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The role of melatonin in preventing ovarian tissue damage in rats exposed to magnetic fields

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Objectives: We observed the efficacy of melatonin in preventing ovarian tissue damage in rats exposed to magnetic fields.

Materials and methods: Forty rats were divided into four treatment groups: Group 1, control group (n = 10); Group 2, melatonin administration only (n = 10); Group 3, magnetic field exposure only (n = 10); Group 4, magnetic field exposure with melatonin administration (n = 10). The magnetic field was applied at a dose of 20 μT for 30 min/day for 10 days. Melatonin was orally administered at a dose of 10 mg/kg. We evaluated follicle count, degree of fibrosis, amount of adhesion, amount of apoptosis, ovarian dimensions, and follicular degeneration by dissecting the ovaries of the rats on day 11, and differences among the groups were evaluated.

Results: Group 3 had an increased amount of follicle degeneration, more fibrosis, and more adhesion than Group 4, but these findings were not statistically significant. The apoptosis scores in Groups 1 and 2 were significantly lower than in the other groups. Ovarian dimensions were significantly decreased in Group 3. Follicular degeneration was significantly increased in Group 3.

Conclusion: Exogenously administered melatonin, if used at much higher doses orally, may be a noncytotoxic, antiapoptotic agent and may also have a protective effect on ovarian tissue damage that radiation can cause at the level of fine structure.

Key words: Magnetic fields, melatonin, ovarian tissue

1. Introduction

As a consequence of technological improvements, exposure to magnetic fields has been increasing. The harmful effects of chronic exposure to magnetic fields can be observed in all tissues of the human body, particularly in the ovaries (1,2).

Damage in ovarian tissues due to magnetic fields is generally associated with an increase in free radicals and oxidant production and a decrease in antioxidant capacity (3–5). With increased exposure to magnetic fields, reactive oxygen species (ROS) increase in the ovaries and most other tissues of the body (4). ROS affect cell proliferation and differentiation and cause apoptosis and necrosis (6,7). ROS also have direct toxic effects on cells, leading to lipid peroxidation, protein oxidation, and DNA damage. As a result, neurodegeneration, cellular aging, cancer, chronic inflammatory pathologies, and ovarian pathologies occur (8–11).

Melatonin (N-acetyl-5-methoxytryptamine), an important nonenzymatic antioxidant hormone produced by the pineal gland, has a circadian rhythm that is generated by the circadian pacemaker situated in the suprachiasmatic nucleus (SCN) of the hypothalamus, synchronized to 24 h primarily by the light/dark cycle via the SCN (12). Once synthesized, melatonin is quickly released into the bloodstream and then into other body fluids, such as the cerebrospinal fluid, semen, amniotic fluid, and ovarian follicular fluid (13). Melatonin has many functions in organisms, such as regulating seasonal reproduction (14).

It has been shown that melatonin is a powerful free radical scavenger and a broad-spectrum antioxidant (15). Melatonin crosses all cell membranes easily because of its small size and highly lipophilic nature and is found at high concentrations in subcellular compartments, including mitochondria and nuclei (16,17). Melatonin plays a role in preventing lipid peroxidation and damage to protein and DNA, and in maintaining optimal mitochondrial function (18,19) and homeostasis by reducing or preventing mitochondrial oxidative stress, which causes subsequent apoptotic events and cell death (20,21). Melatonin and its metabolites (i.e. cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, and N1-acetyl-5-methoxykynuramine) are excellent direct

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scavengers of toxic reactants and free radicals including superoxide, hydroxyl, singlet oxygen, hydrogen peroxide, hypochlorous acid, nitric oxide, and peroxynitrite anion (18). In addition, melatonin plays an important role in activating antioxidant markers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSH-Rd), and glucose-6-phosphate dehydrogenase (G6PD) (22).

This study was designed to investigate whether damage to ovaries induced by exposure to a magnetic field is inhibited by the administration of melatonin, a protective antioxidant that has a role in regulating ovarian function.

2. Materials and methods

Forty Wistar albino rats, ranging in weight from 150 to 200 g, were included in this study. During the experiment, the rats were kept in wire mesh cages with a grid size of 30 × 60 × 30 cm, each of which housed 10 rats. The rats were given ad libitum access to food (Feeding Foundation Standard Pellet Rat Feed) and tap water in a 12-h light/dark cycle. Standard room temperature (22 ± 1 °C) and humidity were maintained.

Permission to perform the study was obtained from the local ethics committee of Ankara Education and Training Hospital. The 40 rats were categorized into four treatment groups:

Group 1: Control group (n = 10)

Group 2: Melatonin administration (10 mg/kg) only (n = 10)

Group 3: Magnetic field exposure (20 μT/day) only (n = 10)

Group 4: Melatonin administration (10 mg/kg) + magnetic field exposure (20 μT/day) (n = 10)

We generated the magnetic field by passing current through a bobbin. The magnitude of the magnetic field generated was calculated with the formula B = k × I/R, where B = magnetic field, k = constant, I = current, and R = wire length. The ammeter was set at 2.40 A with a rheostat while the applied magnetic field was produced. In the assembly, the coil, rheostat, voltmeter, and ammeter were connected in a series. The coil was placed at the midpoint of the rat cages to provide the same amount of exposure to all animals. Thirty minutes after the administration of the melatonin, the magnetic field was applied for 30 min at a dose of 20 μT for 10 days.

Melatonin was added to a solution consisting of 16.2 mL of saline and 1.8 mL of alcohol. This mixture was applied orally at a dose of 10 mg/kg via nasogastric tube 30 min prior to exposure to the magnetic field.

After the rats were euthanized on day 11, their abdomens were opened with a vertical incision and the ovaries were removed under sterile conditions. We collected the extracted materials in saline-containing containers to perform histological examinations. After detoxification in 5% formic acid solution, tissues were processed and sections were taken from the prepared paraffin blocks with a thickness of 4 mm and stained with hematoxylin and eosin. The sections were evaluated under an Olympus BX51 microscope for the number of mature follicles, adhesion, fibrosis, apoptosis, ovarian size, and follicular degeneration.

2.1. Histopathological examination

Histomorphological characteristics were evaluated using the following scoring system:

Number of mature follicles: 1–5 = 1 point, 6–10 = 2 points, ≥11 = 3 points.

Adhesion: None = 0 points, partial = 1 point, significant = 2 points.

Fibrosis: None = 0 points, partial = 1 point, common = 2 points.

Apoptosis: None = 0 points, partial = 1 point, common = 2 points.

Ovarian size: Atrophied = 1 point, normal = 2 points.

Follicular degeneration: None = 0 points, partial = 1 point, common = 2 points.

2.2. Statistical analysis

All data are expressed as median (min–max). Differences between the arithmetic means of the parameters studied in the biological material obtained from the rats were compared statistically using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences among the groups were assessed using Kruskal–Wallis analysis. Increases in weight were assessed using the Wilcoxon signed rank test. Significance was set at $P < 0.05$ for all statistical comparisons.

3. Results

The number of mature follicles was relatively lower in Group 3 than in Group 1 ($P < 0.001$). The control group (Group 1) and melatonin-only group (Group 2) had less adhesion than the groups exposed to the magnetic field (Groups 3 and 4; $P < 0.005$). Group 3 had the highest adhesion score. Fibrosis scores were significantly lower in Groups 1 and 2 than in Groups 3 and 4 ($P < 0.001$) (Table 1).

Apoptosis scores were significantly lower in Groups 1 and 2 than in Groups 3 and 4 ($P = 0.001$). The ovarian sizes were higher in Groups 1 and 2 than in the other groups. The ovarian size of Group 3 was significantly smaller than that of the other groups ($P < 0.001$; Table 1).

Follicular degeneration was significantly lower in Groups 1 and 2 than in the other groups, but no significant differences were observed among the other groups (Figure 1). When we examined the groups in terms of follicular degeneration, we observed the greatest amount of degeneration in Group 3 (Figure 2). When compared to the group receiving only radiation,

Table 1. Histomorphometric scores of groups.

Group	Number of mature follicles	Adhesion score	Fibrosis score	Apoptosis score	Ovarian dimension	Follicular degeneration score
Group 1	3 (3–3) [a,b]	0 (0–0) [a,e]	0 (0–0) [a,b]	0 (0–0) [a,e]	2 (2–2) [a,g]	0 (0–0) [a,e]
Group 2	3 (3–3) [c,d]	0 (0–0) [c,f]	0 (0–0) [c,f]	0 (0–0) [c,f]	2 (2–2) [c,h]	0 (0–0) [c,f]
Group 3	1 (1–2) [a,c]	1 (1–2) [a,c]	2 (1–2) [a,c]	2 (1–2) [a,c]	1 (1–1) [a,c]	2 (1.5–2) [a,c]
Group 4	2 (2–2) [b,d]	1 (1–1) [e,f]	2 (1–2) [b,f]	1 (1–1.5) [e,f]	1 (1–2) [g,h]	1 (1–2) [e,f]
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] The difference between Group 1 and Group 3 was statistically significant (P < 0.001).
[b] The difference between Group 1 and Group 4 was statistically significant (P = 0.004).
[c] The difference between Group 2 and Group 3 was statistically significant (P < 0.001).
[d] The difference between Group 2 and Group 4 was statistically significant (P = 0.004).
[e] The difference between Group 1 and Group 4 was statistically significant (P < 0.001).
[f] The difference between Group 2 and Group 4 was statistically significant (P < 0.001).
[g] The difference between Group 1 and Group 4 was statistically significant (P = 0.018).
[h] The difference between Group 2 and Group 4 was statistically significant (P = 0.018).

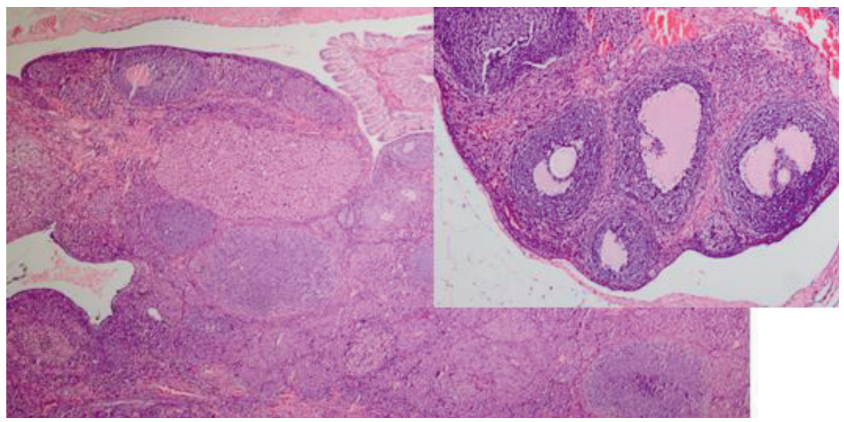

Figure 1. Normal ovarian tissue.

it was observed that the application of melatonin caused an increase in the number of follicles (Figure 3).

4. Discussion

Prolonged exposure to magnetic fields has become part of daily life in recent years owing to technological developments and the increasing use of these technologies. However, such exposure may have adverse effects on the reproductive system. Although the mechanism underlying the toxic effects of magnetic fields on tissues is not yet clearly understood, it is thought to cause damage by increasing tissue temperatures and inducing oxidative stress, which leads to a breakdown in communication between cells (23).

In a study assessed in 2008, the long-term application (2 hours/day for 10 months) of magnetic fields of 100 and 500 μT, which are considered to be the safety limits for the public and employees, respectively, were investigated for oxidative DNA damage and the low-frequency magnetic field was shown to cause oxidative DNA damage (24). The safety margin in Turkey is 200 μT. In our study, the applied magnetic field was 20 μT considering the body area of the rats. In our study, even at this low level, the significant follicle degeneration, size reduction, and increased apoptosis in cells suggests that this safety limit value should be further reduced. We applied the magnetic field for 10 days. Although it is very difficult to indicate the tendency to transform into cancer during this process, as follicle degeneration increases, the appearance of cancer cells is a predictable parameter according to the studies performed.

In studies that suggested that magnetic fields lead to ovarian tissue damage, exposure to magnetic fields caused ovarian atresia, shrinkage and changes in shape

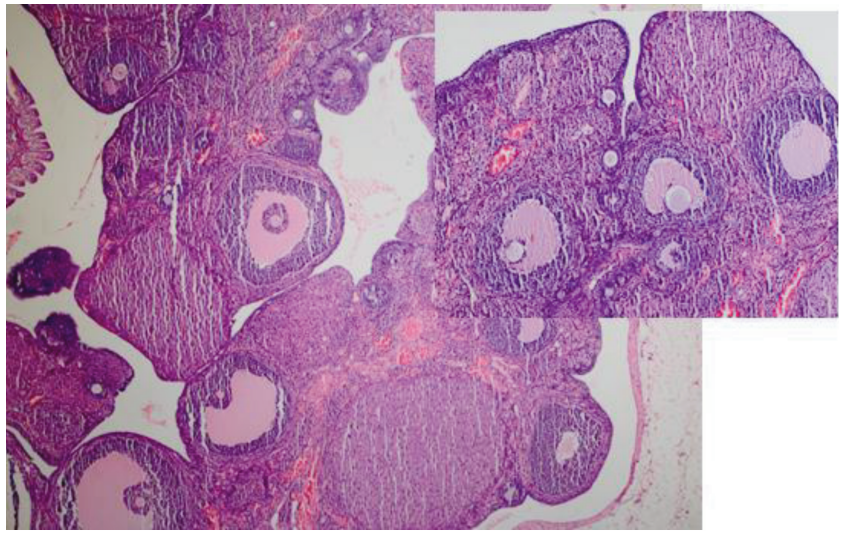

Figure 2. Ovarian tissue from the group that received radiation exposure only. The atrophic appearance of the ovary and the lack of follicles are remarkable.

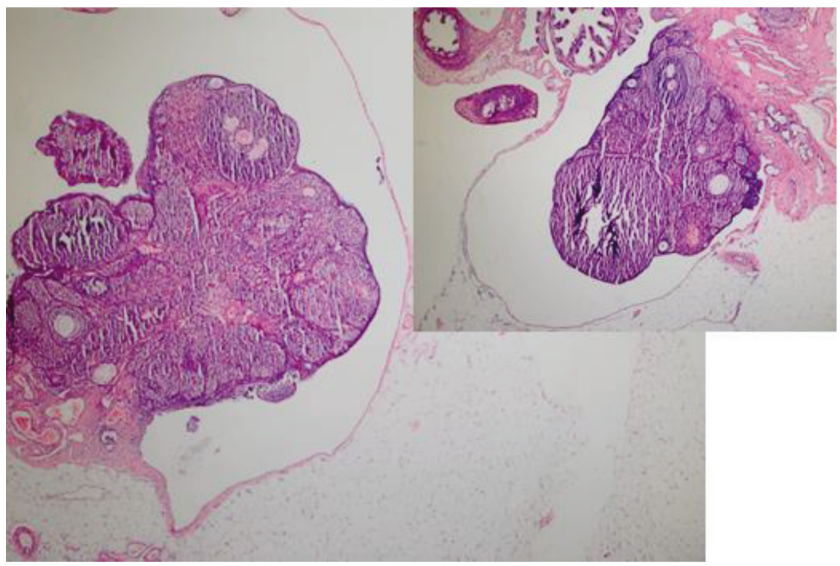

Figure 3. Ovarian tissue from the group that received melatonin before radiation exposure. When compared to the group receiving only radiation, it is observed that the application of melatonin caused an increase in the number of follicles.

of oocyte nuclei, decreased numbers of ovarian follicles, and DNA damage (1,2,25,26). In our study, we similarly found significant degeneration in mature follicles in the group exposed to radiation. We also observed a significant decrease in the number of ovarian follicles and ovarian atresia. Recent studies have found that magnetic fields increase apoptosis by inducing oxidative stress in uteri and ovaries (27,28). In an in vitro study, magnetic fields caused DNA damage in rat granulosa cells and significantly decreased ovarian size (29). Ferchichi et al. also showed in their study that ovarian sizes significantly decreased in rats exposed to magnetic fields (30). In 1999, Kim et al. showed that radiation caused cell apoptosis and impaired ovarian functions (31). In our study, we observed a significant increase in follicular degeneration, apoptosis, fibrosis, and adhesion scores and a significant decrease in ovarian size in groups exposed to magnetic fields. When we applied melatonin as an antioxidant, we found that there was not any significant recovery.

Melatonin is a powerful antioxidant that eliminates the damaging effects of free radicals that are formed in parallel with the metabolic rhythm (32). Because it is small enough

to easily pass through biological membranes, melatonin can access all of the organelles of a cell and easily prevent injuries to lipids, proteins, and DNA. Recent studies have shown that melatonin significantly prevents follicular degeneration, follicular atresia, oxidative stress, and apoptosis and reduces mitochondrial dysfunction in the ovaries. It also protects granulosa cells from oxidative stress (33–36). In a study by Vijayalaxmi et al., it was determined that the use of 300 mg of oral melatonin prevented DNA damage, reduced chromosomal aberrations, and reduced micronuclei development (37). In our study, we administered 10 mg/kg melatonin to rats 30 min before radiation exposure to prevent damage induced by the magnetic field. Melatonin was dissolved in solution and applied orally with nasogastric tube. We adjusted the dose of melatonin by adapting the doses used in humans and the study of Onal et al. about the effect of melatonin on bowel mucosal damage. In humans, melatonin is used orally in doses of 3–6 mg for sleep disorders. In the study carried out by Onal et al. in 2010, it was assessed that 15 mg/kg i.p. applied melatonin prevented bowel damage (38).

In our study, follicular degeneration, apoptosis, fibrosis, and adhesions were significantly increased and ovarian size was significantly reduced in the group receiving radiation. The number of mature follicles in the melatonin-receiving group was not different from that of the control, indicating that melatonin did not corrupt the ovarian tissue and had no degenerative effect. Ovarian damage was less severe in rats that received melatonin compared to those that did not, but it was seen that radiation-induced damage was not prevented to a significant extent.

In our study, there was a significant increase in immature follicles and a significant degeneration of mature follicles in the radiation-receiving group. Although there was a partial decrease in immature follicles with the administration of melatonin with radiation, there was no statistically significant result. Kaya et al. (39) also showed a 42% and 12% increase in the number of primordial and antral follicles, whereas the precentral staining was not significant, 16 h after radiation. Jarrell et al. (40) and Ataya et al. (41) also reported that preantral follicles showed nearly normal morphology after radiation applications.

Although it was not statistically significant, the number of mature follicles was higher in the group receiving melatonin and radiation than in the group receiving only radiation. Adhesion scores were significantly increased in the group receiving radiation. Adhesion, fibrosis, and apoptosis scores of the melatonin and radiation group were decreased when compared with the radiation-receiving group, but this was not statistically significant. Although we assessed the protective effect of melatonin in the ovary, it was not statistically significant. The reason for this may be that we used a low dosage of melatonin or had an insufficient sample size. Oral bioavailability of melatonin is considered to be lower than intraperitoneal use. Because the effect of radiation on ovarian tissue is very high, we believe that if preradiation melatonin is to be used orally, it should be used at much higher doses (100–300 mg) than the 2 mg used in routine settings.

In conclusion, we found that melatonin, an exogenous agent, is a noncytotoxic, antiapoptotic agent. There may also be a protective effect by reducing the impact of free radicals on the ovarian tissue damage at the fine structure level that radiation application can generate. Prophylactic melatonin may be useful for preventing ovarian tissue damage and consequent development of female infertility that may occur as a result of increased exposure to magnetic fields. However, melatonin should be used at a much higher dose than the oral dose. Our work should be supported by immunohistochemical studies with new antibodies to demonstrate cellular degeneration.

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