RADIO-PROTECTIVE EFFECTS OF MELATONIN ON THE SKIN HISTOLOGY IN THE RAT

Zelal Unlu Cakır[1], Can Demirel[1], Pelin Ugurlu[2], Berna Guney Saruhan[3], Sennur Ketani[4], Ugur Seker[3], Seyit Burhanedtin Zincircioglu[5]*

1. Gaziantep University, Faculty of Medicine, Department of Biophysics, Gaziantep, Turkey
2. Dicle University, Institute of Natural and Applied Sciences, Diyarbakır, Turkey
3. Dicle University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Diyarbakır, Turkey
4. Dicle University, Ziya Gokalp Faculty of Education, Department of Biology Education, Diyarbakır, Turkey
5. Dicle University, Faculty of Medicine, Department of Radiation Oncology, Diyarbakır, Turkey

Abstract

In this study, the protective effect of melatonin on the radiation-damaged skin was investigated and effectiveness of the protective effect of melatonin was compared with amifostine. Fort this purpose, 40 female Sparague Dawley rats were used. Animals were divided into 5 groups. Control group (C), radiation group (R), and 2 experimental groups (radiation + melatonin 25 mg/kg (R+M) and radiation + amifostin 200 mg/kg (R+WR). A single dose of 20 gy gamma radiation were exposed to the left legs of the rat groups of R, R+M,and R+WR.

At the end of a four-week of experiment period, the skin on the left femur of each rat was dissected and routine histological procedures were applied. Nikon Eclipse 400 research microscopy was used for histological observations.

All histological structures of the skin were found to be normal in the control group. However, we observed that reduction in thickness of the epidermis and the dermal papillae have disappeared, sweat glands, sebaceous glands and hair follicles atrophy was observed in radiation groups. Our findings showed that R+M group, the thickness of the epidermis, collagen synthesis, R and R+WR group was better with histological structure.

As a result, the histological abnormalities of radiation is shaped to protect in the skin, it has been found that melatonin has a significant effect compared to Amifostine (WR2721).

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Introduction

Recently application of radiation science in different settings (e.g., radiotherapy, biomedical research, military and space research) is increased and therefore protecting humans against the harmful effects of radiation is a major challenge that needs an urgent solution. During radiotherapy, ionizing irradiation interacts with biological systems to produce free radicals or reactive oxygen species (ROS), which attack various cellular components including DNA, proteins and membrane lipids, leading to serious cellular damage.[1] The X-rays (electromagnetic ionizing radiation) are composed of massless particles of energy (photons) that disrupt the electrons of atoms within cells and therefore affect cellular functions. X-ray irradiation (XRI) can affect both normal and neoplastic cells especially the rapidly growing ones such as the epidermal cells. Although X-rays are widely used for both imaging and therapeutic purposes, our knowledge about their possible injurious effects on the skin is incomplete. Morphologically XRI can produce epidermal loss, cristolysis, cytoplasmic vacuolization, appearance of euchromatic nuclei, altered microvasculature, hyperkeratinization, redistribution of biometals, as well as basal and squamous cell carcinoma.

Corresponding author:
Dr. Sennur Ketani
Dicle University, Ziya Gokalp Faculty of Education,
Department of Biology Education,
Diyarbakir, Turkey

The type and extent of these changes depends on the dose, duration and frequency of XRI.[2-5]

Melatonin (N-acetyl-5-methoxytryptamine), which was discovered about 40 year ago, is a ubiquitously acting molecule with several functions. It was initially recognized as a molecule related to neuroendocrine physiology, especially reproductive physiology. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone which is secreted from cells called pnealosits in the pineal gland and regulates biorhythm.[6,7] Because Melatonin (M) easily passes through cell membrane due to its high lipophilic nature and reaches mitochondria and cell DNA; and has an antioxidant feature, It is thought that melatonin is effective on the reducing oxidative damage caused by radiotherapy.[8] For reducing the effects of such damage melatonin is included the use of antioxidant agents.[9,10] As many plants are major sources of melatonin, cherries and there are high concentrations in the pineal gland in the brain.[11] However, in recent years, using experimental models of the protective effect of melatonin against radiation damage studies showing a protective effect.[12,13,14] To date, the radioprotective role of melatonin against X-ray induced skin damage is still unknown.

Various studies about decreasing normotensive tissue demolition and damage of cell after radiation therapy have been carried out. In the pre-clinic studies it was shown that Amifostin (WR-2721) had protective effect on this alterations. It has been determined that Amifostin is an effective agent on the acute and chronic effects of radiation treatment on the esophagus, intestine and colon of experimental animals. Amifostin is a chemical which is transformed into its active form WR-1065 by alkaline phosphatase in capillary endothelium and easly enters into the cell protoplasm. Even the mechanism of cytoprotective effects of amifostin is un known, common idea is that amifostin has reducing feature of free radicals ocurring after radiotehrapy (RT).[15,16,17] Amifostine in clinical protective effect against radiation damage to normal tissues is the only drug proven preservatives used in routine. Radiation therapy has been used for many years.[18]

In this study, It was histologically investigated the effects of melatonin on the alterations caused by radiotherapy treatment in the skin of rats. The effect of melatonin was also compared with that of Amifostine.

Materials and Methods

The experimental protocol was approved by the Medical Sciences Application and Research Center of Dicle University (DUSAM), and use Committee of the Dicle University Experimental Animal Ethics Committee.

In our study 40 Sprague Dawley female rats provided from Medical Sciences Application and Research Center of Dicle University (DUSAM) were used. Prior to the experiments, the experimental animals were acclimated to the laboratory conditions for 1 week before the experiments. In this period, the animals were maintained in 50x80x50 size polycarbonate cages and there were 8 animals in each cages. After acclimation period the animals were separated in to 5 groups which had 8 animals per group. Before experiments the average weight of the animals were calculated as 175±15,5 gr.

Throughout the experiments the temperature and the humidity were 21±2 ºC and %50–70. During the 4 weeks experiments the animals were maintained under 12 hours light (07:00 – 19:00) and 12 hours dark artificial light regime. The experimental animals were supported with standard diet and ad libitum access to water within DUSAM.

For this purpose, 40 female Sparague Dawley rats were used. Animals were divided into 5 groups. These are control group (C; n=8: saline, 1 ml i.p.), radiation (R; n=8; saline, 1 ml i.p.), radiation + melatonin 25 (R+M25; n=8: 25 mg/kg, i.p.), radiation + melatonin 50 (R+M50; n=8; 50 mg/kg i.p.) and radiation + amifostisin (R+WR; n=8; 200 mg/kg i.p.). A single dose of 50 Gy gamma radiation was exposed to the left legs of the rat groups of R, R+M25, R+M50 and R+WR. Radiation groups (R, R + M25, M50 R +, R + WR) single dose of 50 Gy using a cobalt-60 device left leg was irradiated. Dose rate of 50 Gy / min. Dicle University Oncology Hospital, Radiation Oncology Department, the radiation in the group of rats during the first day to the surface 50 Gy facing Dicle University Oncology Hospital, Radiation Oncology Department at the ALCYON-II Co-60 teletherapy device 30x30 cm in size field 42 minutes Gamma radiation was irradiated.

Rats irradiation 4 weeks after intramuscular 10 mg / kg xylazine (Rompun, Bayer, Turkey) and 70 mg / kg Ketamine HCl (Ketalar Eczacıbaşı, Turkey) anesthetized with

an anesthetizing were sacrificed.

4 weeks later after radiation, the animals in all groups were sacrificed and the left legs of skin samples taken from the areas exposed to radiation were fixed in 10% formalin for 24 hours. After routine histological process, the tissue samples were embedded in paraffin, 5 µm thick sections were taken and these sections were deparaffinized on adhesive lams for 24 hours. After xylene treatment and decreasing graded alcohol series, sections were stained with Hematoxylin-Eosin and Crossman modified Mallory Azan and[19] sealed with entella. All sections were examined histologically under light microscope (Nikon Eclipse 400, Nikon, Japan).

Results

All histological structures of the skin were found to be normal in the control group (Figure-1A). It was observed that M group had similar histological findings to C group (Figure-1A,1C). The thickness reduction of the epidermis, the disappearance of dermal papillae, atrophy of sweat glands, sebaceous glands and hair follicles were observed in the radiation groups (Figure-1B).In R+M and R+WR groups, similar to R group, thickness reduction of the epidermis, atrophy of sweat glands and hair follicles were identified (Figure-1B, 1D, 1E), however it was concluded that R+M group was better in histological structure than R and R+WR groups with regard to glands, follicles and collagen synthesis (Figure-2B, 2D, 2E).

Discussion

A lot of studies have been carried out about radioprotective effect of melatonin on experimental animals. Melatonin shows its radioprotective effect with reducing oxidative stress.[6,8,13,19] Therefore, it is known that melatonin takes part in the expression of antioxidant enzymes like Cu/Zn-superoxide dismutase (CuZn-SOD), Mn-superoxide dismutase (Mn-SOD) catalase and glutathione peroxidase (GPx).[10]

Melatonin has been experimentally implicated in skin functions such as hair growth cycling, fur pigmentation, and melanoma control, and melatonin receptors are expressed in several skin cells including normal and malignant keratinocytes, melanocytes, and fibroblasts.[19]

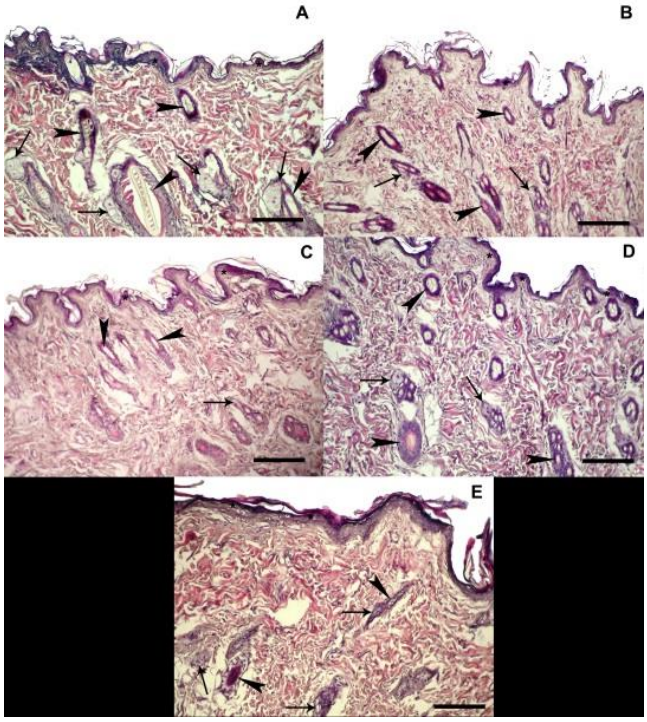

Figure-1. The histological sections of rats in control and experimental groups.
A:Control, B:Radiation, C:Melatonin, D:Radiation + Melatonin, E:Radiation + Amifostin (WR) Groups. Epidermis (X), Hair Follicles (arrowheads), Sebaceous glands (arrows).
(Staining: Hematoxylin -Eosin, Scale bar:50µm)

In a study carried out by Şener et al[20] rats treated with radiation were injected with melatonin and in histopathological examination, loss of bladder epithelium and vacuolization were identified. In an experimental study carried out by Avata et al.[21], the 900 MHz radiation effect of cell phones on the skin was investigated. At the end of the study, researchers found that melatonin treatment had reducing effect on fibrosis and lipid peroxidation levels caused by radiation of cell phones.

In our study, similar to the study carried out by Şener et al[20], epithelium vacuolization was found in the radiation group, however, we concluded that in radiation group treated with melatonin (Radiation + Melatonin group) there was less vacuolization in the tissues.

The protective effect of amifostin on rat kidneys was histopathologically investigated [9] and researchers have been determined that there were atrophy in renal tubules and fibrosis in diffuse intertubules in radiation treated group.

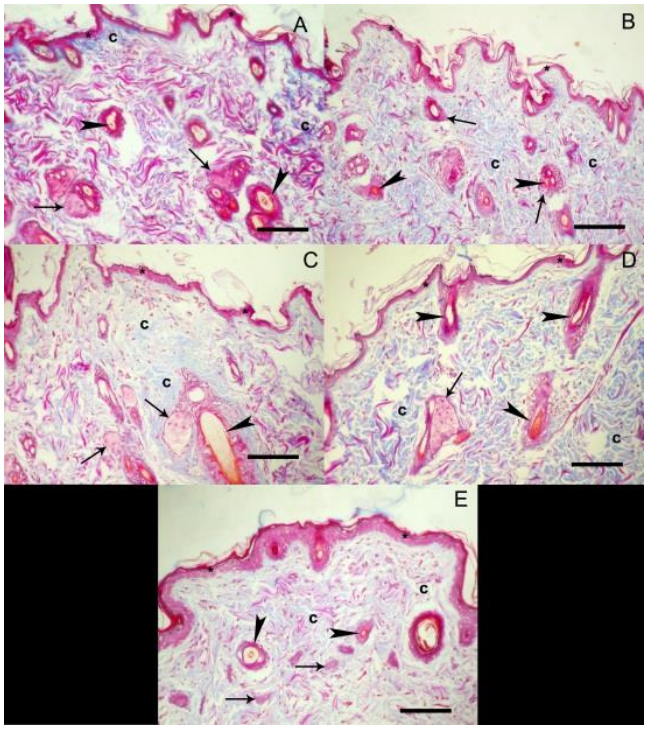

Figure-2. The histological sections of rats in control and experimental groups.
A:Control, B:Radiation, C:Melatonin, D:Radiation + Melatonin, E:Radiation + Amifostin (WR) Groups. Epidermis (X), Hair Follicles (arrowheads), Sebaceous glands (arrows), collagen fibers (c). (Staining: Crossman Mallory Azan, Scale bar:50µm)

However in the same study, in the group which was treated with amifostin and single dose 6 Gy radiation, the histopatohological symptoms were minimal with mild atrophy in tubules. In another study carried out by Topkan et al.[16] It was shown that melatonin and amifostin had radioprotective effect on the experimental animals exposed to 3 fractions 25 Gy radiation. But they determined that there was no protective effect on the group treated with both chemicals.[11]

Conclusion

In our study we determined that melatonin had therapeutic effect on histological alterations in rat skin caused by 20 Gy radiation. Consequently; it has been also determined that melatonin has more remarkable effect than amifostin (WR-2721) on the protection of histological disorders caused by radiation in the skin.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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