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Protective Effects of Melatonin and Vitamin E Against Ovarian Damage Induced by Pelvic Radiotherapy

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ABSTRACT

Object: To investigate the protective effects of melatonin and vitamin E against pelvic radiotherapy-induced ovarian radiation injury. Method: Thirty-two mature female mice were randomly divided into four groups: the irradiation-only group, the vitamin E-administered irradiation group, the melatonin-administered irradiation group, and the vitamin E + melatonin-administered irradiation group. All drug-irradiated groups received intraperitoneal injections of melatonin and/or vitamin E starting 3 weeks before irradiation. Then, the drug-irradiated and irradiation-only groups underwent a total dose of 2 Gy (1 Gy/session * 2 sessions) of local pelvic radiotherapy. Post-radiotherapy, the drug-irradiated groups continued receiving intraperitoneal injections of melatonin and/or vitamin E for 2 weeks. Serum levels of anti-Müllerian hormone (AMH), estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured, along with estrus cycle monitoring and follicle count comparisons. Results: Compared with the irradiation-only group, the vitamin E-administered and vitamin E+ melatonin-administered irradiation groups showed improvements in disrupted estrous cycles ($p<0.05$). All drug-irradiated groups improved hormonal levels of ovaries, with statistically significant differences, and combined use provided superior effects ($p<0.05$). Melatonin and vitamin E administration also increased the number of functional follicles, reduced atretic follicles, and increased total follicle numbers, with synergistic effects being superior ($p<0.05$). Conclusion: Both melatonin and vitamin E provide protective effects against pelvic radiotherapy-induced ovarian damage, with combination therapy offering the best outcomes.

KEYWORDS

Melatonin; Vitamin E; Pelvic radiotherapy; Ovarian reserve function

1. INTRODUCTION

Three major challenges affecting human health in the 21st century include cardiovascular disease, malignant tumors, and infertility. Between 2003 and 2015, the standardized five-year survival rates for most cancer types in China saw increases, particularly for endometrial cancer, thyroid cancer, cervical cancer, and bone tumors [1]. Younger onset ages, improved prognosis, and more demands for quality of life and fertility of patients present new challenges for new strategy of anti-tumor therapy. According to studies, 62% of young women diagnosed with cancer had plans for children before their diagnosis [2].

Pelvic radiotherapy, a key treatment for many pelvic malignancies, often causes irreversible damage to ovarian reserve function, leading to ovary dysfunction, premature ovarian failure, infertility, and

negatively impacting survivors' reproductive capabilities. Additionally, it can lead to osteoporosis, urogenital issues, and cardiovascular diseases due to ovarian insufficiency, severely affecting quality of life.

Recent advances in traditional Chinese medicine show that various herbs or decoctions may mitigate radiation-induced ovarian tissue injuries, offering potential hope for ovarian protection. Our team reviewed literature on antioxidants and selected a safe and effective combination: vitamin E and melatonin. Melatonin has demonstrated protective effects against cisplatin-induced ovarian injury in mice [3], reducing the risk of premature ovarian failure. Supplementation with vitamin E improves symptoms in women with ovarian insufficiency, as indicated by elevated AMH levels and antral follicle counts [4]. No previous reports exist on the protective effects of melatonin and/or vitamin E combinations against radiation-induced ovarian damage. Thus, we aim to explore the roles of melatonin and vitamin E, individually and together, in protecting mouse ovaries from pelvic radiotherapy damage, providing potential drugs for protecting ovarian tissues damaged by pelvic radiotherapy.

2. MATERIALS & METHODS

2.1. Experimental Materials

Experimental Animals: Mice used in this study were C57BL/6J strain, purchased from Shanghai Jihui Laboratory Animal Co., Ltd., aged 6-8 weeks, housed under alternating day-night lighting and constant temperature conditions.

Reagents: Melatonin, α-tocopherol (MedChemExpress Company); Mouse estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and anti-Müllerian hormone (AMH) ELISA kits (Shanghai Zonco Bio-Tech Co., Ltd.); Hematoxylin, eosin, and Giemsa stains (Zhuhai Beso Biotechnology Co., Ltd.)

2.2. Experimental Methods

2.2.1. Experimental Animal Modeling

Irradiation was administered at the Naval Medical University Radiation Center using a 60Co source at room temperature. Before irradiation, mice were anesthetized and secured in a position to minimize positional variation during irradiation (as shown in Figure 1A, 1B). Female mice were randomly divided into the irradiation-only group and the vitamin E-administered irradiation group, melatonin-administered irradiation group, and vitamin E + melatonin-administered irradiation group. All drug-irradiated mice received intraperitoneal injections of melatonin and/or vitamin E (vitamin E dosage: 50 mg/kg, melatonin: 10 mg/kg) starting 3 weeks before irradiation. Following irradiation, they continued receiving intraperitoneal injections of melatonin and/or vitamin E (vitamin E dosage: 50 mg/kg, melatonin: 10 mg/kg) for 2 weeks. The irradiation-only group received an equivalent volume of saline solution. All female mice received a total dose of 2 Gy (1 Gy/session * 2 sessions) of local pelvic radiotherapy.

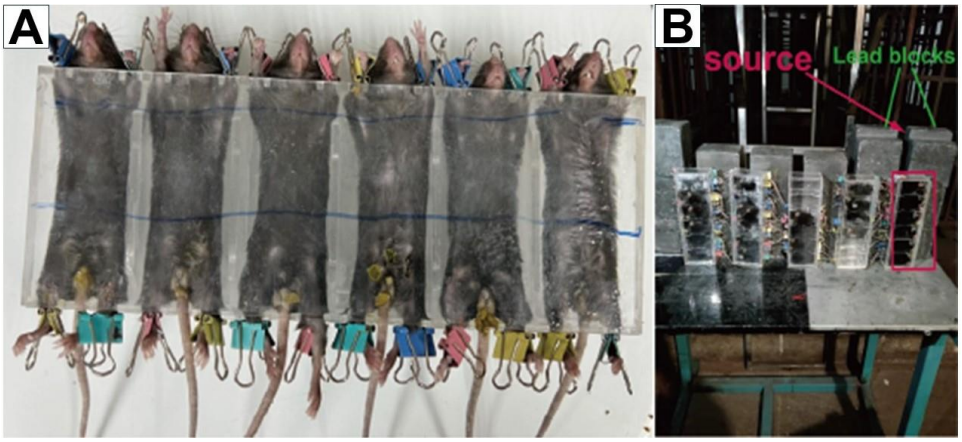

Figure 1. Schematic representation of the modeling process for pelvic radiotherapy-induced ovarian injury in mice

(A) Illustration of the fixation method for mice during the construction of the radiogenic ovarian injury model. (B) Illustration of the radiation conditions involved in the creation of the radiogenic ovarian injury model.

2.2.2. Experimental Schedule

Thirty-two female mice were randomly assigned to four groups. After undergoing treatments including medication administration and irradiation, subsequent experiments such as follicular counting, estrus cycle assessment, and ovarian hormone level detection were conducted.

2.2.3. Estrus Cycle

Beginning 24 hours after completion of irradiation and drug administration, daily evaluations of vaginal cytology were carried out at 08:00 and 20:00 to determine the stage of the estrus cycle. Complete assessments spanning 21 days were performed and statistical analyses were applied.

2.2.4. Hormone Levels

Upon finishing the estrus cycle assessment, mice were anesthetized, blood samples obtained via eye puncture, and serum prepared. The levels of AMH, E2, FSH, and LH were detected utilizing ELISA methods.

2.2.5. Follicle Counting

Ovarian tissues were excised, processed for paraffin embedding, and histological examination was performed to assess and quantify follicles across different stages and the total number of follicles.

2.3. Statistical Analysis

GraphPad Prism 9.0 and SPSS 22.0 software packages were utilized for analysis. Data are presented as mean ± SD. Unpaired t-tests were employed to analyze differences between quantitative data. P-values less than 0.05 indicated statistically significant differences.

3. RESULTS

3.1. Effect of Melatonin and Vitamin E Administration on Estrus Cycle Disruption in Irradiated Mice

Melatonin and vitamin E administration both improved disruption of the estrus cycle in irradiated mice: Among the batches of experiments, some mice exhibited estrus cycle disruptions in the irradiation and drug-irradiation groups. Compared to the 72.22 ± 9.62% estrus cycle disruption rate

in the irradiation-only group, the disruption rate in the drug-irradiated group was 55.56 ± 9.62%. These differences were statistically significant, as detailed in Table 1.

Table 1. Estrus Cycle Disruption Rates Among Different Groups

Group	Rule ratio (%)	Disorder ratio (%)
IR+NS	29.17±4.17	70.83±4.17
IR+Vit E	45.83±4.17	54.17±4.17(*)
IR+Mel	50.00±4.17	50.00±4.17
IR+Vit E +Mel	62.50±4.17	37.50±4.17(*)

3.2. Impact of Melatonin and Vitamin E Administration on Ovarian Hormone Levels in Mice Exposed to Radiation

Melatonin and vitamin E supplementation significantly improve the hormone profile in ovaries affected by radiation, with enhanced effectiveness when used in combination: compared to the simple irradiation group, administering melatonin and/or vitamin E before and after radiation exposure led to a marked increase in anti-Müllerian hormone (Figure 2A) and estrogen (Figure 2B) levels in irradiated mice, simultaneously decreasing levels of follicle-stimulating hormone (Figure 2C) and luteinizing hormone (Figure 2D).

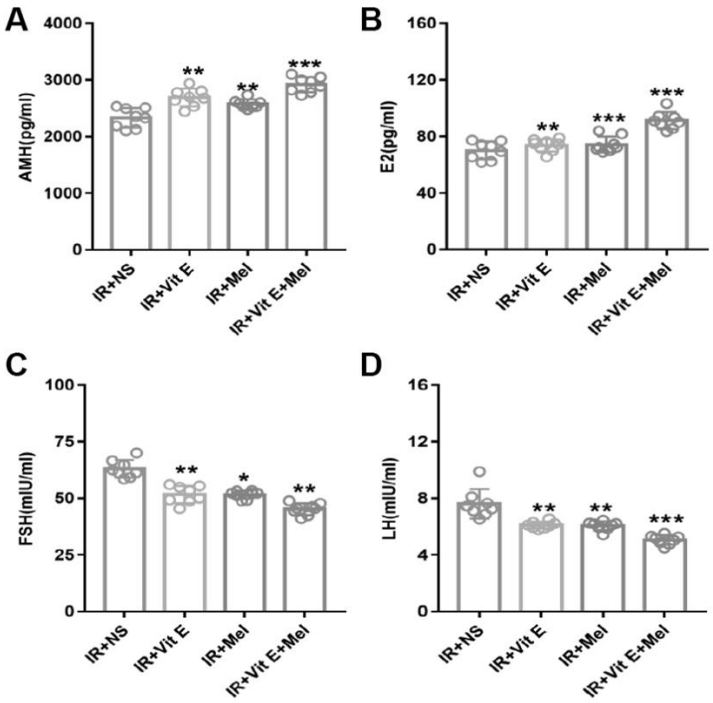

Figure 2. Effects of melatonin and vitamin E administration and combined administration on ovarian hormone levels in mice after pelvic radiotherapy

(A) Anti-Müllerian hormone (AMH) levels in mice; (B) estradiol (E2) levels; (C) follicle-stimulating hormone (FSH) levels; (D) luteinizing hormone (LH) levels. (IR+NS: Irradiation only group; IR+Vit E: Vitamin E treated irradiation group; IR+Mel: Melatonin treated irradiation group; Ir+Vit E +Mel: Vitamin E + Melatonin treated irradiation group) (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$)

3.3. Effects of Melatonin and Vitamin E on Ovarian Follicles in Irradiated Mice

Supplementation with melatonin and vitamin E leads to an increase in primordial, primary, pre-antral, and antral follicle numbers (Figure 3 A/B/C/D), decreases atretic follicle counts (Figure 3 E), and raises total follicle counts (Figure 3 F), with synergistic benefits observed when used concurrently.

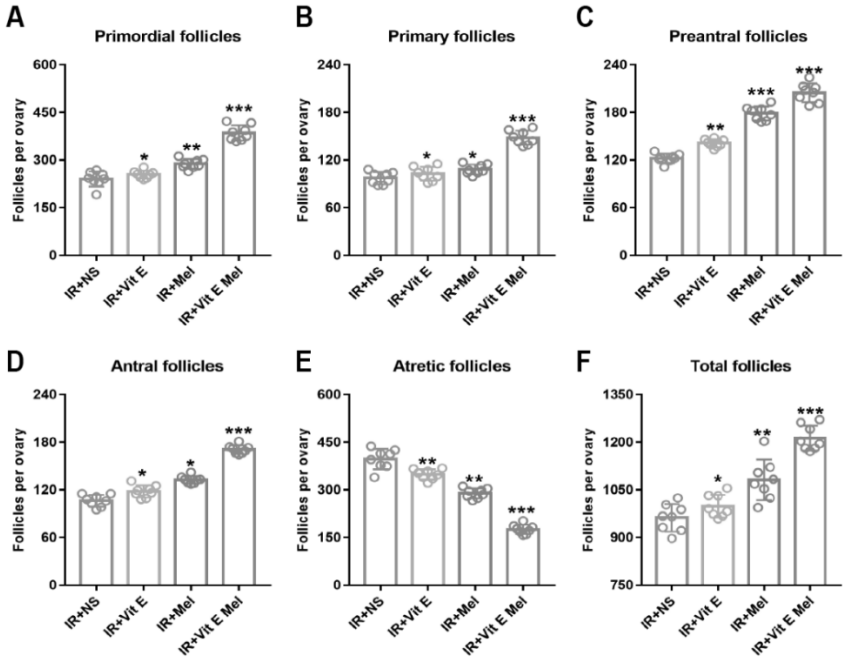

Figure 3. Follicle counts of each group

(A) Primordial follicle counts; (B) Primary follicle counts; (C) Pre-antral follicle counts; (D) Antral follicle counts; (E) Atretic follicle counts; (F) Total follicle counts. (IR+NS: Irradiation only group; IR+Vit E: Vitamin E treated irradiation group; IR+Mel: Melatonin treated irradiation group; Ir+Vit E +Mel: Vitamin E + Melatonin treated irradiation group) (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$)

4. DISCUSSION

In recent years, global fertility rates have been declining, and with the trend towards younger patients among those afflicted with tumors, research into preserving reproductive function in young cancer patients has become increasingly prominent. Pelvic radiotherapy, a crucial component of anticancer therapies, often results in diminished ovarian function and even failure. Urgently needed is an effective strategy to protect ovarian function throughout pelvic radiotherapy procedures.

Currently, measures for ovarian function preservation mainly consist of physical, biological, and pharmacological protections. Physical protection aims to reduce radiation damage through shielding but fails to prevent indirect effects, such as those on the hypothalamic-pituitary-gonadal axis caused by cranial radiation, leading to suboptimal results. Ovary transposition surgery offers a viable option for ovarian function retention in fertile women requiring pelvic radiotherapy, yet it faces limitations such as poor ovarian reserve, high ovarian metastasis risk, and applicability only to chemotherapy-only patients. Biological protections, including embryo freezing and transplantation, oocyte cryopreservation and transfer, ovarian tissue cryopreservation and transplant, and ovary transplantation, are widely practiced in fertility preservation efforts for fertile females with tumors, though these methods face substantial challenges and restrictions—technical difficulties, limited availability, socio-economic factors, and medical contraindications, among others.

Regarding pharmacological defenses, the FDA-approved WR-2721 demonstrates the best efficacy internationally but suffers from considerable toxicity and adverse reactions, limiting its application. Domestic radiation injury preventive medications include estrogens and extracts of Rubia cordifolia, but practical experiences have confirmed their ineffectiveness. Recent studies exploring the mitigation of gonadal toxicities induced by targeted radiotherapy have made progress, such as AS10199 [5] and FTY720 [6], which serve as methods to reduce radiation-induced gonadal toxicity.

Radiotherapy-generated oxygen radicals disrupt antioxidant defense systems within the female reproductive organs, causing oxidative stress and oxidation-related damages. These damages manifest as single-strand breaks (SSBs) or double-strand breaks (DSBs) in DNA, cross-linking of DNA-DNA or DNA-protein complexes, and structural damage to biomolecules, increasing apoptosis in granulosa cells and resulting in follicle atresia. Research shows that melatonin possesses antioxidant properties. Its lipophilicity and hydrophilicity allow easy access to cellular and subcellular compartments, protecting nuclear DNA, membrane lipids, cytoplasmic proteins, and other macromolecules from oxidative damage. Melatonin boosts the production and activity of enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), enhancing its antioxidant effects [7]. Melatonin inhibits nitric oxide synthase (NOS) activity, decreasing NO generation [8], suggesting its potential therapeutic effect against radiation. Vitamin E, an essential antioxidant, terminates radical chain reactions promptly, inhibiting lipid peroxidation, stabilizes biological membranes, and shields cells from free radical damage [9].

5. CONCLUSION

This study innovatively constructs a pelvic radiotherapy-induced ovarian damage mouse model, exploring the protective effects of melatonin and vitamin E against ovarian damage caused by pelvic irradiation. Compare with the irradiation-only group, the vitamin E-administered irradiation group and the combined vitamin E + melatonin-administered irradiation group showed improvements in estrus cycle disturbances, showing statistically significant differences. In the assessment of mouse ovarian hormone levels and follicle count, both vitamin E and melatonin displayed improving trends, indicating their ability to ameliorate ovarian function to varying degrees. Innovatively combining melatonin with vitamin E, using dosages of 10 mg/kg and 50 mg/kg respectively, animal experiments revealed that the joint effect surpassed individual treatments, thus the compound effectively alleviates radiation-induced ovarian tissue damage, offering new hope in protecting against radiation damage to the ovaries.

CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

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