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The Effects of Melatonin on the Bladder in the Application of Low and High Dose Rate Radiotherapy in the Abdominopelvic Region in Rats

Ratlarda Abdominopelvik Bölgeye Radyoterapi Uygulamasında Melatoninin Mesane Üzerine Etkileri

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ABSTRACT

Background: To compare the changes caused by low and high dose rate radiotherapy in the bladder tissue of rats and to examine the effect of melatonin on the bladder tissue.

Materials and Methods: 40 adult rats were randomly divided into five groups with 8 rats in each group. Radiotherapy and melatonin treatment were not applied to group 1 (G1) control group rats. A single dose of 8 Gy and 400 MU/min radiotherapy was applied to G2 and G3 group rats. A single dose of 8 Gy and 1400 MU/min radiotherapy was applied to G4 and G5 group rats. G3 and G5 group (treated groups) rats were given 50 mg/kg melatonin intraperitoneally 15 minutes before the radiotherapy application. Rats were sacrificed under anesthesia and bladder tissues were removed. Histopathological examination was performed on the samples stained with hematoxylin eosin and toluidine blue.

Results: Number of mast cells was increased in G2 and G4 ($p<0.01$). In addition, edema and vascular congestion were observed in these groups. In G3 and G5, acute phase markers decreased compared to radiotherapy received groups.

Conclusion: Bladder tissue degradation was observed in G2 and G4 compared to the control group. However, there was no difference in bladder tissue between the groups given two different doses. The acute phase markers of edema, number of mast cells, inflammatory cell infiltration, and vascular congestion in the bladder tissue of the groups administered melatonin decreased.

Keywords: Abdominopelvic region, melatonin, bladder, low dose rate radiotherapy, high dose rate radiotherapy

ÖZ

Amaç: Düşük ve yüksek doz hızlı radyoterapi uygulamasının ratların mesane dokusunda meydana getirdiği değişikliklerin karşılaştırılması ve melatoninin mesane dokusu üzerindeki etkisinin incelenmesidir.

Gereç ve Yöntemler: Kırk erişkin rat rastgele her grupta 8 rat olacak şekilde beş gruba ayrıldı. Grup 1 (G1) kontrol grubu ratların herhangi bir işlem uygulanmadı. G2 ve G3 grubu ratlara tek doz 8 Gy ve 400 MU/dk radyoterapi uygulandı. G4 ve G5 grubu ratlara tek doz 8 Gy ve 1400 MU/dk radyoterapi uygulandı. G3 ve G5 grubu ratlara radyoterapi uygulamasından 15 dakika önce intraperitoneal 50 mg/kg melatonin verildi. Ratlar sakrifiye edildi ve mesane dokuları çıkarıldı. Hematoksilen ve eozin ve toluidin mavisi ile boyanan örnekler üzerinde histopatolojik inceleme yapıldı.

Bulgular: G2 ve G4'te enflamatuvar hücre infiltrasyonu arttı. Ayrıca bu gruplarda ödem ve vasküler konjesyon gözlendi. G3 ve G5'te akut faz belirteçleri radyoterapi alan gruplara göre azaldı.

Sonuç: Kontrol grubuna kıyasla G2 ve G4'te mesane dokusu bozulması gözlendi. Ancak iki farklı doz verilen gruplar arasında mesane dokusu açısından fark yoktu. Melatonin uygulanan grupların mesane dokusunda ödem, enflamatuvar hücre infiltrasyonu, mast hücre sayısı ve vasküler konjesyondan oluşan akut faz belirteçleri azaldı.

Anahtar Kelimeler: Abdomino-pelvik bölge, melatonin, mesane, düşük doz hızlı radyoterapi, yüksek doz hızlı radyoterapi

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Introduction

Cancer, which is one of the diseases that affect patient survival and quality of life, has not yet been treated with 100% success. In the clinic, radiotherapy treatment in addition to surgery and chemotherapy treatments is applied together with them at a rate of 50-60%. In 2018, approximately 600 thousand patients were diagnosed with bladder cancer, and approximately 200 thousand patients died (1). The risk factors for this disease are multiple. Exposure to carcinogens is one of the biggest causes of bladder cancer. Geography, age, and gender also affect the incidence of the disease.

The most important symptom of bladder cancer is microscopic/gross hematuria. In 75% of cases diagnosed as bladder tumors, it is mucosa-limited (non-muscle invasive) urothelial bladder cancer. In 25% of cases, cancer invades muscle tissue or metastasizes (2). Transurethral resection of bladder tumor is the mainstay treatment in patients with non-muscle-invasive bladder cancer, whereas, in patients with muscle tissue invasiveness, the bladder is removed (3).

In the treatment of various types of cancer, including bladder cancer, treatment using ionized X-rays is called radiotherapy. The aim of radiotherapy treatment is to remove unwanted tissue by applying a radiation dose to the tumor (4). Compared to chemotherapy, the advantage of radiotherapy treatment is that it inhibits tumor growth *in situ* with its local effect. However, the deterioration of healthy tissue exposed to irradiation in the cancer area is inevitable (5). X-rays applied on the cancerous tissue with radiotherapy treatment penetrate the surface tissue layers and reach the deep tissues. Thus, by causing some changes in the DNA structure, it induces apoptosis of the target tissue, and tissue proliferation is prevented (6). As in any treatment, the correct dose is very important in radiotherapy treatment. While the apoptosis of the cancerous tissue is induced, the dose that does not harm the healthy tissue is the most effective dose (7). Many studies have shown that doses of 6-8 Gy and above are effective in produce an effective immunogenic response (8).

As a result of routine medical treatments applied to bladder cancer, patients' quality of life and survival time decrease. For this reason, alternative or complementary treatments are popular in addition to radiotherapy treatment today. One of them is melatonin, a naturally produced hormone in our body that has many beneficial potential effects, including its anticancer and radioprotective properties (9,10). It has been shown that this active substance can suppress urological cancers, including bladder cancer, by affecting basic cellular pathways by applying it to patients together with radiotherapy treatment, which is generally applied together with chemotherapy (11).

Ionizing radiation beams cause some interactions as they pass through the biological layers of the body. Molecules inside the cell, especially DNA molecules, are damaged by ionizing radiation (6,12,13,14). This damage to DNA affects the survival of the cell, especially since it is the main part responsible for cell growth and division. In addition, the increase in free radicals that occur as a result of this interaction and the inability to maintain the balance between the naturally occurring antioxidants in the body causes the vital functions of the organism to come to an end. Melatonin, which has a strong antioxidant property, has a protective effect against oxidative damage caused by free radicals (15). In addition to its antioxidant effect, the melatonin hormone, which plays a role in many physiological events such as the creation of immune responses, aging, sleep, and temperature regulation, also shows a healing effect against the damage caused by radiation with its radioprotective feature (16).

Material and Methods

Experimental Groups

This project was ethically approved by the University of Health Sciences Türkiye Hamidiye Animal Experiments Local Ethics Committee no: 2021-01/07. Before starting the experiment, 40 adult male rats (12 weeks old) weighing approximately 250 grams were fed with tap water and pellet feed at 21-23 °C, in cages suitable for the number of groups, in 12 hours of light/12 hours of darkness.

Fourty rats were randomly divided into 5 groups with 8 rats in each group:

- G1: Control group
- G2: Low dose rate radiotherapy group (LDR)
- G3: Low dose rate radiotherapy + melatonin group (LDR+M)
- G4: High dose rate radiotherapy group (HDR)
- G5: High dose rate radiotherapy + melatonin group (HDR+M)

Radiotherapy and Melatonin Application

A varian brand, trilogy model linear accelerator device, located in the Clinic of Radiation Oncology, University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital, was used for radiotherapy application. Anesthesia of 80 mg/kg/IP ketamine and 20 mg/kg/IP xylazine was administered to rats in G2, G3, G4, and G5 groups. The rats were placed on the platform to be treated with radiotherapy in the supine position. The skin-source distance to the abdominopelvic regions of the rats was adjusted to 100 cm. Ionized X-rays at 6 MV low and high dose rates were applied. A bolus of 10 mm tissue equivalent

was placed in the abdominopelvic region to keep the area where the maximum dose was applied above the bladder tissue. For the 6 MV ionized X-ray, the dose maximum point was calculated at a depth of 1.6 cm from the skin surface, and the dose efficiency of the device was 1 MU =1 cGy. Melatonin (Melatonin Crystalline, Sigma-Aldrich Corporation) was dissolved in 1% ethanol solution and 1 mL was given to each animal 15 minutes before radiotherapy for the groups administered melatonin (17).

No procedure was applied to the G1 control group rats. A single dose of 8 Gy and a low dose rate of 400 MU/min radiotherapy was applied to the abdominopelvic regions of the rats in the G2 and G3 groups. In addition to G3 group rats, 50 mg/kg melatonin IP was given 15 minutes before radiotherapy. A single dose of 8 Gy and a high dose rate of 1400 MU/min radiotherapy was applied to the abdominopelvic regions of the rats in the G4 and G5 groups. In addition to G5 group rats, 50 mg/kg melatonin IP was given 15 minutes before radiotherapy (17).

Collection of Samples

At the 48th hour following the radiotherapy treatment, all rats were sacrificed by exsanguination (euthanasia procedure by taking blood from the heart) by applying 80 mg/kg/IP ketamine and 20 mg/kg/IP xylazine anesthesia. For histological examinations, bladder tissues were placed in 10% formaldehyde and fixed for 72 hours.

Histopathological Assessment

At the end of the fixation, dehydration was performed for one hour in 70%, 80%, 96%, and 99% ethanol solutions, respectively. Then, the bladder tissues were kept in alcohol-xylene mixture for half an hour and in xylene for 1 hour for the transparency phase. The last step is repeated. In the paraffin embedding step, it was first kept in a paraffin-xylene mixture for half an hour and then in paraffin for 2 hours. 5 µm thick sections were taken from the bladder tissues embedded in the paraffin blocks. Finally, all samples were stained with hematoxylin & eosin stain for histopathological evaluation. Then, bladder tissues were examined with a light microscope (Axiocam-Zeiss). The sections were scored: 0: No damage, 1: Mild damage, 2: Moderate damage, 3: Severe damage (18). Mast cells were counted in consecutive Toluidine Blue (TB)-stained lung sections by one or more observers. Mast cells were counted in ten randomly selected fields from each preparation at 40X magnification. The number of mast cells was expressed as the number of cells per unit area. Images were taken using a digital camera (Zeiss, Axiocam 105 Color, Germany) and a Light Microscope (Zeiss, Scope.A1, Germany). Mast cells counted separately for all groups from the samples were statistically analyzed, and $p<0.05$ was considered significant.

Statistical Analysis

Statistical analysis of the data was performed with GraphPad-Prism program. The distribution of variables was analyzed using the Shapiro-Wilk test and non-normally distributed variables were reported as median (minimum-maximum). Non-normally distributed variable groups were examined with the Kruskal-Wallis H test. Within-group differences were examined using the Bonferroni correction. $P<0.05$ was considered statistically significant.

Results

Histopathological Findings

In the G1 group, normal mucosa and the overlying mucosa layer were observed in the bladder wall (Figure 1). Detachment and loss of urothelial cells were observed in both high and low dose rate radiotherapy-treated rats, while urothelial integrity was preserved in melatonin-treated rats and urothelial degeneration was observed only in a local area. In the radiotherapy groups, increased inflammatory cell infiltration, edema, and vascular congestion were observed. Acute phase markers of two treatment groups (G3 and G5) decreased compared to radiotherapy groups. The increased acute phase marker scores of the bladder tissues of radiotherapy-treated rats were significantly reduced by melatonin treatment, but the scores did not approach the levels of control rats.

Number of Mast Cells

The number of mast cells was increased in low and high dose rate radiotherapy-treated groups compared with the control group (Figure 2). Low dose rate radiotherapy-treated group number of mast cells was higher than low dose + melatonin received group and high dose + melatonin received group ($p<0.01$). High dose rate radiotherapy-treated group number of mast cells was higher than low dose + melatonin received group and high dose + melatonin received group ($p<0.01$) (Figure 3).

Discussion

In the clinic, the use of high dose rate based modern radiotherapy devices, which do not use the flattening filter, has started to increase as an upper version of the low dose rate standard linear accelerator devices with a radiation straightening filter (19,20). Long treatment times are shortened with high dose rate technology. In addition, this treatment technique is considered to be better because the scattered radiation is less compared to low dose rate technology, it reduces the risk of secondary cancer in the healthy tissue around the cancerous tissue

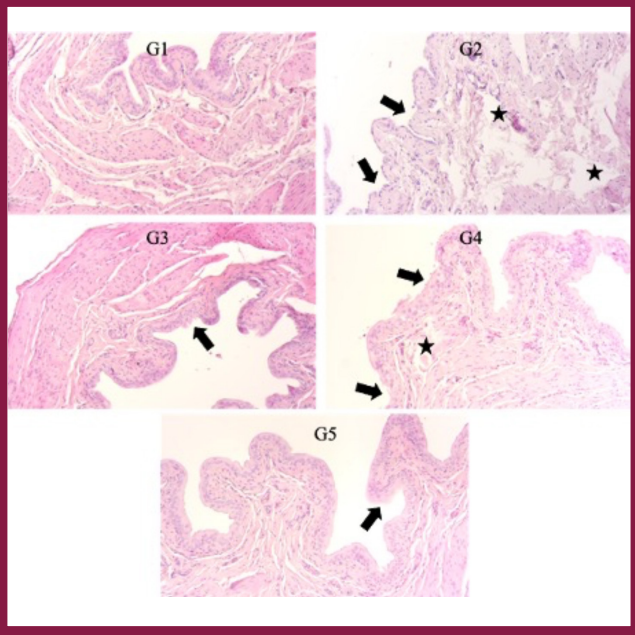

Figure 1. The urinary bladder in experimental groups stained with H&E. G1: Regular morphology of urinary bladder was observed. G2 and G4: Radiotherapy-treated rats' bladder tissue samples degenerated urothelial layer (arrow) and edema (star) were observed. G3 and G5: Healing of the urothelial mucosa (arrow) and mucosa layer was observed in melatonin-treated groups. The light microscope, X10 magnification. G1: Control, G2: LDR, G3: LDR+M, G4: HDR, G5: HDR+M

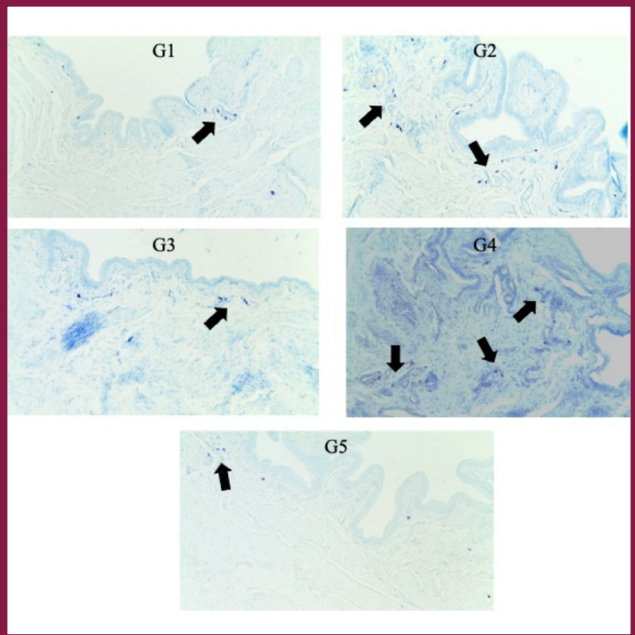

Figure 2. Bladder tissues stained with TB, arrow: Mast cells. The light microscope, X10 magnification. G1: Control, G2: LDR, G3: LDR+M, G4: HDR, G5: HDR+M

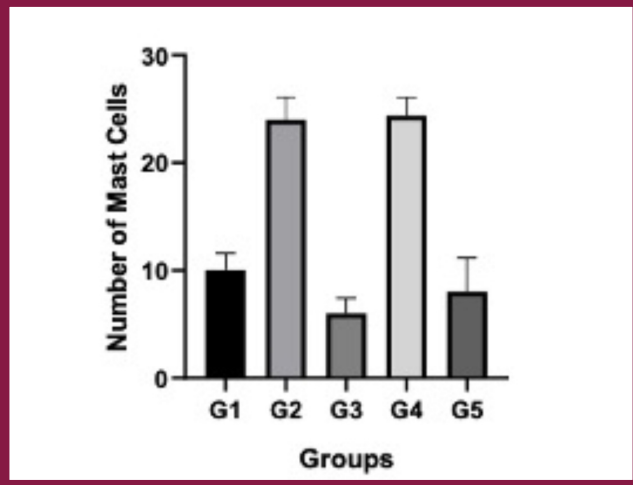

Figure 3. Comparison of the number of mast cells between groups. G2>G3 ($p<0.001$), G2>G5 ($p<0.01$), G4>G3 ($p<0.001$), G4>G5 ($p<0.01$)

and avoids unnecessary dose exposure of healthy tissues. Studies comparing the physical interactions of these two technologies in the healthy bladder tissue of rats are not available in the literature. In addition, in this study, the radioprotective effects of melatonin against cell damage caused by radiotherapy in the bladder tissue were also investigated.

Today, some cell-protecting agents are used to increase the induction level of apoptosis of cancerous tissue by applying ionizing radiation therapy and to minimize the effect of these radiation rays on the healthy tissue around the cancerous tissue. As recent studies have shown, the ability of melatonin to reduce tissue damage by the hydroxyl radical is presented as a justification for testing its radioprotective ability (21). In studies comparing many antioxidant-active substances such as vitamin E, vitamin C, curcumin, mannitol, and glutathione, it has been noted that melatonin is one of the strongest antioxidants (22). Melatonin is lipophilic, so it can reach almost all organelles of the cell and especially the cell nucleus. With this feature, it can be said that it has a protective effect against DNA damage (23).

In light of all this information, we compared the effects of low dose rate and high dose technology on bladder tissue in radiotherapy application in our study. In addition, we evaluated the effects of melatonin application on the damage to healthy tissue as a result of these treatments. Improvement in bladder tissue morphology noted in this study, including improvement of radiation-induced vascular occlusion, healing of the urothelial mucosa and mucosa layer, and preservation of uroepithelial integrity. Mast cells serve to renew the organism and to return to its former healthy state in case of any degradation in the tissue. It has properties such as tissue repair and immune system

support. In this study, mast cells detected by the TB staining method were counted and statistically analyzed. While the number of mast cells in the bladder tissues of the rats in the radiotherapy groups increased statistically, there was no statistical difference between the two different doses of radiotherapy. However, the number of mast cells was found to be statistically lower in the melatonin administered groups. It has been suggested that melatonin is associated with reversal of radiotherapy-treated capillary dilation and edema, and that the combination of melatonin and radiation reverses the immunological toxicity of irradiation (24,25).

Conclusion

As a result of our literature review, we could not find any study comparing the effect of melatonin application on the bladder from 8 Gy, 6MV ionized X-rays, low (400 MU/min), and high (1.400 MU/min) radiotherapy treatment applied to the abdominopelvic region. According to the results of this study, in which the histological examination of the early damage that may occur in the tissue exposed to radiation was performed, healthy bladder tissues were significantly affected by radiation. It can be said that melatonin, which is applied for the protection of healthy tissue, reduces radiation damage in the bladder tissue. However, we believe that the studies on this subject should be detailed, and dose studies should be increased for both radiotherapy application and melatonin treatment.

Ethics

Ethics Committee Approval: This project was ethically approved by the University of Health Sciences Türkiye Hamidiye Animal Experiments Local Ethics Committee no: 2021-01/07.

Informed Consent: Since our study was with experimental animals, we do not have a patient consent form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.S., S.T., K.B., E.E., Concept: T.S., E.E., Design: T.S., E.E., Data Collection or Processing: S.T., K.B., E.E., Analysis or Interpretation: K.B., S.T., Literature Search: S.T., E.E., Writing: T.S., S.T.

Conflict of Interest: There is no conflict of interest between the authors.

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