

## Comparison of Protective Effect of Melatonin and Amifostine on Acute Renal Damage Caused by Ionizing Radiation

*İyonizer Radyasyonun Neden Olduğu Akut Renal Hasara Karşı Melatonin ve Amifostinin Koruyucu Etkilerinin Karşılaştırılması*

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### Abstract

The aim of this study is to compare histopathologically the protective effect of melatonin and amifostine on radiation induced renal damage. 50 female albino rats were divided into five groups: control, radiotherapy alone, radiotherapy+amifostine, radiotherapy+melatonin, radiotherapy+amifostine+melatonin. Intraperitoneal amifostine (200 mg/kg) and intraperitoneal melatonin (10 mg/kg) was administered to 30 minutes before irradiation. Rats were irradiated with a single dose of 8 Gy on whole body. At the end of the follow-up period, percentage of damaged glomeruli was determined by counting damaged glomeruli of kidney cortex as segmental or total necrosis for each animal. The protective effect of each agent has been shown, and there is an advantage in favor of melatonin ( $p = 0.005$ ). Although there is not an advantage of adding amifostine to melatonin ( $p = 0.243$ ), there is statistically significant better protective effect in amifostine+melatonin group when compared with amifostine alone ( $p = 0.003$ ). The protective effect of melatonin on radiation induced acute renal toxicity is histopathologically better than amifostine.

**Key words:** Amifostine, melatonin, acute renal damage, ionizing radiation.

### Özet

Bu çalışmanın amacı, erken dönemde iyonizer radyasyonun neden olduğu akut böbrek hasarı üzerine melatonin ve amifostinin koruyucu etkisinin histopatolojik olarak karşılaştırılmasıdır. 50 dişi albino rat kontrol, yalnız radyoterapi, radyoterapi+amifostin, radyoterapi+melatonin ve radyoterapi+amifostin+melatonin olmak üzere beş gruba ayrıldı. İntraperitoneal amifostin (200 mg/kg) ve intraperitoneal melatonin (10 mg/kg) radyoterapiden 30 dakika önce uygulandı. Tek tek ratların tüm vücutlarına 8 Gy eksternal radyoterapi uygulandı. Takip süresinin sonunda her bir hayvan için segmental veya total nekroz gibi böbrek korteksi glomerul hasarı sayılarak hasarlı glomerul yüzdesi tespit edildi. Amifostin, melatonin ve amifostin+melatonin'in koruyucu etkisi istatistiksel olarak gösterilmekle birlikte melatonin lehine bir avantaj tespit edilmiştir ( $p = 0.005$ ). Melatonin'e amifostin eklenmesinin tek başına melatonin'e göre üstünlüğü olmamasına karşın ( $p = 0.243$ ) amifostine melatonin eklenmesinin tek başına amifostine göre istatistiksel üstünlüğü gösterilmiştir ( $p = 0.003$ ). Sonuç olarak melatonin'in iyonizer radyasyonun neden olduğu akut böbrek hasarı üzerine koruyucu etkisi amifostin'den histopatolojik olarak daha iyidir.

**Anahtar kelimeler:** Amifostin, melatonin, akut renal hasar, iyonizer radyasyon.

### Introduction

The aim of radiation therapy is to deliver sufficient dose to tumor tissue to provide cure without of inducing damage in surrounding normal tissue. Kidneys can be exposed to radiation during radiation therapy of gastrointestinal tumors, and total-body irradiation in preparation for bone marrow transplantation. Radiation induced nephropathy

includes vascular permeability, perfusion disturbance, inflammatory reactions and fibrosis [1,3]. Segmental fibrinoid necrosis of the glomerular tufts, occlusive intracapillary accumulations of periodic acid positive material with enmeshed erythrocytes, mesangiolysis, endothelial swelling, and splitting of glomerular capillary basement

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membranes, fibrinoid changes of the terminal afferent arterioles are the abnormalities seen by light microscopy [4,6].

Amifostine, an amino thiol, is a pro-drug that is dephosphorylated by alkaline phosphatase to the active metabolite into the cell. It prevents radiation induced cellular injury through free-radical scavenging, hydrogen donation, and inhibition of DNA damage [3]. Several studies have demonstrated its protective effect on normal tissues against the toxic effects of radiation [7,9]. Melatonin (N-acetyl-5-methoxytryptamine), an endogenous compound synthesized by pineal gland, is a potent antioxidant as in scavenging hydroxyl radicals and increases some antioxidant enzymes such as superoxide dismutase and glutathione peroxidase [10,11].

In this study, we aimed to compare histopathologically the protective effect of melatonin and amifostine on radiation induced renal damage in early phase. And also no functional endpoints were tested.

## Methods

### *Animals and experimental design*

All experiments were conducted adhering to the guidelines of the institutional animal ethics committee (TUHDYK-2012/77). 50 female albino rats, 3–4 months old, weighing  $200 \pm 25$  g, maintained under standard temperature and humidity conditions, were used in the study. The animals had free access to sterile water and food, and were housed in a polypropylene cage containing sterile paddy husk for bedding throughout the experiment.

The animals were divided into five groups (with ten rats each) and treated as follows:

- Group 1: control (Cont),
- Group 2: radiotherapy alone (RT),
- Group 3: radiotherapy + amifostine (RT+AMI),
- Group 4: radiotherapy + melatonin (RT+MEL),
- Group 5: radiotherapy + amifostine + melatonin (RT+AMI+MEL).

Animals in the RT group were treated with 0.9% saline solution (SS) 30 minutes before irradiation. Amifostine was administered to the rats in the RT+AMI and RT+AMI+MEL groups 30 minutes before irradiation. Animals in the RT+AMI group received amifostine (200 mg/kg, ER-KIM Ilac, Istanbul, Turkey) by intraperitoneal injection before irradiation [12]. Melatonin was administered to the rats in the RT+MEL and RT+AMI+MEL groups 30 minutes before irradiation. Animals in the RT+MEL and RT+AMI+MEL groups received melatonin (10 mg/kg, Sigma Chemical Co, St. Louis, USA) by intraperitoneal injection before irradiation [13]. All experimental procedures were performed on

anesthetized rats. Anesthesia was maintained via intramuscular ketamine (100 mg/kg, Pfizer Ilac, Istanbul, Turkey) and xylazine (3.9 mg/kg, Interhas A.S., Istanbul, Turkey) during irradiation. The follow-up period was 72 hours in all groups. At the end of this follow-up period after all rats were anesthetized scarification has been done using cervical dislocation method.

### *Irradiation*

Rats were anesthetized and fixed on their blocks across a blue Styrofoam (Med-Tec, Orange City, IA, USA) treatment couch in prone position. RT, RT+AMI, RT+MEL and RT+AMI+MEL groups were irradiated individually with a single dose of 8 Gy using a  $^{60}\text{Co}$  treatment unit (Cirrus, cis-Bio Int., Gif-sur-Yvette, France). Dose rate was 1.15 Gy/min.

### *Histopathologic analysis*

Tissue specimens were prefixed in formaldehyde for 24 h. All tissues were embedded in paraffin wax and processed through hematoxylin-eosin, in 4- $\mu\text{m}$  sections. Tissue samples were evaluated by a light microscope. All samples were analyzed and percentage of damaged glomeruli was determined by counting damaged glomeruli of kidney cortex as segmental or total necrosis for each animal.

### *Statistical Analysis*

Conventional methods were used to generate descriptive statistics. Data between groups were compared using Mann-Whitney U-test (post hoc with Bonferoni correction). p-values  $< 0.05$  were considered significant between compared two groups.

## Results

The percentage of damaged glomeruli is presented in Table I. We demonstrated pathological changes which concentrate in focal areas of the cortex of the kidney glomeruli in radiotherapy group. The main finding was a significant segmental glomerular necrosis (mesangiolysis). In addition to this, fibrinoid changes in the glomeruli and accumulation of fibrinous material in Bowman's space was detected (Figure 1). There was no significant pathological change in tubules. The protective effect of amifostine, melatonin, and amifostine plus melatonin on radiation induced renal toxicity was statistically meaningful ( $p < 0.001$ ,  $= 0.003$ ,  $< 0.001$ , respectively). There was an advantage in favor of melatonin when compared with amifostine ( $p = 0.005$ ). Although there was not an advantage of adding amifostine to melatonin when compared with melatonin alone ( $p = 0.243$ ), there was statistically significant better protective effect in amifostine plus melatonin group when compared with amifostine alone group ( $p = 0.003$ ). As there was no significant damage following 8 Gy whole body irradiation on the

kidney tubules, the protective effect of any agent could not be assessed.

**Table 1:** The percentage of damaged glomeruli

	Groups				
	Cont	RT	RT+AMI	RT+MEL	RT+AMI+MEL
	0	40	30	20	20
	0	40	40	20	30
	0	50	40	30	30
	0	40	30	25	30
Glomeruli damage (%)	0	50	20	20	20
	0	40	30	20	15
	0	40	30	25	20
	0	50	40	30	15
	0	40	40	20	15
	0	40	30	30	20

## Discussion

The capillary endothelium and the tubular epithelium in the kidneys are conditional cell renewal systems, but they have a limited proliferation potential when they are stimulated by injury. The histopathological findings depend on the time interval between the radiation and the tissue examination. Tissue damage can be seen in both the glomeruli and the tubules. Glomerular capillary endothelial damage is seen within a few days after radiation. Chronic mesangiocapillary or membranoproliferative glomerular injury is the most consistently observed changes in kidney after radiation. Segmental fibrinoid necrosis of the glomerular tufts, occlusive intracapillary accumulations of periodic acid-Schiff-positive material with enmeshed erythrocytes, mesangiolysis, endothelial swelling, and splitting of glomerular capillary basement membranes, fibrinoid changes of the terminal afferent arterioles are the abnormalities seen by light microscopy [4-6].

Tubular changes are late findings, observed after months [14,15]. Tubular loss and atrophy may occur in the absence of glomerular solidification in animals subjected to irradiation. Nonspecific accumulations of mononuclear leukocytes can be seen. The interstitium may show no changes or only a slight apparent increase may occur in interstitial tissue. Tubular cell vacuolization and desquamation are other nonspecific change. Some studies have suggested an important and perhaps primary toxic effect on the tubules by radiation as the basis for

subsequent radiation nephropathy [16,20]. Evidence indicates an acute tubular injury corresponding to acute tubular necrosis that may occur after high-dose irradiation [6]. We showed radiation damage on glomeruli using histopathological examination after 72 hours after irradiation. Our main finding was mesangiolysis but we could not find any damage on kidney tubules.

Amifostine and melatonin have similar action mechanisms [11,21]. There are lots of studies in literature which show the protective effects of amifostine and melatonin on different tissues. Although experimental examinations were done in first three days after radiation in molecular studies, they were done after first week in histopathological studies. We think that our study is very important, because we showed the protective effect of each amifostine and melatonin against radiation on kidney tissue using histopathological examination after a very short time from irradiation such as three days [8,13,22,24].

There is no study in literature which compares the protective effect of amifostine and melatonin on radiation induced renal toxicity. Also, this is another importance of this study. Topkan et al. has observed a superior radioprotective function of melatonin over amifostine in preventing radiation-induced epiphyseal growth plate injury, without any increase in radioprotective effect by adding amifostine to melatonin [25]. Similar to this study, we found that there is an advantage in favor of melatonin when compared with amifostine. In addition to this result, there is not an advantage of adding amifostine to melatonin when compared with melatonin alone and there is statistically significant better protective effect in amifostine plus melatonin group when compared with amifostine alone group.

## Conclusion

This study is important because it shows that melatonin is more effective when compared with amifostine which is commonly being used as a radioprotective agent. In the future, the clinical uses of melatonin like amifostine, further studies are necessary which investigate primarily the functional protective effect of melatonin against radiation damage.

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