Effect of Jogyeongbohyeoldan (JBD) on the restoration of ovarian function in aged female mice

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Figure

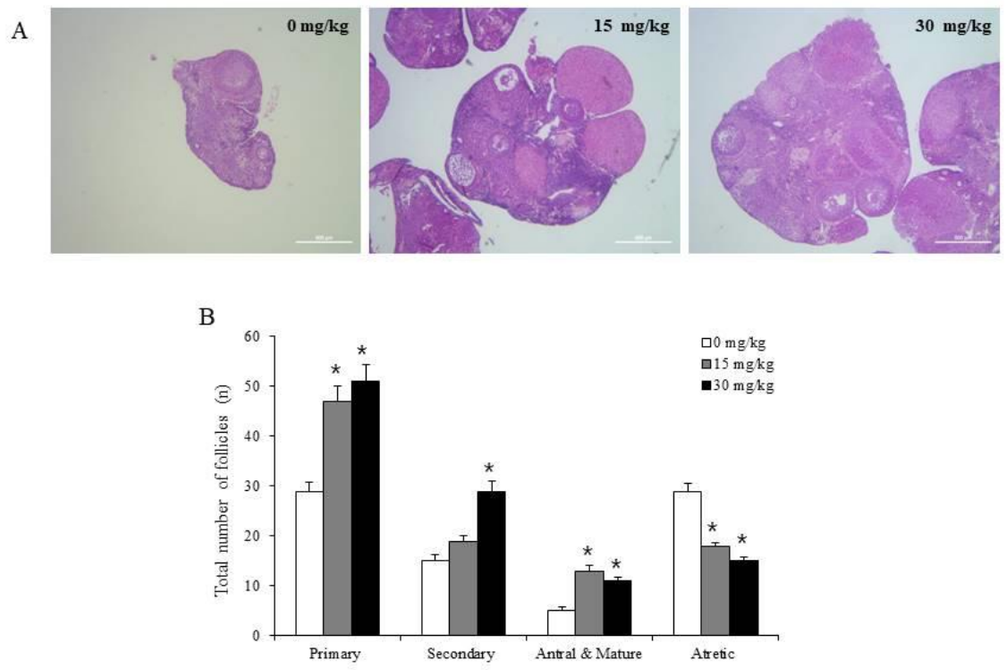

*Fig. Effect of JBD on follicular development. Whole ovaries were collected the day after 4 JBD treatment for 4 weeks and provided in the histological analysis by H&E staining. (A) The representative histological characteristics of follicles at each development stage. (B) The distribution of follicles at different stages JBD increases the number of primary, secondary, and antral/mature follicles, while the number of atretic follicle was decreased. *P < 0.05 (versus controls)*

Introduction

Ovarian aging remains a representative unmet demand of infertility treatment. Jogyeongbohyeoldan (JBD) is an oriental herbal medicine used to improve female fertility. This study investigated whether JBD restores ovarian function and simulates gene expression related to activation of primordial follicle, ovarian stem cells and angiogenesis in aged female mice.

Methods

C57BL/6 female mice aged 12 months (natural ovarian aging, NOA) were administered every day with JBD of 15 mg/kg (n=7) and 30 mg/kg (n=7) of body weight for 4 weeks using oral zoned needle. The control group (n=7) was treated with normal saline. After final treatment of JBD, ovaries were collected, and follicle counts were evaluated by histological study and ovarian mRNA expressions of genes related to PI3K/mTOR (4E-BP1, S6K1, RPS6) and Hippo (MST1, LATS1) signaling pathway for the activation of primordial follicle and ovarian stem cells, and angiogenesis (VEGF, Visfatin, and SDF-1α) were evaluated by quantitative real-time PCR. In the second experiment, NOA and premature ovarian failure (POF) mice were treated with JBD in the same manner as above, (n=7 each), and they were superovulated with PMSG and hCG, followed by mated with male mice. Zygotes were retrieved and cultured for 4 days, and numbers of zygotes and embryo development rate were examined.

Results

JBD significantly increased numbers of surviving follicles (primary, secondary, and antral/mature) and ovarian mRNA expression of 4EBP1, S6K1, RPS6, MST1, LATS, VEGF, and SDF-1α. Also, JBD significantly increased numbers of zygotes retrieved and embryo development rate to blastocyst in both NOA and POF mice compared to the control group (P < 0.05).

Conclusion

These results show that JBD can restore ovarian aging and improve ovarian function and oocyte quality in aged mice. Also, this study suggests that JBD can activate PI3K/mTOR and Hippo signaling, ovarian stem cells, and ovarian angiogenesis.

Effect of JBD on ovarian function and oocyte quality

The histological characteristics of follicles at each development stage were shown in Figure A and B. A significantly increased number of surviving follicles including primary, secondary, and antral/mature follicles were observed in JBD treatment of 15 mg/kg and 30 mg/kg (Figure A) compared to the control group. The mean numbers (±SD) of primary, secondary and antral/mature follicles were 47±3.0, 19±1.2, and 13±1.2 in 15 mg/kg JBD, respectively, and 51±3.4, 29±2.1, and 11±0.8 in 30 mg/kg JBD, which were significantly increased about two times compared to the control group (29±1.8, 15±1.4, and 5±0.8, respectively). In contrast, the number of atretic follicles was decreased by about half in JBD treatment compared with the control group (Figure B).

Effect of JBD on ovarian function and embryo developmental competency in NOA and POF mouse model

In NOA mouse model, the mean numbers of zygotes retrieved per mouse and embryo development rate to blastocyst were 14.0 and 37.8% in the JBD 15 mg/kg group and 16.3 and 41.4% in the JBD 30 mg/kg group which was significantly increased compared to 6.0 and 8.8% in the control group (P < 0.05). This effect of JBD was similar between the treated concentrations, 15 mg/kg and 30 mg/kg. On the other hand, the fragmentation rate of retrieved zygotes was slightly higher in the 15 mg/kg of JBD, but not significantly different from the control group and the 30 mg/kg of JBD (Table 1) .

Table 1. Effect of JBD treatment on the number and embryo development of zygotes retrieved in NOA mice

JBD (mg/kg)	No. of mice provided	No. of zygotes retrieved	No. of zygotes			No. of 2-cell embryos (%)	No. of blastocyst (%)
			retrieved /mouse	fragmented (%)	cultured		
0	7	42	6.0	8 (19.0)	34	7 (23.5)	3 (8.8)
15	7	98	14.0[a]	24 (24.5)	74	45 (60.8)[a]	28 (37.8)[a]
30	7	114	16.3[a]	15 (13.2)	99	58 (58.6)[a]	41 (41.4)[a]

[a]P < 0.05 (vs controls)

Table 2. Effect of JBD treatment on the number and embryo development of zygotes retrieved in POF mice

JBD (mg/kg)	No. of mice provided	No. of zygotes				No. of 2-cell embryos (%)	No. of blastocyst (%)
		retrieved	retrieved /mouse	fragmented (%)	cultured		
0 (Normal)	7	175	25[a]	30 (17.1)	145	122 (84.1)[a]	100 (68.9)[a]
0 (POF)	7	68	9.7	23 (33.8)	45	8 (17.8)	0 (0)
15 (POF)	7	98	14.0[a]	8 (8.2)	90	74 (82.2)[a]	62 (68.9)[a]
30 (POF)	7	143	20.4[a]	17 (11.9)	126	102 (80.9)[a]	83 (65.9)[a]

[a]P < 0.05 (vs POF, negative control)

In POF mouse (negative control), the mean numbers of zygotes retrieved per mouse and embryo development rate to blastocyst were 9.7 and 0%, which were significantly decreased compared to those (25.0 and 68.9%) of normal mouse (positive control) (P < 0.05). However, JBD treatment significantly increased the numbers of zygotes retrieved and embryo development rate of POF mouse at both concentrations (15 mg/kg and 30 mg/kg) to a level similar to that of the positive control (P < 0.05). The mean numbers of zygotes retrieved per mouse and blastocyst formation rates were 14.0 and 68.9% in 15 mg/kg JBD treatment, and 20.4 and 65.9% in 15 mg/kg JBD treatment (Table 2).

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