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Full Length Research Paper

Protective role of melatonin on blood parameters following irradiation in rat

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The aim of this study was to determine the preventive role of melatonin on several blood parameters after irradiation exposure in rats. A total of 100 adult Wistar albino rats were divided into five groups. One group was used as control and other groups were treated with 60, 90, 120 and 160 cGy/min of radiation, respectively. A dose of 4 mg/kg of melatonin was administered subcutaneously to 10 rats from each group exposed to different amounts of radiation. Saline was administered to 10 rats from the control group. 20 days after the completion of the 10-day experiment, the rats were sacrificed and blood samples were collected for determination of erythrocytes, leukocytes, hemoglobin, hematocrit and thrombocyte levels. In the group that received 60 cGy-radiation, the number of erythrocytes were similar to the control group, whereas the leukocyte and thrombocyte counts were increased. In the 90 cGy-radiation exposed rats, erythrocytes, hemoglobin and hematocrit values were low as compared to the controls. In the 120 to 160 cGy-radiation treated rats, leukocyte, hemoglobin, hematocrit and thrombocyte numbers were significantly different from the control groups (P<0.01). In the radiation-melatonin administrated rats, no difference was seen in the blood parameters as compared to the control group, except for the thrombocyte numbers. The thrombocyte numbers in all experimental groups were significantly higher than the controls (P<0.05). Melatonin was effective to reverse the deleterious effects of radiation on the blood parameters in rats and this effect was found to be dose-dependent.

Key words: Melatonin, blood parameters, radiation.

INTRODUCTION

In the treatment of cancer, radiotherapy has an important role alongside or in replacement of other treatment methods. However, the application of radiotherapy is restricted due to the side effects of radiation on normal tissues. Cystine and cysteine application before radiotherapy has been determined to protect rats from the lethal effects of x-ray irradiation (Becq et al., 1951; Sweeney et al., 2006). Amifostine has been clinically tested. However, optimum protective dose has been found to cause other toxicities (Yuhas, 1980). It appears that reliable, effective and useful agents need to be developed for human protection from radiation which may be a result of radiotherapy, occupational or accidental exposure.

Melatonin (5-methoxy-N-asetiltriptamin) has been found to play an important role in the regulation of several physiologic and neuroendocrine functions in mammals (Larios-Arceo et al., 2008). It is used for prevention of sleep disorders and fatigue such as jetlag. Melatonin, which is a strong antioxidant (Reiter et al., 1999), inhibits the secretion of dopamine from rational omicron cells (Dubocovich, 2007). Experimental studies have shown that melatonin inhibits tumor growth (Blask et al., 1988). Studies performed *in- vivo* and *vitro* have shown that exogenous melatonin (Mel) exerts oncostatic effects on melanoma cells (Izykowska et al., 2009). Furthermore, it has been reported that patients with colon and rectum cancers have reduced levels of circulating melatonin. In

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another study, it has been shown that melatonin and octreotide potently protected against radiation-induced intestinal injury in rats, but melatonin was significantly more effective in preserving the histological structure of the intestines (Onal et al., 2010). Experimental studies have clarified that melatonin played protective and/or ameliorative role against deleterious effects of gamma radiation (Assayed and Abd El-Aty, 2009).

It has been shown that agents protect against the damages of radiation by preventing formation of free radicals (Zhao et al., 2007). Melatonin is one of the most effective compounds that can remove free radicals (Reiter, 1991, 1999). In the skin, melatonin acts as an antioxidant which scavenges and inactivates free radicals arising due to UV irradiation (Izykowska et al., 2009). The aim of this study was to investigate effects of melatonin on various hematological parameters in female rats exposed to radiation at different doses.

MATERIALS AND METHODS

A total of 100 Wistar albino female rats weighing 200 to 250 g were used in the study. 20 rats comprised the control group and the rest were divided equally into four groups, each exposed to various doses of radiation in Radiation Oncology Department, Medical School, Erciyes University. 760 Cobalt 60 instrument was used to apply 60, 90, 120 and 160 cGy/min γ rays. Each experimental group was further divided into two groups: a) group treated with radiation (R), b) groups treated with radiation plus melatonin (RM). In the RM group, 4 mg/kg melatonin was given subcutaneously to 10 rats daily for 10 days. Due to its radioprotective effect, the ideal lowest dose of melatonin was chosen as 4 mg/kg (Vijayalaxmi et al., 1995). 10 rats from the control group (C) were not given anything for radiation and the other 10 rats were given physiological saline (CSP). During the experimental period, all groups were kept under the same conditions but were housed in different cages. Due to the fact that one rat from CSP group was injured by the others and two rats from the 60 cGy radiation group as well as one rat from each of the 90 and 160 cGy radiation groups were not able to complete the experimental period, the experimental numbers were reorganized to 18 (9 + 9) in control group, 16 (8 + 8) in 60 cGy/min, 18 (9 + 9) in 90 cGy group, 20 (10 + 10) in 120 cGy group and 18 (9 + 9) in 160 cGy group. Following the intra group comparison of the irradiated rats with and without melatonin, the control group was compared with the group with radiation and then with the group with radiation plus melatonin.

20 days following the completion of the 10-day experiment, all rats were sacrificed and blood (2 ml) was obtained from each mouse and placed into test tubes containing ethylene diamine tetra-acetic acid (EDTA). All samples were mixed very well and all blood parameters such as erythrocytes, leukocytes, hemoglobin, hematocrit and thrombocyte were measured by Max M Blood Cell Counter system. The statistical analysis was done using SPSS program and Mann Whitney U test was used for statistical comparison. Significant P value was taken as $P<0.05$ and $P<0.01$.

RESULTS

In groups not exposed to radiation, no significant difference was found in the blood parameters between those that received serum physiologic solution and those that did not receive the solution (Table 1).

In the group that received 60 cGy radiation, the rats that were given 4 mg/kg of melatonin for 10 days showed similar blood parameters with those that did not receive radiation, while the group that did not receive melatonin had statistically different thrombocyte and leukocyte counts ($P<0.05$) (Table 2).

In the group that received 90 cGy radiation, the animals that were given 4 mg/kg of melatonin for 10 days showed statistically different erythrocyte, hemoglobin, hematocrit and leukocyte counts as compared to the control rats ($P<0.05$) (Table 3). When the results of the blood parameters obtained from the rats treated with 120-cGy radiation and the rats treated with 120 cGy radiation plus 4 mg/kg of melatonin for 10 days were compared, the values of erythrocytes, hemoglobin and hematocrit were found to be statistically different in the group that received radiation ($P<0.01$) (Table 4).

Comparison of results obtained from both the rats treated with 160 cGy radiation and the rats treated with 160 cGy radiation plus melatonin show that in those that received only radiation, only the leukocyte numbers were high but those that received radiation plus melatonin had higher erythrocytes, leukocytes, hemoglobin and hematocrit values ($P<0.01$) (Table 5).

In terms of erythrocyte, RM 60 and 90 cGy, RM, 120 and 160 cGy RM groups, as compared to control, all of them had no significant difference (P values, respectively, 0.935, 0.06, 0077 and 0.705).

In terms of leukocyte, RM 60 and 90 cGy, RM, 120 and 160 cGy RM groups, as compared to control groups, each of them had a higher mean level than the control group (P values of 0.0246, 0.007, 0.00003 and 0.0004).

In terms of hemoglobin, RM to RM, and 90 and 60 cGy groups in the control group differ significantly (P values of 0.3285 and 0066, respectively), whereas in 120 and 160 cGy RM RM groups, the mean is significantly lower in the control group (P values of 0.0006 and 0.0007, respectively).

In terms of hematocrit, 60 and 90 cGy RM groups were not significantly different from the control group (P values of 0.5910 and 0.068), whereas in 120 and 160 cGy RM RM groups, the average is significantly lower in the control group (P values of 0.0008 and 0.0077, respectively).

In terms of thrombocyte, 90 cGy R and 160 cGy R groups were not different from the control group (P values of 0.061 and 0.2481, respectively), whereas in R and 120 cGy, 60 cGy, the average of the R groups were significantly higher than the control group (P values of 0.0006 and 0.0412, respectively) (Table 6).

In the radiation and melatonin group, the blood parameters were similar to the control group, except for the thrombocyte numbers ($P<0.01$, $P<0.05$) (Table 7).

DISCUSSION

This study shows that melatonin inhibits the effects of

Table 1. Comparison of the hematological parameters of the control groups.

Parameter	C- Group (n = 9)	CSP- Group (n = 9)	P value
Erythrocytes	6.69 ± 0.83	6.75 ± 0.78	NS
Leukocytes	9.96 ± 1.62	9.71 ± 1.84	NS
Hemoglobin	14.01 ± 1.84	13.81 ± 1.65	NS
Hematocrit	38.36 ± 4.75	39.11 ± 3.94	NS
Thrombocyte	578.3 ± 101.45	551.6 ± 98.23	NS

C, Control; CSP, control + serum physiologic solution; NS, not significant.

Table 2. The comparison of the hematological parameters of the rats treated with 60 cGy radiation.

Parameter	R Group (n = 9)	RM Group (n = 9)	P value
Erythrocytes	6.84 ± 1.44	7.62 ± 0.64	0.05
Leukocytes	14.59 ± 1.62	9.93 ± 0.064	0.0123*
Hemoglobin	11.99 ± 2.38	13.5 ± 0.93	0.1851
Hematocrit	38.23 ± 3.1	39.7 ± 6.65	0.111
Thrombocyte	682.67 ± 145.43	878.43 ± 83.24	0.005*

R group, Radiation group; RM group, radiation + melatonin group.

Table 3. Comparison of the hematological parameters of the rats treated with 90 cGy radiation.

Parameter	R Group (n = 9)	RM Group (n = 9)	P value
Erythrocytes	5.03 ± 0.66	7.51 ± 0.66	0.0177*
Leukocytes	15.51 ± 3.96	9.73 ± 1.23	0.0123*
Hemoglobin	11.91 ± 1.01	14.09 ± 0.91	0.02 *
Hematocrit	35.39 ± 2.79	40.59 ± 2.64	0.0035*
Thrombocyte	702.22 ± 123.23	821.88 ±106.96	0.06

R group, Radiation group; RM group, radiation + melatonin group.

Table 4. Comparison of the hematological parameters of the rats treated with 120 cGy radiation.

Parameter	R Group (n = 9)	RM Group (n = 9)	P value
Erythrocytes	4.44 ± 1.05	7.3 ± 0.94	0.007*
Leukocytes	16.64 ± 3.56	9.56 ± 2.81	0.4529
Hemoglobin	9.32 ± 146	13.81 ± 1.58	0.0003*
Hematocrit	27.47 ± 3.84	38.79 ± 4.17	0.0003*
Thrombocyte	726.44 ± 215.84	791.33 ± 119.29	0.5078

R group, Radiation group; RM group, radiation + melatonin group.

whole body radiation on the blood parameters in rats and that the effect is radiation dose dependent.

Radiation directly affects the cell nucleus causing atomic ionization and indirectly reacts with water molecules to form free radicals that lead to chromosome breakage and genetic damage (Bomford et al., 1993). This study has shown that rats exposed to various dosages of radiation (60 to 160 cGy) experience different effects in blood parameters (erythrocytes, hemoglobin, hematocrit, leukocyte and thrombocyte) depending on radiation dosages. Increasing the radiation dosage results in an increased leukocyte and thrombocyte number but a decrease in other blood parameters. Exogenous melatonin with its anti-apoptotic and antioxidant properties additively increased the immunity of the squirrels, by protecting their hematopoietic system and lymphoid organs against X-ray radiation induced cellular toxicity (Sharma et al., 2008). Increasing the leukocyte number due to the increasing radiation dosage is explained by activation of the immune system.

It has been reported that there is a relationship between cancer and melatonin and that melatonin

Table 5. Comparison of the hematological parameters of the rats treated with 160-cGy radiation.

Parameter	R Group (n = 9)	RM Group (n = 9)	P value
Erythrocytes	4.6 ± 1.08	6.88 ± 1.17	0.0389*
Leukocytes	17.63 ± 2.02	9.47 ± 2.15	0.0004*
Hemoglobin	8.71 ± 1.39	14.05 ± 2.63	0.0007*
Hematocrit	27.92 ± 6.78	40.0 ± 6.29	0.0045*
Thrombocyte	647.62 ± 223.72	758.3 ± 128.3	0.2665

R group, Radiation group; RM group, radiation + melatonin group.

Table 6. The comparison of the hematological parameters of the control group and those receiving differing doses of radiation

Parameter	Erythrocyte	Leukocyte	Hemoglobin	Hematocrit	Thrombocyte
Control	6. 69 ± 0.83$^{¥}$	9. 96 ± 1.62$^{\phi}$	14.01 ± 1.84$^{\xi}$	38.36 ± 4.75$^{\omega}$	578.3 ± 101.45$^{\gamma}$
60 cGy R	6. 84 ± 1.44$^{¥}$	14. 59 ± 1.62$^{\gamma}$	11.99 ± 2.38$^{\xi}$	38.23 ± 3.1$^{\omega}$	682.67 ± 145.43$^{\alpha}$
90 cGy R	5. 03 ± 0.66$^{¥}$	15.51 ± 3.96$^{\gamma}$	11.91 ± 1.01$^{\xi}$	35.39 ± 2.79$^{\omega}$	702.22 ± 123.23$^{\gamma}$
120 cGy R	4. 44 ± 1.05$^{¥}$	16.64 ± 3.56$^{\gamma}$	9.32 ± 1.46$^{\varphi}$	27.47 ± 3.84$^{\Psi}$	726.44 ± 215.84$^{\alpha}$
160 cGy R	4. 6 ± 1.08$^{¥}$	17.63 ± 2.02$^{\gamma}$	8.71 ± 1.39$^{\varphi}$	27.92 ± 6.78$^{\Psi}$	647.62 ± 223.72$^{\gamma}$

In each column means bearing different symbols are significantly different from the control group. R group, Radiation group.

Table 7. The comparison of the hematological parameters of the control group and those receiving differing doses of radiation plus melatonin.

Parameter	Erythrocyte	Leukocyte	Hemoglobin	Hematocrit	Thrombocyte
Control	6.69 ± 0.83$^{¥}$	9.96 ± 1.62$^{\gamma}$	14.01 ± 1.84$^{\phi}$	38.36 ± 4.75$^{\xi}$	578.30 ± 101.45$^{\gamma}$
60 cGy RM	7.62 ± 0.64$^{¥}$	9.93 ± 2.20$^{\gamma}$	13.50 ± 0.93$^{\varphi}$	39.70 ± 6.65$^{\xi}$	878.43 ± 83.24*
90 cGy RM	7.51 ± 0.66*	9.73 ± 1.23$^{\gamma}$	14.09 ± 0.91$^{\phi}$	40.59 ± 2.64$^{\xi}$	821.88 ± 106.96$^{\alpha}$
120 cGy RM	7.30 ± 0.94$^{¥}$	9.56 ± 2.81$^{\gamma}$	13.81 ± 1.58$^{\phi}$	38.79 ± 4.17$^{\xi}$	791.33 ± 119.29$^{\Psi}$
160 cGy RM	6.88 ± 1.17$^{¥}$	9.47 ± 2.15$^{\gamma}$	14.05 ± 2.63$^{\phi}$	40.00 ± 6.29$^{\xi}$	758.30 ± 128.30$^{\omega}$

In each column means bearing different symbols are significantly different from the control group.
R group, Radiation group; RM group, radiation + melatonin group..

suppresses the immune response leading to successful treatment of cancer patients either alone or in combination with chemotherapy, radiotherapy or immunotherapy (Solar, 1999). A superior radioprotective function of melatonin over amifostine in preventing radiation-induced epiphyseal growth plate injury was found, without any increase in radioprotective effect by adding amifostine to melatonin (Topkan et al., 2008). Our results show a similarity with Solar's (1999) studies even though there was a short time between giving radiation and sacrificing the animals. Solar's (1999) studies also showed that melatonin has direct or indirect inhibitory affect on some types of neoplasia and that it's antioxidant effects play an important role on its oncostatic properties (Solar, 1999). In our study, melatonin-treated group showed similar results with the control group. This led us to believe that melatonin has a protective effect against the radiation damage on blood parameters in rats. The efficacy of melatonin has been assessed as a treatment for ocular diseases, blood diseases, gastrointestinal tract diseases, cardiovascular diseases, diabetes, rheumatoid arthritis, fibromyalgia, chronic fatigue syndrome, infectious diseases, neurological diseases, sleep disturbances, aging and depression (Sánchez-Barceló et al., 2010). Melatonin has also been used as a complementary treatment in anaesthesia, hemodialysis, *in vitro* fertilization and neonatal care (Sánchez-Barceló et al., 2010). Using melatonin for treatment supports our study.

It has been reported that melatonin has protective effects in gamma radiation-induced DNA damage in human lymphocytes (Reiter, 1996). It also prevented mortality of rats exposed to high dose radiation (Vijayalaxmi et al., 1999).

The results of this study show that unwanted effects of radiation on blood parameters whether due to treatment or exposure may be eliminated by melatonin. Melatonin may also have effects on other parameters such as blood factors. Vijayalaxmi et al. (1999) has shown that

melatonin has a protective affect on damages caused by all types of radiation. It has also been reported that melatonin prevents radiation caused by lipid peroxidation in female rats (Kaya et al., 1999).

Some of the findings that were not included in the statistical analysis are also consistent with the results. In the rats treated with radiation, hair loss and skin lesions were seen, whereas those treated with radiation plus melatonin, very few showed these side effects. Five rats were found to be pregnant at the completion of the experiment. Among these, three received radiation only and two received radiation plus melatonin. Among the pregnant rats, only one that received radiation only gave birth to a live baby with head and facial anomalies. All others had healthy babies. These results are for observation only and are not considered in the statistical analysis.

Various mechanisms may be involved in prevention of radiation damage to blood cells by melatonin. These mechanisms include antioxidant activity, free radical and oxidative stress preventive activity of melatonin (Reiter, 1996; Reiter et al., 1999).

In conclusion, melatonin may prevent harmful effects of radiation on the blood parameters in rats, and the effect appears to be dose-dependent.

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