RESEARCH ARTICLE

Protective Effect of Melatonine Against Radiation Induced Nephrotoxicity in Rats

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Abstract

Purpose: The degree of radiation injury to kidneys which are located within the limits of radiotherapy area is determined by the volume and the dose of radiation to which the organ is exposed. When the tolerance dose of the kidney is exceeded after a latent period of 6 months acute nephritis develops and after 18 months chronic nephritis ensues. Melatonin is known to prevent the oxidative injury of toxins and radiotherapy with its free radical scavenging capacity. Methods and materials: In this study 8 weeks old 24 Sprague –Dawley rats were allocated into 4 groups: Control group; Radiotherapy group (20 Gy bilaterally in 5 fractions); Melatonin group (10 mg/kg intraperitoneally), and Melatonin+radiotherapy group (20 Gy Radiotherapy in 5 fractions+ melatonin 10 mg/kg intraperitoneally). After a follow-up period of 6 months BUN was determined in all groups. After rats were euthanized the kidneys were removed for histopathological examination under both light and electron microscopes. Results: After 6 months follow-up, both at light and electron microscopy levels, the rats in radiotherapy+melatonin group were significantly protected against the radiation injury comparing to radiotherapy group ($p<0.05$). Conclusion: It was shown in this experimental model that melatonin has protective effects against radiation injury to kidneys.

Keywords: Radiotherapy - nephrotoxicity - melatonine - rat model

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Introduction

Kidneys are frequently included into the therapy area during radiotherapy applications. The extent of radiation damage depends on both dose and volume of radiation (Perez et al., 2008). Renal tolerance dose is 20 Gy in bilateral irradiation, while in unilateral exposure renal dysfunction starts in 15 Gy and renal function is completely lost in doses of 25-30 Gy. Concomitant use of cisplatin some chemicals such as BCNU and actinomycin further lowers the tolerance dose. When renal tolerance dose is exceeded after a latent period of 6 mounts acute nephritis develops, after 18 mounts on chronic nephritis and hiperreninemic hypertension is observed. In histopathologic examination interstial edema secondary to increased capillary permeability is evident in acute phase, in chronic phase glomeruler sclerosis, interstisial fibrosis and glomeruler mesensiolysis are the common findings (Zaki et al., 2003).

Rapid multiplication of the tumoral cell leaves hypoxia and necrotic areas at the center of the tumors. Since radiation is ineffective at these regions, higher radiation doses are usually required at the tumor center to control tumoral multiplication. The normal tissue surrounding the tumor is rich in blood vessels and well oxygenated, so that it is more prone to radiation injury and needs to be protected. Radiation oncologists and radiation biologists use many chemical and biological protectors to minimize radiation injury to surrounding normal tissue and radioprotectors are among the topics of research interest (Nair et al., 2001).

Melatonin is a hormone which is secreted from the pineal gland. Its synthesis in pineal gland follows a circadian rhythm (Snyder and Axelrod, 1967; Claustrat et al., 1995). After its synthesis in pineal gland it rapidly passes the blood stream and detected in bile, CSF, saliva, ovarian follicle fluid, semen and amnion fluid (Hedlund et al., 1976; Pardridge and Meitus, 1980; Vakkuri, 1985; Reiter 1986; Brzenzinski et al., 1987; Nowak et al., 1987; Bornman et al., 1989; Shaw et al., 1989; Kennaway and Voultsios, 1998; Rousseau et al., 1999; Tan et al., 1999). Minimal amount of non metabolized form is excreted in the urine. Melatonin reduces oxidative damage in cases free radicals and their products are generated (Reiter et al., 2001).

In studies carried out to determine it, potential toxicity both in physiologic and pharmacologic concentration its acute and chronic toxicity found to be minimal (Wang et al., 2012). The protective capacity of melatonin in renal toxicity is studied mainly with chemotherapeutic

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agents. Hara et al. in their studies concerning the effects of melatonin in cisplatin induced renal toxicity reported that melatonin preserved GSH/GSSH ratio, prevented lipid peroxidation and normalize glutathione peroksidase levels (Hara et al., 2001). Melatonin is reported to prevent oxidative injury induced by radiotherapy and toxin via its free radical scavencing capacity (Blickenstaff et al., 1994). Vijayalaxmi et al reported that peripheral lymphocytes exposed to 1, 5 Gy radiation dose induced high micronucleus ratio and addition of melatonin 20 minutes prior to incubation decreased micronucleus formation (Vijayalaxmi et al., 1995).

Melatonin may have a positive effect on preventing radiotherapy induced progressive nephrosclerosis and renal failure.

Materials and Methods

In this study, 8 weeks old female Sprague Dawley rats were used. They were purchased from Başkent University research center and the rats were certified that they didn't have any infections disease and had not received any antibiotic or nephrotoxic substance. The study was carried out in KTU surgical research center and the rats were irradiated with linear accelerator in KTU department of radiation oncology.

24 rats were randomly allocated into 4 groups 6 rats in each. The groups were as follows control; Intraperitoneal 0.9% NaCl solution was given in 5 consecutive days. Radiotherapy group; 20 Gy radiotherapy was given in 5 fractions in 5 consecutive days. Melatonin only group; melatonin was given in dose of 10 mg/kg intraperitoneally in 5 consecutive days. Melatonin+radiotherapy group; melatonin in dose of 10 mg/kg intraperitoneally was given 30 min prior to radiotherapy for 5 days.

Prior to radiotherapy both kidneys were visualized after IV injection of iohexol (Omnipaque, Opakim-İstanbul) and radiation plan was carried out with safety margin of 5 mm in Simulix-X Oldeft Simulator.

Radiotherapy was given to bilateral kidney location in a total dose of 20 Gy, in 400 Gy per fraction 6 MV photons, using linear accelerator. The rats were restrained on a straphore after ether inhalation anesthesia.

Blood BUN analysis were carried out in Roche Modular biochemical analysis systems (D-P) its original commercial kits. After 6 mount of follow up, under ketamine anesthesia a median abdominal incision was made and blood sample from abdominal aorta drawn for biochemical analysis of blood BUN both kidneys were removed for pathological evaluation.

Light microscopy; 24 kidneys specimens from 4 different group were fixed in 10% formaldehyde for 24 hours. Paraphin blocks were prepared and tissue samples of 4-5 micron thickness were prepared by cutting the paraphin blocks. They were stained hemotoxylene-eosin and masson-tricrom staining and evaluated by single pathologist under light microscope (Nikon 200). Intertisial expansion (expansion due to interstitial edema, increment in vascularisation), tubuler atrophy (shrinking of proximal and distal tubuler ephitelial cells and resulting tubular diameter narrowing), basal labyrant expansion (basal cell membrane expansions) parameters were scored.

Electron microscopy; Kidney specimens of 1-2 mm^3 in size were fixed in 3% gluteraldehyte solution for 24 hours. They were passed through alcohol series 1% osmium tetraoxide for 2 hours and embedded in Epon 812. Thick slices were cut by an Ultratom and stained toluidin blue. From toluidin stained specimens 60-70 mm thick fine slices were cut using the same microtome and stained uranyl acetate and lead citrate. The specimens were evaluated under electron microscope (JEOL 1010). Vacuolisation (increase of vacuole number) microvillus degeneration (tearing of the microvillus membrane,apicall loss of microvillus, actin filament breakage) cytoplasmic electron dense deposits,basal lamina ondulations and basal laminar thickenings were scored. Scoring; not increased (0 point), mildly increased (1 point), moderately increased (2 points) and significantly increased (3 points) şeklinde yapıldı.

Comparisons among groups were done with Kruskal Wallis analysis of variance (as post hoc with Bonferroni correction Mann Whitney U test) for biochemical parameters (P=0.001). For electron and light microscopy results chi-square test was used. Results were given as mean±standard deviation. P<0.05 was accepted as significant.

Results

The rats tolerated the experimental protocol well. During the 6 mount follow up period no rat was died.

BUN values in melatonin+radiotherapy group was significantly lower comparing to radiotherapy group (p=0.001). In control group BUN was significantly different comparing to radiotherapy group (p=0.001). BUN levels in melatonine+radiotherapy group was significantly higher comparing to control group (p=0.001). BUN levels were higher in melatonine only group comparing to control, although it was not statistically significant. Elevation of BUN in these two groups did not reach the elevation in radiotherapy group (Figure 1).

Light microscopy: In all groups tubular atrophy, basal labyrinth expansion and interstitial expansion were evaluated. These findings were severe in radiotherapy group comparing to melatonin+radiotherapy group. These differences were statistically significant (p<0.05). Light microscopy scores were similar in melatonine+radiotherapy and melatonine only groups comparing to control group.

Electron microscopy; In electron microscopy examination microvillus degeneration, decreases in microvillus, increase in vacuolization, cytoplasmic electron

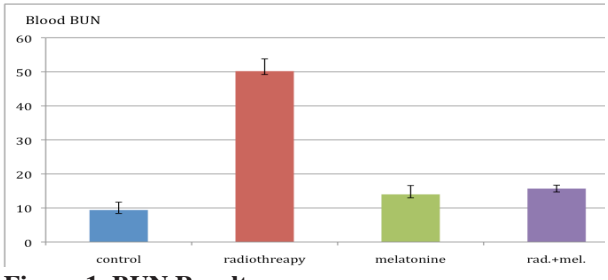

Figure 1. BUN Results.

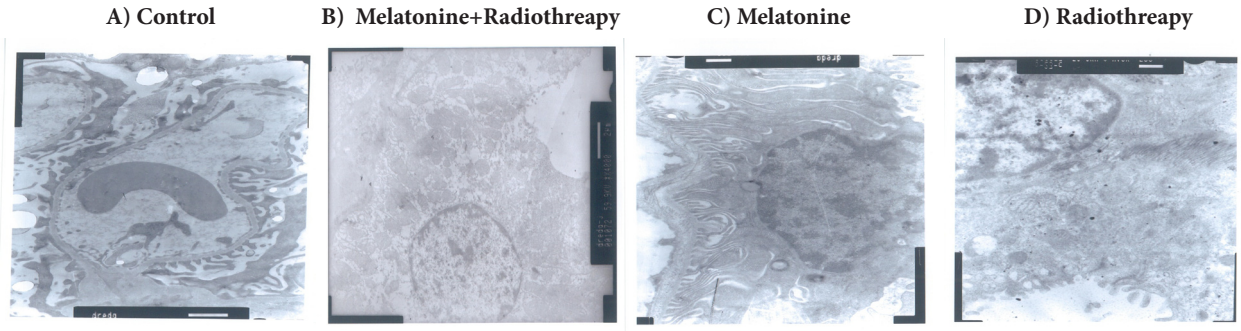

Figure 2. Electron Microscopic Appearances. A) Control, B) Radiothreapy, C) Melatonin and D) Melatonin+Radiothreapy.

dense deposits, basal lamina ondulations, basal lamina thickening were evaluated. In melatonin+radiotherapy group these parameters were better comparing to radiotherapy group ($p<0.05$). Electron microscopy scores were similar in melatonine+radiotherapy and melatonine only groups comparing to control group (Figure 2).

Discussion

It has been shown in many clinical and experimental studies that kidneys are highly sensitive to radiation injuries. In many clinical and experimental studies, kidneys are shown to be highly sensitive to radiation injuries. Radiation nephropathy develops mount even years after radiotherapy. Development of radiation induced nephropathy takes mounts, even years after radiothrepy. Since clinical findings and time elapsed for the development of radiation nephropathy differs it is more important for children and patients with longer life expectancy. Radiation nephropathy is even more important in children and patients with longer life expectancy since clinical findings and the time elapsed changes in a wide spectrum. The radiation injury develops earlier and with increased in severity with higher radiation doses Severity of the radiation injury is dose dependent and it develops earlier with higher doses (Luxton and Kunkler, 1964). Radiation nephropathy was well documented in a large case series published by Kunkler et al. over 50 years ago. These were men who had undergone therapeutic irradiation for seminomas Kunkler et al. published findings of radiation nephropathy in a large case series of seminoma patients over 50 years ago (Kunkler et al., 1952). Radiation nephropathy occurred in about 20% of sufficiently irradiated subjects, and could take various clinical forms; acut radiation nephritis, chronic radiation nephritis, malignant hypertension, benign hypertension Radiation nephropathy presented itself in 20% of patients after sufficient irradiation, the clinical presentation were in various forms; acut radiation nephritis, chronic radiation nephritis, malignant hypertension, benign hypertension (Perez et al, 2008).

The pathogenesis of the radiation nephropathy remains controversial. Controversies exists over the pathogenesis of the radiation nephropathy. The direct functional relationships between tubuli, glomeruli and blood vessels will not enable conclusions on damage developing in separate compartments. Jongejan and coworkers results showed a comparable and simultaneous decline GFR and urine osmolality and hence do not permit conclusions about differences in radiation sensitivity between tubules and glomeruli Jongejan et al. reported a simultaneous decline in GFR and urine osmolality after radiation but did not describe any difference in radiation sensitivity between tubules and glomeruli (Jongejan et al., 1987). Glatstein et al. suggested the glomeruli as the site of initial pathologic changes Glatstein and coworkers determined the glomeruli as the site of initial pathological changes (Glatstein et al., 1977). In literature many studies reported radiation induced glomerular changes appear diffuse and they precede tubular alterations (Madrazo and Churg, 1976; Robbins et al., 1991; Stephens et al., 1995). However, chronic renal failure is observed primarily in those animals in which glomerular injury is combined with severe tubular injury and tubulointerstitial fibrosis. However, chronic renal failure develops in animals when glomerular injury coincides with tubuler injury and tubulointerstisial fibrosis (Robbins et al., 1994). In clinical chronic renal disease the degree of renal dysfunction (Bohle et al., 1990) no such correlation is seen with glomerular changes (Ong and Fine, 1994). In our study,in radiotherapy given group the narrowing of proximal and distal tubules were observed with glomerular damage. Our study showed complex damage of glomerular, tubuler and interstial cells in acute nephropathy period and supported the findings of Cohen and coworkers (Cohen and Robbins, 2003).

Production of reactive oxygen species (ROS) is one of the early effects of ionizing radiation which induces the cellular antioxidant defense enzymes such as superoxide dismutase and glutathione peroxidase (Zhang et al., 2005). ROS and free radicals react with cellular macromolecules (i.e. nucleic acids,lipids and carbohydrates) and causes damage. Oxidative damage to living cells can be estimated with measurable major biomarkers of lipid peroxidation such as penthane, isoprostane and aldehytic products measurable in tissue and body fluids; DNA-hydroxylation products and microscopic indices of damage such as chromosomal aberrations and micronuclei; protein hydroxylation products such as oxidized amino acids can also be detected (Shirazi et al., 2007).

If radiation induced free oxygen radicals can be cleared in nano seconds radiation hazards can be minimized. Many radioprotectors reacting with free radicals can prevent radiation induced cellular death. Radioprotectors should have certain properties to be used in clinical settings. Any compound protecting normal tissue has the risk to protect the tumor tissue as well. Their protective

effect both in the normal and the tumor tissue should be known quantatively and the therapeutic gain should be calculated. Radioprotectors have potential riscs of protecting tumor tissue from the effects of radiation as protecting the normal tissue. The protective effects on both tumor and normal tissue of any radioprotector should be quantatively known and a therapeutic gain can be calculated. Ideally the dose-response effect of the compound should be evaluated both in the normal and the tumor tissue and ideal dose be determined (Andreassen et al., 2003). At both physiological and pharmacological concentrations, melatonin acts as a differentiating agent in some cancer cells and lowers their invasive and metastatic status through alterations in adhesion molecules and maintenance of gap junctional intercellular communication. In other cancer cell types, melatonin, either alone or in combination with other agents, induces apoptotic cell death (Blask et al., 2002; Casado et al., 2010; Rodringuez-Garcia et al., 2012). Melatonin reduces tumor growth in experimental models in vivo and proliferation and invasive properties of cancer cells in culture (Cos et al., 2002; Manda and Bhatia, 2003).

Melatonin (N-acetyl-5-metoxytriptamine) is a known agent preventing oxidative damage of the toxins and radiotherapy with its free radical scavenging capacity (Martinez-Cayuela, 1955; Edwards et al., 1984; Verma and Sonwalker, 1991). In in vitro experiments it was shown that melatonine was 5 and 14-fold more potent than glutathione and mannitol, respectively, in scavenging hydroxyl radicals. Also, melatonin decreases the activity of nitric oxide synthase, a pro-oxidative enzyme (Majsterek et al., 2005). Additionally melatonine increase to activity of some important antioxidant enzymes at molecular level including superoxide dismutase and glutathione peroxidase (Rodriguez et al., 2004). Vijayalaxmi et al. observed in an *in vitro* study in peripheral lymphocytes that micronutrients ratio increases when they are exposed to 15.6 Gy of radiotherapy. They observed that melatonine caused a drop of micronutrient count when added to the cell cultures in concentrations 0.5-2.0 mMol 20 minutes before the incubation (Vijayalaxmi et al., 1999). They also compared the effects of melatonin given in doses of 5mg/kg and 10 mg/kg doses given 1 hour before radiothreapy and observed that melatonin in 10 mg/kg dose had better results. In a randomized double blind clinical trial conducted by Sebra et al, melatonine when administered orally to healthy adult males in dose of 0.5-2.0 mMol for 28 days caused no toxicity (Vijayalaxmi et al., 2004). In this study melatonine 10 mg/kg intraperitoneally was used and provided sufficient protection. But in this experimenal study melatonine itself was found to be a cause of kidney function elevation when added to the radiotherapy protocol. In previous kidney studies with chemotheuropotic agents and melatonine this effects of melatonine was not mentioned (Hara et al., 2001). This condition may be the results of s low doses of melatonine (5 mg/kg) used in these studies. In our study it was shown that, despite BUN elevations caused by melatonine, it also had protective effects of kidney histopathology both in light and electron microscopic level.

Further pharmacological investigations are necessary on BUN elevation caused by melatonine administration (Russcher et al., 2012). Altough there was an elevation in BUN levels in melatonine+radiotherapy group, this elevation was thought not to be related with radiotherapy since there was also an elevation in melatonine only group. As it was shown in a study carried out by Sebra et al oral use of melatoninemay prevent this BUN elevation as it prevented other toxicities of melatonine. Our study is the first in the literature that melatonine had radioprotective effects on kidneys, in terms of histopathology when administered concomitant with radiothrepy. If in further studies, it can be shown to be nephroprotective when administered orally and minimal BUN elevations can be evaluated pharmacologically, melatonine may become a drug of choice with its anticancer and antioxidant properties

As a conclusion, melatonin, a known antioxidant agent has a radioprotective effect on irradiated kidneys. After a 6 mounts of follow-up period light and electron microscopic findings showed that kidneys in melatonin+radiotherapy

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