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Protective Effect of Low Dose Melatonin on Radiation-Induced Damage to Rat Liver

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Abstract

Background: Melatonin is a free radical scavenger. It has also antioxidant effects through its stimulatory actions on antioxidant system.

Objective: We conducted this study to examine the effect of different oral doses of melatonin on liver tissue.

Methods: We used 42 rats—6 rats in each group. Group I served as the control group; group II rats were pretreated with only melatonin; group III was exposed to radiation only; and groups IV to VII were pretreated with different doses of melatonin (10, 100, 200, 400 µg/kg, respectively) and exposed to 6 Gy whole body gamma irradiation.

Results: Whole body irradiation induced liver tissue damage by increasing malondialdehyde (MDA) concentrations and decreasing glutathione (GSH) levels. Hepatic MDA levels in irradiated rats that were pretreated with oral administration of melatonin in Groups V, VI and VII, were significantly decreased, while the GSH levels were significantly increased.

Conclusion: Oral administration of melatonin may prevent radiation-induced liver damage. This radio-protective effect is dose-dependent.

Keywords
Melatonin; Irradiation; Lipid peroxidation; MDA; GSH

Introduction

Ionizing radiation produces free radicals or reactive oxygen species (ROS) that attack various cellular components including DNA molecules, proteins, and membrane lipids, leading to a significant cellular damage [1]. Since these radicals initiate lipid peroxidation, it is expected that those who undergo whole body irradiation will have higher levels of lipid peroxidation [2]. Lipid peroxidation involves oxidative conversion of polyunsaturated fatty acids to several products including malondialdehyde (MDA) and lipid peroxides. For its cytotoxicity and inhibitory actions on protective enzymes, MDA acts as a co-carcinogen [3].

Antioxidant system consists of several low molecular weight molecules such as glutathione (GSH) and various antioxidant enzymes [4]. GSH is one of the most important molecules in the cellular defense against oxidative stress. Depletion of tissue GSH is one of the primary factors that permits lipid peroxidation to occur [5]. Decreased cellular GSH concentrations and reduced capacity for GSH synthesis make cells susceptible to radiation injury [5, 6].

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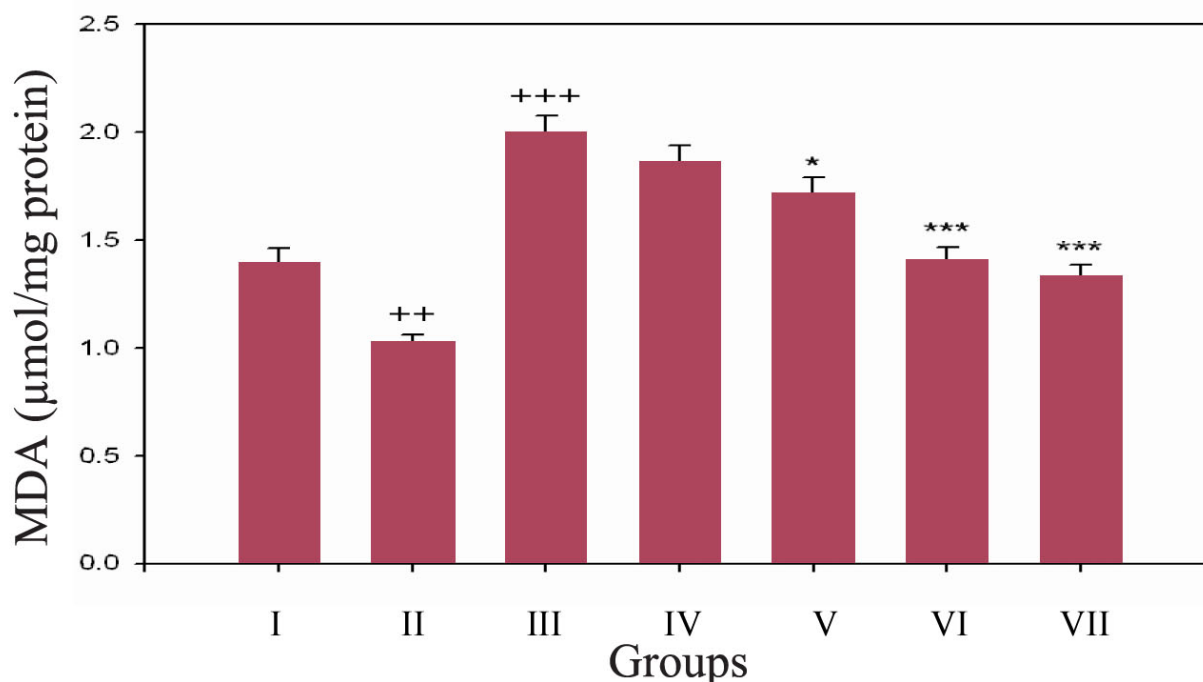
Figure 1: Mean MDA levels in rat livers 72 h post-irradiation (6 Gy) in the presence and absence of melatonin. (Group I: Control group; II: Melatonin only; III: Irradiation only; IV: Irradiation + 10 µg/kg melatonin; V: Irradiation + 100 µg/kg melatonin; VI: Irradiation + 200 µg/kg melatonin; and VII: Irradiation + 400 µg/kg melatonin). Error bars represent SEM for six animals in each group. Statistical difference with irradiated only animals (group III): ***p<0.001; **p<0.01; *p<0.05. Statistical difference with the control group: +++p<0.001; ++p<0.01; +p<0.05.

Melatonin, the major product of pineal gland, has been shown to be a direct free radical scavenger and an indirect antioxidant acting through its stimulatory actions on the antioxidant system [7-9]. The objective of this study was to investigate the antioxidant effect of melatonin, at different oral doses, on radiation-induced oxidative damage to liver tissue.

Materials and Methods

Chemicals
All reagents were of the highest quality available. Melatonin was obtained from Sigma Chemical Co. (St. Louis, MO, USA); other chemicals used in this study were obtained from both Sigma (St. Louis, MO, USA) and Merck (Germany).

Animals
Forty-two Wistar albino male rats weighing 170–220 g, were used for the experiment. All animals were housed in stainless steel cages and supplied with wood chips, in a temperature controlled room (22 °C) and a 12:12 h light:dark cycle. The experimental protocol was in accordance with the guidelines for care and use of laboratory animals as adopted by the Ethics Committee of the School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Experimental design
On the day of the experiment, the rats were divided into seven groups of six rats housed in different cages. Group I rats served as the control group and received isotonic saline, no radiation; group II rats were pretreated with 400 µg/kg melatonin, no radiation; group III was administered isotonic saline and exposed to radiation; and groups IV to VII were administered 10, 100, 200 and 400 µg/kg body weight
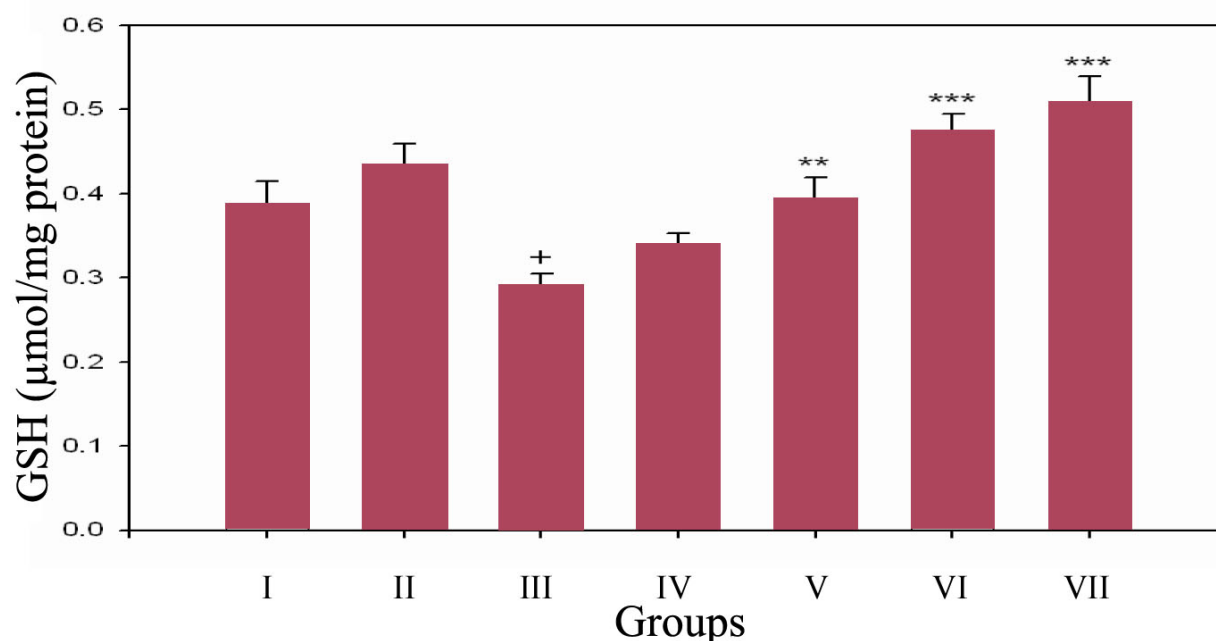
Figure 2: Mean GSH levels in rat livers 72 h post-irradiation (6 Gy) in the presence and absence of melatonin. (Group I: Control group; II: Melatonin only; III: Irradiation only; IV: Irradiation + 10 µg/kg melatonin; V: Irradiation + 100 µg/kg melatonin; VI: Irradiation + 200 µg/kg melatonin; and VII: Irradiation + 400 µg/kg melatonin). Error bars represent SEM for six animals in each group. Statistical difference with irradiated only animals (group III): ***p<0.001; **p<0.01; *p<0.05. Statistical difference with the control group: +++p<0.001; ++p<0.01; +p<0.05.

of oral melatonin (Sigma Chemical Co.), respectively, and exposed to radiation. The melatonin was dissolved in water and administered to animals. All of the drugs and isotonic saline were administered orally by a gavage. An hour after administration of the drugs, animals were anesthetized with HCL, 50 mg/kg, and exposed to 6 Gy whole body irradiation. The melatonin only and control group animals were anesthetized, but not irradiated.

Irradiation

A ^{60}Co teletherapy unit (Theratron 780, Atomic energy of Canada limited, Canada) at SSD of 78.5 cm was used to deliver 6 Gy whole body irradiation to rats. The dose was calculated along the central axis at a depth of 2 cm, with a dose rate of 178.6 cGy/min. Animals in all groups were sacrificed 72 hours after irradiation. Liver tissue concentrations of MDA and GSH were measured.

Tissue preparation

Seventy-two hours after irradiation, animals were anesthetized by 50 mg/kg of sodium thiopental. The rats' livers were excised and homogenized in a 10-fold physiological saline solution in a homogenizer (Omni accessory pack international homogenizer, USA). The homogenate was centrifuged at 10,000 g for one hour to remove debris. The supernatant was taken for biochemical analysis. All procedures were performed at a temperature of +4 °C throughout the experiments.

Biochemical analysis

Concentrations of MDA, an index of lipid peroxidation, and GSH, an indicator of antioxidant capacity, were estimated in liver tissue. Lipid peroxidation was determined in liver tissue homogenate according to the thiobarbituric acid (TBA) method [10]. MDA levels were measured at 535 nm and expressed as µmol/mg

protein. GSH was measured as described by Ellman [11]. Protein content was determined by the Bradford method [12]. GSH concentration was measured at 412 nm and expressed as μmol/mg protein. Biochemical measurements were carried out at room temperature with a visible ray spectrophotometer (CECIL CE 3041, Cam-bridge, UK).

Statistical analyses

Statistical analysis was performed by SPSS® ver 13 for Windows®. Each data point represents mean±standard error of the mean (SEM) of at least six animals per group. One-way analysis of variance (ANOVA) was performed to compare different groups. Tukey's HSD was used as *post hoc* test. A p value <0.05 was considered statistically significant.

Results

MDA levels

Figure 1 shows MDA levels (μmol/mg protein) in rat liver 72 h post-irradiation in the presence and absence of melatonin. In the radiation only group (group III), MDA level was significantly (p<0.001) higher than that in the melatonin only or control group. MDA level of melatonin only group was significantly (p<0.01) lower than the control group. MDA levels in the irradiated animals that received 200 and 400 μg/kg of oral melatonin (groups VI and VII) were significantly lower (p<0.001) than that in group III (only irradiated). Also, MDA level in irradiated rats that received 100 μg/kg of oral melatonin (group V) was significantly (p<0.05) lower than that in group III (only irradiated). Administration of 10 μg/kg of oral melatonin did not have a significant effect on MDA level after irradiation.

GSH levels

Figure 2 shows GSH levels (μmol/mg protein) in the presence and absence of melatonin in rat liver after 72 h of irradiation. The level of GSH in the group III (only irradiated) was significantly (p<0.05) lower than that in the control and melatonin only group. In irradiated rats that received 100, 200 and 400 μg/kg of oral melatonin (groups V, VI, VII), GSH levels were significantly higher (p<0.01) than that in group III (only irradiated). However, administration of 10 μg/kg of oral melatonin did not have a significant effect on GSH level after irradiation.

Discussion

Various chemical compounds such as amifostine and other sulfhydryl compounds have so far been investigated as potential radio-protective agents [4]. However, the inherent toxicity of these agents at the radio-protective doses warranted further search for safer and more effective radioprotectors [4, 13]. A wide dosage range for melatonin, from physiological to pharmacological concentrations, has been tested in various animal studies. The results of these studies indicated that the acute and chronic toxicity of melatonin is extremely rare [14]. Pretreatment with 0.1 mg/kg of melatonin given orally for 15 consecutive days afford potential protective effect against radiation-induced damage to mouse cerebellum [15]. It has been shown that melatonin at doses as high as 250 mg/kg is non-toxic and that high doses of melatonin are effective in protecting mice from lethal effects of acute whole body irradiation [16].

The results of the present study showed that whole body irradiation would cause tissue damage to rat liver as indicated by increased MDA levels and decreased GSH levels. The increase in MDA levels in the irradiated group demonstrated the role of oxidative mechanisms in irradiation-induced tissue damage. As mentioned earlier, GSH provides major protection in oxidative injury by participating in the cellular defense system against oxidative stress. The decrease in tissue GSH levels after whole body irradiation may reflect its consumption during the oxidative stress. On the other hand,

melatonin administration before irradiation alleviated the radiation-induced damage to liver by reversing the radiation-induced effects. The mechanisms of inhibition of lipid peroxidation by melatonin probably include the direct scavenging of the initiating radicals, especially ⋅OH and ONOO⁻ [17]. Also, melatonin, by indirect antioxidative property, can increase the level of GSH.

Several studies have shown that tissue injuries induced by various stimuli are coupled with GSH depletion and high levels of lipid peroxidation [18-20]. Moreover, researches in the last decade demonstrated that melatonin, by its free radical scavenging and antioxidative properties, ameliorates the radiation toxicity in various tissues. El-Missiry, *et al*, showed that treatment with 10 mg/kg/day melatonin for four days before irradiation (2 and 4 Gy) significantly abolished the radiation-induced elevations in MDA and protein carbonyl levels—the oxidative stress markers—in the liver and significantly maintained hepatic GSH content, glutathione-S-transferase (GST), and catalase (CAT) activities close to those of the control group [21]. In another study, we showed that radiation exposure decreased GSH level and increased MDA level in the lens; these values however remained within normal limits when melatonin had been administered prior to irradiation [22].

In our current study, we came to similar results reported previously, which showed that treatment with melatonin increased GSH level and decreased MDA concentration compared to the irradiated only group. Nonetheless, we found there was no significant difference in GSH and MDA levels in irradiation plus 10 μg/kg group compared to irradiation only group. These findings are in agreement with previous studies [3, 13, 15, 17, 21, 23-27]. It seems that radio-protective effects of melatonin are dose-dependent. Melatonin at doses of 200 and 400 μg/kg was more protective against whole body irradiation (p<0.001) compared to other dosages.

Considering all results [22, 25, 28, 29], it seems that administration of melatonin may enable the use of higher doses of irradiation during radiotherapy and may be beneficial in alleviating the complications of cancer treatment. However, further experiments and clinical trials on this issue are imperative.

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