's fixative for 24 h at room temperature, and stored in 70% ethanol at 4 °C until use. Fixed ovaries were embedded in paraffin and 5-μm-thick sections were mounted on slides. They were stained with Harris's hematoxylin and eosin (HE).

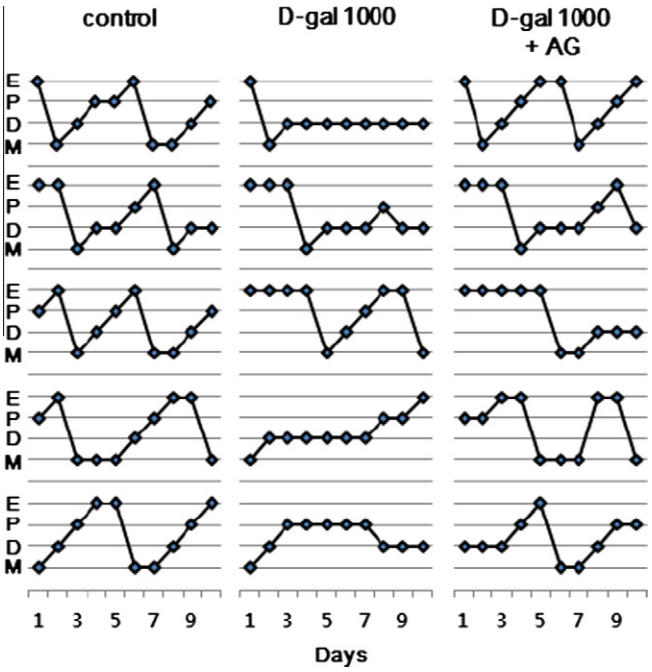

Fig. 3. Estrous cycles in D-gal-treated mice. Most control mice displayed a normal 4 or 5-day cycle. Most D-gal 1000-treated mice showed abnormal estrous cycles. The abnormal estrous cycles tended to return to normal upon treatment of aminoguanidine (AG).

2.5. Statistical analyses

Statistical differences between groups were analyzed using a one-way analysis of variance (ANOVA). Data are shown as the mean ± standard error of the mean (SEM) and significance was defined as $P < 0.05$.

3. Results

3.1. Plasma AMH and testosterone levels

First, we used mice treated with two different dosages of D-gal (100 or 500 mg/kg). AMH levels in blood plasma were investigated (Fig. 1A). The estimated AMH levels are averages for the five mice in each group (Fig. 1B). Although there is some variation in AMH levels among individual mice, the levels tend to increase according to D-gal dosage. Based on these results, we fixed the D-gal concentration at 1000 mg/kg and included another AG and D-gal-treated group. AMH and testosterone levels in the blood plasma of D-gal-treated mice were higher than control mice, and these increases were reversed by AG treatment (Fig. 2A and B).

3.2. Estrous cycle disruption

Most normal mice had normal, four or five-day estrous cycles, whereas only one of five D-gal-treated mice showed normal cycles (Fig. 3). The irregular estrous cycle was recovered to some extent upon AG treatment.

3.3. Ovarian morphology of D-gal treated mice

The ovaries in the control mice were normal, with follicles in different stages and corpora lutea (Fig. 4A). However, the ovaries in D-gal-treated mice showed typical PCOS-like changes with many small follicles (B) and ovarian cysts (C). These specific phenotypes were observed in 4 out of 12 D-gal-treated mice.

4. Discussion

The D-gal-induced aging model has been commonly used for aging research because it results in accelerated aging that is similar to that observed as a result of normal aging processes [1,8]. It is known that excessive formation of ROS and accumulation of AGEs by the Maillard reaction as a result of D-gal administration accelerates aging in this animal model [1,3,4,7]. To date, several animal studies have shown age-related changes in brain, kidney, liver, and blood cell function with D-gal treatment. However, female reproductive organ aging in this animal model has not been reported. Anti-Müllerian hormone (AMH) is a useful marker for ovarian reserve in aging females [11,13]. The level of AMH in blood plasma declines in humans at approximately 35 years of age and is undetectable after spontaneous menopause in women [12]. In addition, AMH levels decline over the course of aging in mice [18]. To address whether ovarian aging is induced in D-gal-treated mice, we investigated AMH levels in blood plasma. The levels of AMH were significantly higher than in normal control mice. We tested other predictive markers of PCOS, as high levels of AMH are known to be a PCOS marker [15,16,19].

In this study, we found high blood plasma AMH and testosterone levels in D-gal treated-mice compared to control mice. These increases were reversed upon AG treatment, and may be caused by AGEs from additional D-gal. Additionally, we found irregular estrous cycles and ovarian cysts in the D-gal-treated mice. We suggest that the D-gal-treated mouse model is similar to PCOS, rather than aging, in terms of female reproduction.

PCOS affects 5–10% of women of reproductive age and is one of the most common endocrine disorders. It is characterized by

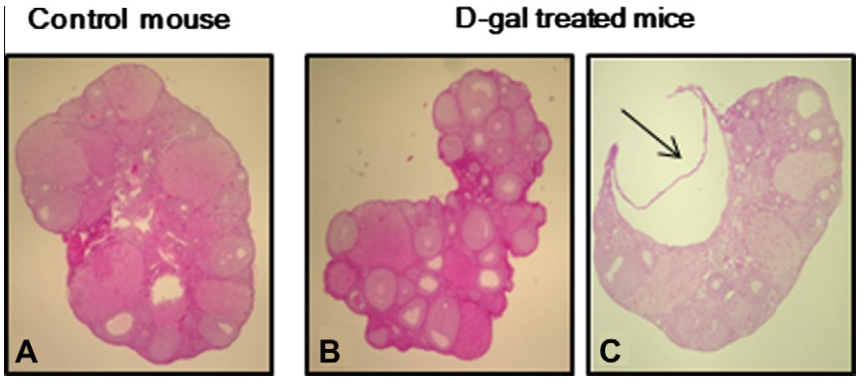

Fig. 4. Morphology of control and D-gal-treated ovaries. (A) Most D-gal treated-mice showed normal phenotypes similar to control mice. Ovaries from 10 D-gal treated mice were investigated. Two D-gal-treated mice had cystic ovaries (arrow) (C) and two mice had large numbers of follicles (B).

excessive androgen levels, polycystic ovaries, and oligo/anovulation [20]. The causes of PCOS are not known, but Type 2 diabetes is strongly associated with PCOS. Recent studies have shown that AMH is a good surrogate marker for PCOS [15,16,20]. During ovarian folliculogenesis, AMH is expressed in pre- and small-antral follicles and may participate in regulating terminal follicular development by reducing follicle sensitivity to follicle stimulating hormone (FSH) [21]. Therefore, abnormally high AMH levels inhibit normal maturation of ovarian follicles and increase the numbers of pre-antral follicles [22]. The exceptionally large number of follicles in ovaries of D-gal-treated mice may be due to high AMH levels (Fig. 3B). In this regard, high levels of AMH in PCOS patients may involve formation of ovarian cysts and oligo/anovulation.

At present, the widely used animal models for PCOS have been developed by prenatally or postnatally exposing mice to various androgens or estrogens [23–26]. In addition, ob/ob and db/db mice, which are common models of obesity and diabetes, exhibit some PCOS-specific phenotypes [26]. However, these animal models, which are induced by exposure to sex hormones or are genetically mutated, may not be suitable for the study of PCOS, because these conditions may not commonly occur in pre-PCOS women. Recent studies have examined the connection between PCOS and increased AGEs in the body. Serum AGE levels were increased in women with PCOS [27] and AGEs and its receptor (RAGE) were highly expressed in granulosa cells of the PCOS ovary [28]. In our study, PCOS phenotypes induced by the accumulation of AGEs may be caused by repeated exposure to D-gal, although we did not directly examine AGEs in blood or ovarian tissue of D-gal-treated mice. Therefore, D-gal-treated mice may be a more useful animal model for PCOS research than previous models.

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