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Original Article

Premature ovarian insufficiency induced by tripterygium glycoside: does melatonin offer protection?

Min Ma[1,2*], Ruixia Li[1,2*], Xiuying Chen[1,2], Bin Li[1,2]

[1]Department of Obstetrics and Gynecology, Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China; [2]Shanghai Key Laboratory of Female Reproductive Endocrine Related Diseases, Shanghai, China. [*]Equal contributors.

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Abstract: Oxidative stress is a key mechanism of ovarian damage. This study was aimed to evaluate whether melatonin (MT) could protect premature ovarian insufficiency (POI) induced by tripterygium glycoside (TG) from oxidative stress. The POI mice were treated by different dosage of MT. The main characteristics and histomorphological changes of mice were determined when finished the administration. Serum and ovarian homogenates levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and anti-mullerian hormone (AMH) were assessed. The expression of MDA-5, SOD-1 and AMH in the ovaries was detected. The results showed MT significantly downregulated MDA and upregulated SOD, GSH-Px and AMH levels in serum and ovarian homogenates, and decreased MDA-5 and increased SOD-1 and AMH expression in the ovaries. We also found that MT remarkably increased number of developing follicles and corpus luteum. Our data suggest low dosage of MT could improve ovarian reverse function and protect ovarian structures of POI by an extent by ameliorating oxidative stress.

Keywords: Premature ovarian insufficiency, tripterygium glycoside, oxidative stress, melatonin

Introduction

Premature ovarian insufficiency (POI), generally irreversible, is a common cause of infertility, which is a syndrome defined as the cessation of ovarian function before the age of 40 and characterized by the occurrence of oligomenorrhea or amenorrhea, elevated gonadotrophins and low estrogen levels, affecting approximately 1% women [1-4]. The known etiologies of POI include genetic disorders, enzyme deficiencies, autoimmune diseases, iatrogenic causes like surgery, chemotherapy and radiotherapy, infections, environment toxins, metabolic factors, social psychological factors and so on [5-10]. However, the cause of most POI patients remains unknown. So far, due to poor feasibility of human study, the mechanism, early diagnose and therapy of POI has still been a significant issue, which need further and deeper research.

The mechanisms of ovarian damage vary from different causes. Previous studies have suggested that follicle cell apoptosis, oxidative stress, ovarian atrophy, cortical fibrosis and blood-vessel injuries were the main mechanisms of ovarian damage [11-14]. Oxidative damage induced by reactive species likely plays a prominent role in the development of the disorder that significantly influences female fertility [15]. In our previous study, a mouse model of POI induced by tripterygium glycoside (TG) via subcutaneous injection was successfully established [2]. In the subsequent study, it's found that oxidative stress was involved in POI and might be the potential pathogenesis of POI induced by TG [3], which was in accord with the literature [12].

Melatonin (MT), a hormone mainly secreted by pineal gland, is an effective endogenous antioxidant against oxidative stress, which plays an essential role in the pathogenesis of many reproductive processes because of its amphiphilic nature that allows for crossing morphophysiological barriers [15-21]. Evidence has accumulated showing that MT is involved in the protection of the oocyte and granulosa cells within the follicle, and MT appears to have varied functions at different stages of follicle development, oocyte maturation and luteal

stage [15, 16, 22-25]. Then, whether MT could play an important role in protecting ovarian structures and improving ovarian reverse function of POI by its strong antioxidant activity, which is precisely what we want to elucidate.

Therefore, we hypothesized that MT could protect ovarian structures and improve ovarian reverse function of POI by attenuating TG-induced oxidative stress-related alterations. To examine this hypothesis, we assessed serum and ovarian homogenates levels of MDA, SOD, GSH-Px and AMH, investigated the expression of MDA-5, SOD-1 and AMH in the ovaries, and analyzed ovarian histomorphological changes.

Materials and methods

Chemicals

TG was purchased from Shanghai Fudan Fuhua Pharmaceutical CO., Ltd., China, whose batch number was Z31020415. MT (Sigma, St. Louis, USA) were bought from Shanghai Haoran Biotechnology CO., Ltd., China. MT was first dissolved in absolute ethanol and then diluted with 0.9% saline at the final concentration of 20, 40 and 80 mg/ml, respectively; the final ethanol concentration was less than 0.5%. The choice of the dosage of MT was based on the references [26, 27]. The enzyme-linked immune sorbent assay (ELISA) kits for MDA, SOD, GSH-Px and AMH were obtained from Shanghai Huayi Biotechnology CO., Ltd., China.

Animals

A total of 72 female KM mice (6 weeks old) were bought from Department of Laboratory Animal Science of Fudan University (Shanghai, China). They were randomly assigned to six groups: control (Group C), TG (Group T), pure melatonin (Group MT), low, moderate and high dosage of melatonin protective group (Group L, M and H, respectively). The mice were kept in a constant temperature conditions (22°C-25°C), an equal light and dark (12 hours for light, 12 hours for dark), and were fed with a proper diet chow and water ad libitum. The study was approved by the Ethical Committee of Fudan University (Shanghai, China).

Treatment

All the mice had been acclimatized for 4 days under standard laboratory conditions before administration. After that, the methods of treatment in six groups were as follows: Group C: the mice were treated with 0.9% saline via subcutaneous injection for 38 days. Group T: the mice were subjected to 0.9% saline from the 5th day to the 7th day and then TG (50 mg.Kg^{-1}.d^{-1}) via subcutaneous injection for 35 days. Group MT: the mice were fed with MT (20 mg.Kg^{-1}.d^{-1}) by oral for 38 days. Group L: the mice were fed with MT (20 mg.Kg^{-1}.d^{-1}) by oral from the 5th day to the 7th day, and in the next 35 days, they were first treated with MT (20 mg.Kg^{-1}.d^{-1}) by oral and then TG (50 mg.Kg^{-1}.d^{-1}) via subcutaneous injection. Except for the dosage of MT (40 mg.Kg^{-1}.d^{-1} for Group M and 80 mg.Kg^{-1}.d^{-1} for Group H), the methods of treatment of Group M and Group H was the same as Group L. Body weight was monitored weekly. Blood samples were obtained in day 15, 22, 29, 36 and 43. None of the mice died during drug administration and all the mice were sacrificed to harvest associated samples in day 43.

ELISA

The left ovaries of mice were homogenized in ice-cold phosphate buffer solution (PBS) to be homogenates at the final concentration of 10%. Blood samples and ovarian homogenates were centrifuged at 3,000 rpm for 10 min to separate the supernatants. Afterwards, the supernatants were collected and stored at -80°C for detection. The supernatants levels of MDA, SOD, GSH-Px and AMH were measured by ELISA. All the procedures were strictly done according to the protocols.

Immunohistochemistry

The expression of MDA-5, SOD-1 and AMH in the ovaries was detected by immunohistochemistry. Above all, the sections were incubated at 60°C for 1 hr, and then routinely deparaffinized in xylene and rehydrated in a graded series of ethanol. After that, antigen retrieval was performed by incubating the sections in 0.01 M citrate buffer (pH 6.0) and applying high microwave irradiation for 30 min. Following, sections were incubated with 10% H_2O_2 for 30 min to eliminate endogenous peroxidase activity and then blocked with 10% normal goat serum for 1 hr at room temperature. Afterwards, all the sections were separately incubated with primary antibodies against MDA-5 (1:300, Abcam, UK), SOD-1 (1:800, Santa Cruz, USA) and

Table 1. Serum levels of MDA, SOD and GSH-Px in six groups in day 15 ($\bar{x}$±S, n=12)

Groups	Day 15		
	MDA (nmol/L)	SOD (U/L)	GSH-Px (pg/ml)
Group C	1.528±0.050	62.002±9.732	61.991±5.679
Group MT	1.473±0.071	60.172±4.065	60.172±2.263
Group T	1.527±0.035	41.173±5.053*	41.273±2.205*
Group L	1.567±0.073	54.547±5.952$^{\#}$	54.347±2.708$^{*,\#}$
Group M	1.609±0.083	48.870±7.492*	48.979±2.114$^{*,\#,\&}$
Group H	1.583±0.101	46.208±7.642$^{*,\&\&}$	46.304±1.584$^{*,\#,\&}$

*P<0.01, compared with Group C; $^{\#}$P<0.01, compared with Group T; $^{\&\&}$P<0.05, $^{\&}$P<0.01, compared with Group L.

Table 2. Serum levels of MDA, SOD and GSH-Px in six groups in day 22 ($\bar{x}$±S, n=12)

Groups	Day 22		
	MDA (nmol/L)	SOD (U/L)	GSH-Px (pg/ml)
Group C	1.695±0.095	63.009±8.490	60.312±8.182
Group MT	1.693±0.120	74.257±5.397*	71.581±6.852*
Group T	1.961±0.355**	52.094±4.061*	48.958±5.987*
Group L	1.487±0.260$^{\#}$	55.460±5.278**	53.283±7.053
Group M	1.629±0.115$^{\#}$	57.485±1.909	55.485±1.612
Group H	1.513±0.103$^{\#}$	54.927±6.408**	52.927±7.271**

**P<0.05, *P<0.01, compared with Group C; $^{\#}$P<0.01, compared with Group T.

Table 3. Serum levels of MDA, SOD and GSH-Px in six groups in day 29 ($\bar{x}$±S, n=12)

Groups	Day 29		
	MDA (nmol/L)	SOD (U/L)	GSH-Px (pg/ml)
Group C	2.107±0.217	67.506±6.480	65.301±6.388
Group MT	1.962±0.099	74.941±6.623	72.736±6.591
Group T	2.237±0.161	55.694±6.870**	53.489±5.887**
Group L	1.961±0.102$^{\#}$	59.060±6.929	56.855±5.729
Group M	2.077±0.163$^{\#\#}$	56.319±6.726**	54.114±6.136**
Group H	1.921±0.124$^{**,\#}$	52.640±5.093*	50.435±5.693*

**P<0.05, *P<0.01, compared with Group C; $^{\#\#}$P<0.05, $^{\#}$P<0.01, compared with Group T.

AMH (1:100, Abcam, UK) for overnight at 4°C and then were placed for 45 min at room temperature. The sections were rinsed with PBS, and then biotinylated secondary antibodies were added for 10 min. After washed in PBS, all the sections were incubated with horseradish peroxidase (HRP) for 10 min at room temperature. Following washing sections were incubated with 3,3-diaminobenzidine (DAB) to visualize the final product, and finally counter-stained with hematoxylin, dehydrated and mounted. Simultaneously, breast cancer sections were used as positive control, and negative control was treated by substituting PBS for primary antibodies.

Histomorphology

The right ovaries of mice were harvested by carefully separating surrounding adipose tissue and fascia and fixed in 4% paraformaldehyde for 48 hours. Then, they were routinely embedded in paraffin and serially cut into 4 μm sections. The sections were stained with hematoxylin and eosin for histomorphological examination under optical microscope to count the number of developing follicles and corpus luteum according to the reference [28].

Ovarian and uterine index

Ovarian and uterine index were calculated on the basis of the literature [1, 2].

Statistical analysis

The results are expressed as mean ± standard deviation. Comparisons of continuous data between two groups were performed by independent sample t-test for parametric variables. A one-way analysis of variance was used for parametric variables for comparisons of three or more study groups. The Bonferroni's test was used for post hoc testing. A *P*-value of <0.05 was considered as statistically significant.

Results

MT alleviated oxidative stress induced by TG

The changes of MDA, SOD and GSH-Px, some related substances of oxidative stress, were clearly shown in **Tables 1-6**. **Table 1** demonstrates serum levels of MDA, SOD and GSH-Px in day 15. Serum MDA levels were similar in six groups. However, the concentrations of SOD and GSH-Px in Group T were significant lower than those in Group C, which increased by the

Table 4. Serum levels of MDA, SOD and GSH-Px in six groups in day 36 ($\bar{x}$±S, n=12)

Groups	Day 36		
	MDA (nmol/L)	SOD (U/L)	GSH-Px (pg/ml)
Group C	1.498±0.228	66.430±3.249	65.328±3.479
Group MT	1.524±0.181	84.411±6.794*	83.016±6.488*
Group T	2.000±0.297*	61.668±4.479	60.100±4.993
Group L	1.692±0.248$^{\#}$	66.349±6.834	64.887±6.903
Group M	1.729±0.102$^{\#\#}$	63.961±3.935	62.476±4.208
Group H	1.871±0.157*	63.878±3.874	62.214±4.059

*P<0.01, compared with Group C; $^{\#\#}$P<0.05, $^{\#}$P<0.01, compared with Group T.

Table 5. Serum levels of MDA, SOD and GSH-Px in six groups in day 43 ($\bar{x}$±S, n=12)

Groups	Day 43		
	MDA (nmol/L)	SOD (U/L)	GSH-Px (pg/ml)
Group C	1.785±0.113	71.523±7.167	69.989±4.757
Group MT	1.531±0.049*	89.117±4.867*	87.071±1.566*
Group T	1.822±0.074	54.358±4.145*	52.048±1.572*
Group L	1.653±0.060$^{**,\#}$	71.729±7.069$^{\#}$	69.901±1.099$^{\#}$
Group M	1.639±0.117$^{*,\#}$	62.035±6.170$^{**,\&\&}$	60.150±1.251$^{*,\#,\&}$
Group H	1.602±0.055$^{*,\#}$	62.718±3.785$^{**,\&\&}$	60.465±0.671$^{*,\#,\&}$

**P<0.05, *P<0.01, compared with Group C; $^{\#}$P<0.01, compared with Group T; $^{\&\&}$P<0.05, $^{\&}$P<0.01, compared with Group L.

Table 6. Ovarian homogenates levels of MDA, SOD and GSH-Px in six groups in day 43 ($\bar{x}$±S, n=12)

Groups	Day 43		
	MDA (nmol/L)	SOD (U/L)	GSH-Px (pg/ml)
Group C	2.039±0.091	79.136±1.931	73.879±2.244
Group MT	1.629±0.056*	79.757±1.778	74.447±1.941
Group T	2.148±0.148**	65.415±1.620*	61.058±1.336*
Group L	1.891±0.086$^{*,\#}$	73.441±1.380$^{*,\#}$	68.609±2.496$^{*,\#}$
Group M	2.189±0.117$^{**,\&}$	74.063±1.078$^{*,\#}$	69.084±1.729$^{*,\#}$
Group H	2.025±0.082$^{\#\#,\&\&,\$}$	65.261±1.912$^{*,\&,\$}$	60.915±2.715$^{*,\&,\$}$

**P<0.05, *P<0.01, compared with Group C; $^{\#\#}$P<0.05, $^{\#}$P<0.01, compared with Group T; $^{\&\&}$P<0.05, $^{\&}$P<0.01, compared with Group L; $^{\$}$P<0.01, compared with Group M.

treatment of MT, especially for Group L. Serum levels of MDA, SOD and GSH-Px in day 22 were illustrated in **Table 2**. Significantly increased MDA levels and decreased SOD and GSH-Px levels were seen in Group T than in Group C. Compared with Group T, MDA levels were significantly downregulated, while SOD and GSH-Px levels had no difference in the three different dosages of MT protective groups. The alterations of serum MDA, SOD and GSH-Px in day 29 were almost in consistent with those changes in day 22 (**Table 3**). The results in Group L were better than those in Group M and H. **Table 4** describes serum levels of MDA, SOD and GSH-Px in day 36. Serum MDA levels in Group T were significant higher than those in Group C, which was obviously decreased after MT administration, particularly in Group L. **Table 5** summaries serum levels of MDA, SOD and GSH-Px in day 43. Significantly decreased SOD and GSH-Px levels were seen in Group T than in Group C. However, these parameters including MDA, had a significant improvement after the treatment of MT, especially for Group L. Ovarian homogenates levels of MDA, SOD and GSH-Px in day 43 were shown in **Table 6**. The data were similar to those presented in **Table 5**, which suggested that low dosage of MT could clearly alleviate oxidative stress and the effect was better than that of moderate and high dosage of MT.

The expression of MDA-5 and SOD-1 in ovarian tissues was detected (**Figures 1** and **2**, respectively). According to the two figures, it could be found that MDA-5 and SOD-1 were located in cytoplasm. The alterations of MDA-5 and SOD-1 were in accord with the changes of serum and ovarian homogenates levels of MDA and SOD, which meant that TG could cause increased MDA levels and decreased SOD and GSH-Px levels, and those parameters could be alleviated by the treatment of MT, especially for Group L.

MT protected ovarian structures

The main features of mice in six groups were shown in **Table 7**. As for body weight, there was no difference in six groups when they were enrolled into groups and before they were administrated. After administration, body weight of mice in Group T had a significant reduction compared with Group C. However, no significance of body weight of mice in MT protective groups was found when compared with

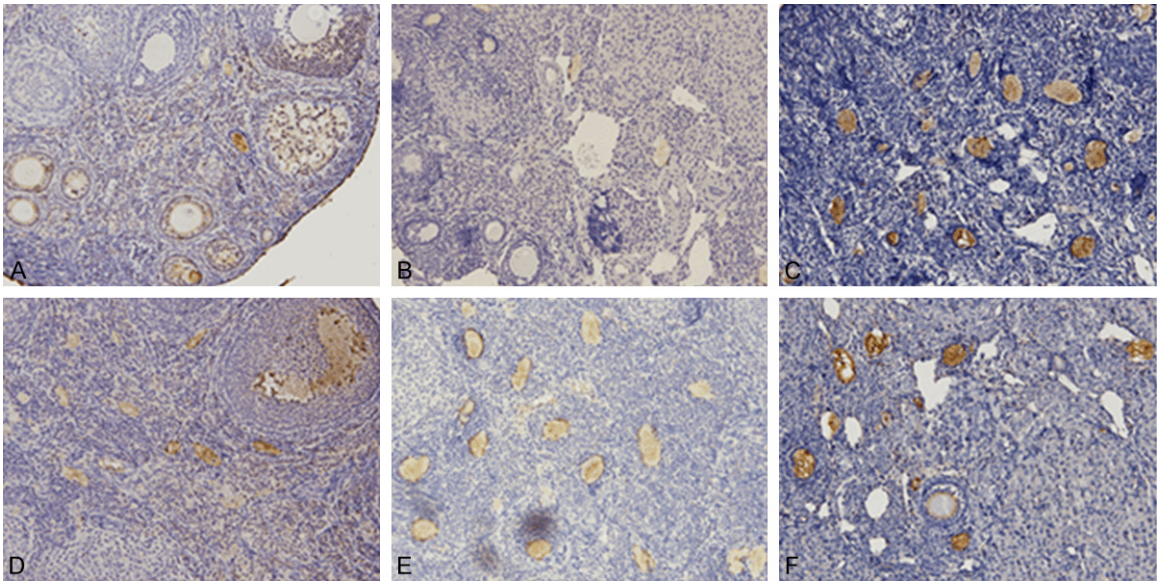

Figure 1. The expression of MDA-5 in the ovaries by immunohistochemistry in six groups. A: Group C, B: Group MT, C: Group T, D: Group L, E: Group M, F: Group H. The levels of MDA-5 in Group T were significantly higher than that in Group C and Group MT. After the treatment of MT, the levels of MDA-5 decreased significantly, especially for Group L. Original magnification: ×200.

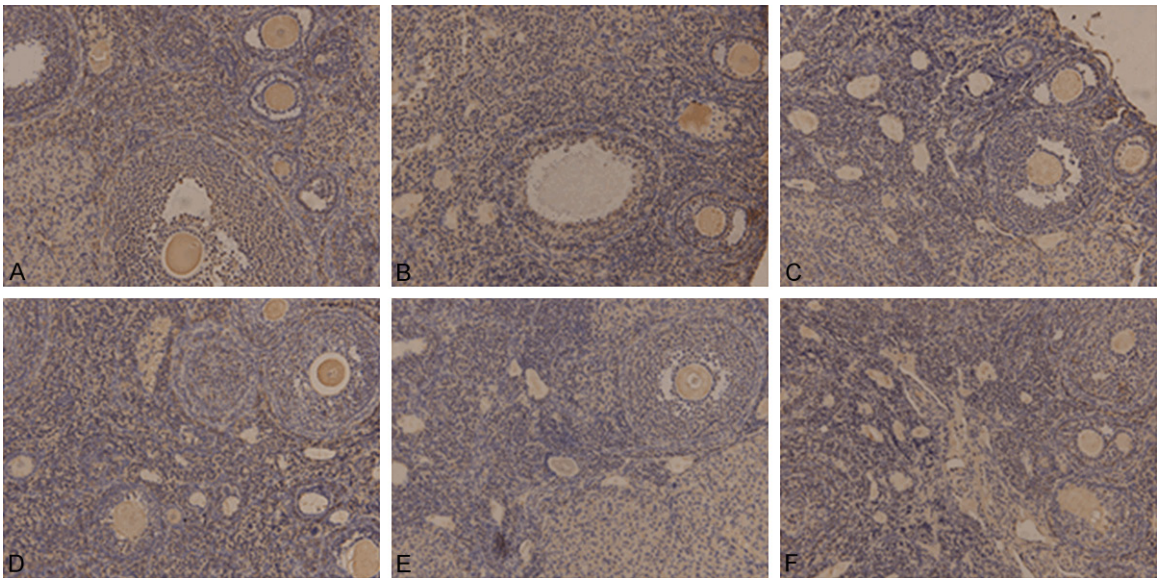

Figure 2. The expression of SOD-1 in the ovaries by immunohistochemistry in six groups. A: Group C, B: Group MT, C: Group T, D: Group L, E: Group M, F: Group H. The levels of SOD-1 in Group T were significantly lower than that in Group C and Group MT. After the treatment of MT, the levels of SOD-1 increased significantly, especially for Group L. Original magnification: ×200.

Group T. Ovarian and uterine index in Group T decreased significantly compared with Group C, which were not alleviated after the treatment of MT. Developing follicles and corpus luteum were counted under optical microscope. Representative patterns of ovarian histomorphology were shown (**Figure 3**). It's found that developing follicles and corpus luteum reduced significantly in Group T. Oral administration with MT significantly increased the number of developing follicles and corpus luteum by an extent, especially for Group L.

Table 7. The manifestations in six groups ($\bar{x}$±S, n=12)

	Group C	Group MT	Group T	Group L	Group M	Group H
Body weight into groups (g)	23.44±1.34	23.69±1.47	23.23±1.65	23.49±1.38	23.64±1.78	23.50±1.43
Body weight before administration (g)	29.76±2.41	29.32±0.94	28.61±2.05	29.18±2.00	29.24±1.47	29.12±1.69
Body weight after administration (g)	38.51±4.41	36.53±2.38	34.40±3.85**	33.65±3.61*	32.66±2.12*	32.43±3.41*
Ovarian index (%)	0.062±0.011	0.060±0.011	0.042±0.007*	0.035±0.007*	0.042±0.008$^{*,\&\&}$	0.037±0.007*
Uterine index (%)	0.364±0.118	0.376±0.248	0.199±0.042*	0.136±0.040*	0.223±0.121**	0.171±0.109*
Developing follicles number	42.83±10.18	50.67±6.83**	19.83±3.04*	39.58±9.39$^{\#}$	33.00±5.92$^{*,\#,\&\&}$	30.00±6.59$^{*,\#,\&}$
Corpus luteum number	6.00±2.56	8.08±4.54	0.83±0.83*	1.67±2.87*	1.33±1.61*	1.00±1.65*

**P<0.05, *P<0.01, compared with Group C; $^{\#}$P<0.01, compared with Group T; $^{\&\&}$P<0.05, $^{\&}$P<0.01, compared with Group L.

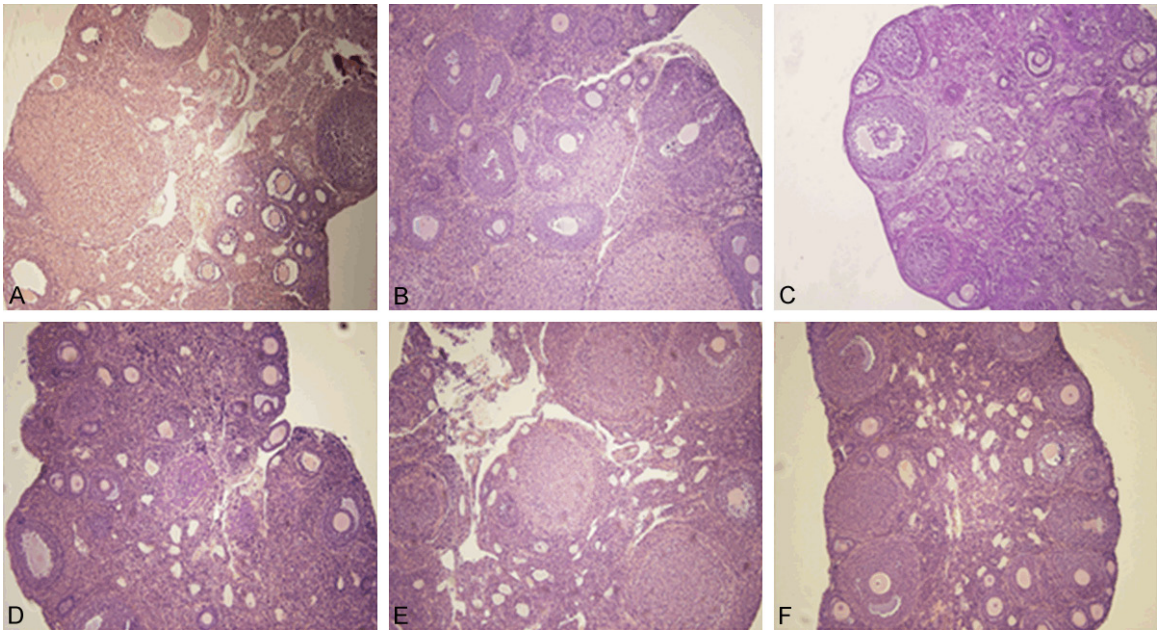

Figure 3. The changes of ovarian histomorphology in six groups. A: Group C, B: Group MT, C: Group T, D: Group L, E: Group M, F: Group H. A and B: Normal histomorphology with normal stroma, types of follicles in the cortex (primordial, primary, secondary and tertiary) and corpus luteum in Group C and Group MT. C: Abonormal ovarian histomorphology with reduced developing follicles, rare corpus luteum, increased atretic follicles in Group T. D-F: Different recovery status after the treatment of MT. The recovery status in Group L was better than another two groups. Original magnification: ×100.

MT improved ovarian reverse function

AMH, a hormone, reflects ovarian reverse function. Serum and ovarian homogenates levels of AMH and the expression of AMH in ovarian tissues were indicated in **Tables 8**, **9** and **Figure 4**, respectively. Compared with Group C, serum and ovarian homogenates levels of AMH in Group T were significantly reduced. After the treatment of MT, especially for low dosage group, AMH levels of serum and ovarian homogenates increased obviously, although the statistical significance was not always existed. AMH in ovarian tissues was located in cytoplasm. The immunohistochemistry expression of AMH was almost the same as the changes of serum and ovarian homogenates AMH levels.

Discussion

MDA, a marker of lipid peroxidation, was estimated to indicate oxidative stress damage to membrane [29]. SOD and GSH-Px, primary antioxidant enzyme, were believed to be necessary for life in all oxygen-metabolizing cells [30]. So, these parameters could also be used to reflect the effect of MT on oxidative stress. In this study, we have shown that MT significantly downregulated MDA and upregulated SOD, GSH-Px and AMH levels in serum and ovarian

Table 8. Serum AMH levels in six groups ($\bar{x}$±S, n=12)

Groups	AMH (ng/ml)				
	Day 15	Day 22	Day 29	Day 36	Day 43
Group C	7.082±0.084	8.166±1.884	7.399±0.882	4.814±0.238	7.281±1.405
Group MT	7.071±0.090	10.253±1.018*	7.890±0.756	5.438±0.765	7.472±1.122
Group T	6.172±0.400*	6.359±0.265**	6.664±0.386**	4.085±0.412**	6.734±0.661
Group L	6.717±0.392#	7.849±0.553	6.899±0.763	4.304±0.436	7.186±0.504
Group M	6,674±0.396**,#	7.868±0.902	6.652±0.583	4.013±0.473**	6.777±0.646
Group H	6.721±0.336#	7.392±0.884	6.339±0.675	4.012±0.514**	6.881±0.372

**P<0.05, *P<0.01, compared with Group C; #P<0.01, compared with Group T.

Table 9. Ovarian homogenates AMH levels in six groups in day 43 ($\bar{x}$±S, n=12)

	Group C	Group MT	Group T	Group L	Group M	Group H
AMH (ng/ml)	11.027±0.349	11.252±0.999	7.898±0.592*	9.790±0.373*,#	7.839±0.278*,&	8.373±0.417*,&

*P<0.01, compared with Group C; #P<0.01, compared with Group T; &P<0.01, compared with Group L.

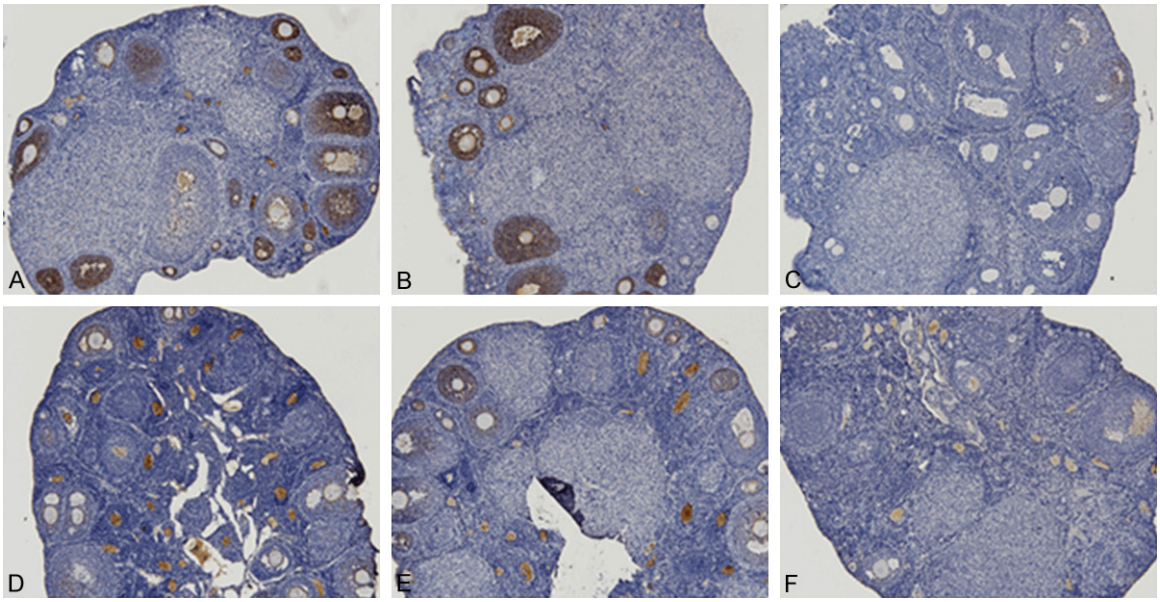

Figure 4. The expression of AMH in the ovaries by immunohistochemistry in six groups. A: Group C, B: Group MT, C: Group T, D: Group L, E: Group M, F: Group H. A and B: Strong AMH expression in normal ovaries in Group C and Group MT. C: Weak AMH expression in ovaries in Group T. D-F: Different AMH expression after the treatment of MT. The AMH expression in Group L was better than another two groups. Original magnification: ×100.

homogenates, and decreased MDA-5 and increased SOD-1 and AMH expression in the ovaries. We also have found that MT remarkably increased number of developing follicles and corpus luteum. The results presented in the tables demonstrate oxidative stress is involved in POI and low dosage of MT may improve ovarian reverse function and protect ovarian structures of POI in some degrees by ameliorating oxidative stress. The changes indicate that MT may probably be as a candidate drug for preventing POI.

Oxidative stress is a key mechanism of ovarian damage [12, 15]. A large number of researchers conclude that MT could protect ovary from oxidative damage [15, 16, 26, 27, 31, 32]. A review summarized MT could become an important medication for improving ovarian function and oocyte quality, and open new opportunities

for the management of several ovarian diseases [15]. Cruz et al reviewed the cytoprotective properties of MT on the ovary and considered that MT had different functions at different stages of follicle development, oocyte maturation and luteal stage [16]. The presence of serotonin, a precursor of melatonin, in granulosa cells and oocytes was consistent with local MT synthesis [22-24]. Protective mechanisms against oxidative stress, such as increased activity of SOD and GSH, control the balance between ROS and antioxidants in the follicle and seem to be critical to the function of the oocyte and granulosa cells [32]. The protective role of MT on the production and preservation of gametes and embryos, and human reproduction were also concluded [23, 24, 31]. Feng et al reported that early MT supplementation could alleviate oxidative stress in a transgenic mouse of Alzheimer's disease [26] and long-term MT supplementation also could improve oxidative stress in ovariectomized adult rats [27]. Therefore, we could consider that our results were consistent with previous studies.

MT is a highly effective antioxidant and protects cells against oxidative stress caused by reactive species [33]. Moreover, metabolites of MT that are formed when the indole functions as a scavenger are likewise equally as good or better than the parent molecule in neutralizing toxic oxygen- and nitrogen-based reactants [17]. The cytoprotective effects of MT and its metabolites are associated with its direct and indirect antioxidant properties. The direct activities of MT and its metabolites involve the scavenging of both ROS and RNS [18], whereas the indirect actions involve the stimulation of antioxidative enzymes and inhibition of pro-oxidative enzymes probably through epigenetic mechanisms [19, 34].

Except for MT, other antioxidants like vitamin E, vitamin C, simvastatin and edaravone also have powerful antioxidative actions [35-38]. A new study investigated the effects of vitamin E supplementation against oxidative stress induced by pure CYN in tialpia (Oreochromis niloticus), which concluded that vitamine E could be considered as a useful chemoprotectant that reduces hepatic and renal oxidative stress and could be used in the prophylaxis and treatment of CYN-related intoxication in fish [35]. Another study found that vitamin C compound mixtures could prevent Ozone-induced oxidative damage in human keratinocytes by the abolishment of NF-kB nuclear translocation, as well as activation of Nrf2 nuclear translocation activating the downstream defense enzymes involved in cellular detoxification process [36]. In a rat model of ischemic heart failure, oxidative stress was attenuated by the treatment of simvastatin [37]. Prophylactic treatment with edaravone was also found to be useful to prevent ischemia/reperfusion (I/R)-induced ovarian damage during pneumoperitoneum [38]. However, MT is typically more effective in protecting against oxidative damage than other antioxidants due to its amphiphilic nature, which allows it to enter every organ and all cells, thereby reducing oxidative and nitrosative damage in both the lipid and aqueous cellular environments [39]. In this study, these antioxidants were not investigated, which were perhaps useful to attenuate oxidative stress in TG-induced POI. That's an open research.

In summary, oxidative stress is actually involved in the development of POI induced by TG. MT, as an effective antioxidant, could protect ovarian structures and improve ovarian reverse function in some degrees by ameliorating oxidative stress, especially for low dosage, which indicates that MT may probably be as a candidate drug for POI. In fact, more biomarkers to reflect ovarian endocrine function should be detected and more methods should be used. However, the samples were not enough for these operations. Although MT is found to be useful and helpful, the reproductive ability of mice after the treatment of MT and the healthy state of its offspring are not investigated in this paper, which is the main limitation. That's what we want to further elucidate.

In conclusion, this is the first study showing the potential of MT to ameliorate oxidative stress induced by TG via subcutaneous injection in a mouse model of POI and the protective properties of MT on ovarian structures and function. Our findings demonstrate that treatment with MT could reduce oxidative damage induced by TG and ameliorate ovarian histomorphological structures, especially for low dosage of MT.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bin Li, Department of Obstetrics and Gynecology, Obstetrics and Gynecology Hospital of Fudan University, 419 Fangxie Road, Huangpu, Shanghai 200011, China. Tel: +86-21-63455050; Fax: +86-21-33189900; E-mail: Drbinli782@163.com

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