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Original Article

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Abstract

Exposure to ionizing radiation produced radiation sickness that is a collection of short and long term complications. The aim of the present study was to investigate the potential neuroprotective effect of melatonin (MLT) on radiation-induced cell death and hippocampus injury. Rats were divided into seven groups namely control, two melatonin treated groups (MLT-1 and MLT-7) treated with melatonin for one and 7 days, two irradiated groups (Rad-1 and Rad7) sacrificed after one and seven days respectively, and two melatonin treated and irradiated groups (MLT-Rad-1 and MLT Rad-7) sacrificed after one and seven days post irradiation. Control rats did not received any treatment. Melatonin treated groups were injected melatonin at a dose of 10mg/kg. Rats in the Irradiated groups, rats were subjected to a single dose of 4Gy γ-radiation from cobalt-60 source .The present results showed that melatonin protected against body weight loss, nestin up-regulation and protected the histological structure of the hippocampus against irradiation. This will enable to the development of radio-preventive strategies for human under radiotherapy as well as occupational and professional exposure to ionizing radiation.

1. Introduction

The radiotherapy and nuclear accidents require in depth understanding to radiation effects in biological system. Ionizing radiation exerts it effect through direct and indirect effects. IR can interact directly with biomolecules particularly DNA and phospholipid, While indirect effect of IR is via water radiolysis and generation free radical including reactive oxygen and nitrogen species (ROS & RNS). These free radicals include superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($\cdot OH$), and hydrogen peroxide (H_2O_2), that overwhelm the levels of antioxidants, resulting in oxidative stress [1].

Brain is vital organ and is subjected for irradiation from several sources in patients under radiotherapy, during occupational and space exploration [2]. However, the effect of irradiation on brain physiology remains unclear. Brain is the more sensitive organ to reactive oxygen spices due to its high oxygen utilization and extraordinary lipid content.

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Many studies have examined both the neuroprotective effects of some antioxidants as well as the damaging impacts of ionizing radiation. In term of this point, the efficiency of melatonin (Mel) to induce repair or otherwise interfere with the damaging effects of gamma irradiation is important [3]. Research shows that Mel is an antioxidant agent. As a free radical scavenger, Mel has a high degree of lipophilicity and therefore cross blood brain barrier. It is suggested that oxidative stress and inflammatory response are linked because irradiation generates free radicals and activates microglia and enhances immune cells to infiltrate brain which lead to generation of more ROS [4]. Therefore, involvement of antioxidants is worthy approach to reduce oxidative stress and protect hippocampus structure and function against γ-radiation. There is no reported evidence for a mitigating effect of MLT administration against radiation-induced damage and apoptosis in hippocampus.

Therefore, the present study will investigate the potential neuroprotective effect of MLT on radiation-induced changes in body weight, nestin levels and hippocampus injury.

Materials and Methods

1. Chemicals:

Melatonin was purchased from Sigma Company for chemical. All other chemicals are of analytical grades.

2. Experimental animal groups:

Adult male albino rats of Wistar strain, weighing 180-200 g were used. Rats were randomly divided into 4 groups, six animals each.

1- The first group considered as control group in which animals received normal laboratory diet without treatment until the end of study.

2- The second group received ip treatment with MLT at a dose of 10 mg/kg/day, for one and 7 consecutive days.

3- The third group was exposed to 4 Gy γ-radiation from the biological irradiator gamma cell-40, Cobalt-60 source (Atomic Energy Agency, Canada), belonging to National Center for Radiation Research and Technology (NCRRT), Cairo, EGYPT.

4- The fourth group received ip treatment of MLT at a dose of 10 mg/kg/day, 30 min before irradiation and continued for one and 7 days after gamma radiation exposure.

Radiation exposure:

Rats were exposed to whole body γ-radiation at a single dose level of 4 Gy delivered at a dose rate of 0.657 Gy/sec. Radiation source was Cs137 (GC-40, Nordion-Canada), at the national center for radiation research and technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

The experimental procedures for the treatment of rats were accomplished according to the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and also approved by the Institutional Animal Ethics Committee of Mansoura University.

Tissue and blood sampling

After the end of experimental period, rats were anaesthetized with ketamine-xylazine [5]. Blood samples were withdrawn directly from the hearts by cardiac puncture on EDTA as anticoagulant, followed by separation of plasma after centrifugation at 1000 xg. The plasma was kept at -20 °C till biochemical investigation. Meanwhile, brains were obtained and the midbrain regions were separated then homogenized in normal saline solution to form 10% (w/v) homogenates. The homogenates were centrifuged to obtain supernatants after cool centrifugation at 1000 xg, and then stored at -20 °C for biochemical analysis.

Determination of body weight

Body weights of rats in the experimental groups were determined before and after the experimental period.

Determination of nestin in plasma

Plasma nestin levels were measured by an enzyme-linked immunosorbent assay (ELISA) kit (YH-Biosearch, Cat.No:YHB3353Hu), based on the sandwich principle, as described in the manufacturer's instructions.

Histological examination

Hippocampuses from different rats were fixed in 10% saline-paraformaldehyde (pH 7.6) for 24 hours for histopathological examinations. Brains were passed ascending concentrations of ethanol, cleared in toluene, embedded in paraffin then sectioned at 6 µm followed by routinely staining with Hematoxylin and Eosin (H&E). The sections were examined by light microscopy for histopathological changes, and photomicrographed by light Olympus microscope (Amscope mu1000 camera).

Results:

From the data presented in Table 1 and Figure 1, rats that treated with 10 mg/Kg melatonin for one and seven days (MLT-1 and MLT-7 groups respectively) did not produce significant change in body weights compared to control group. Moreover, the data showed that there is no change occurred in body weight in MLT-7 group compared to MLT-1 group. On the other hand, exposure to γ radiation caused a significant decrease in body weight one and seven day (R-1 and R-7 groups respectively) post irradiation compared to control group. The data showed also that the decrease in body weight in R 1 group remarkably higher than the R 7 group. For MLT+R 1 and MLT-7+R-1groups, the data showed pronounced improvement in the body weight as compared to R 1and R-2 group, but it is still lower than control group. The data showed that the improvement in body weight gain in R7 group is higher than that in R1 group.

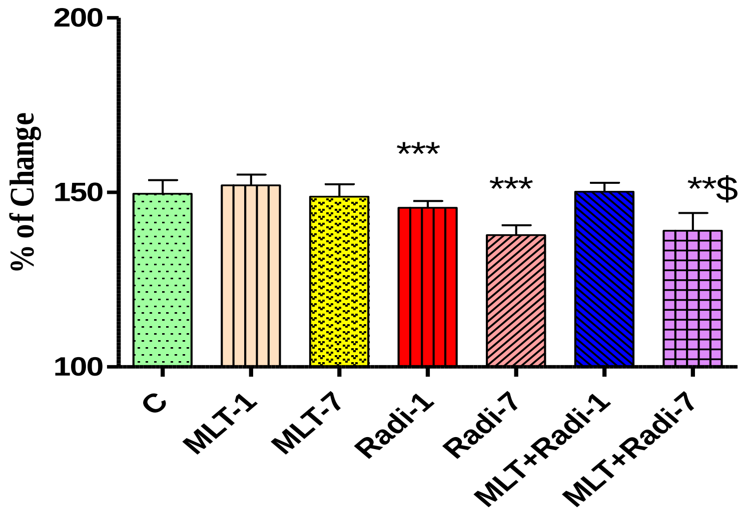

Figure 1. Changes in body weigh in all studied groups expressed as percent of changes from the original weight.

The effect of melatonin nestin levels in rats exposed to 4 Gy gamma radiations is presented in Figure 2. Rats that received 10mg MLT/Kg showed insignificant change in nestin levels compared to the control rats. Radiation exposure induced a significant increase in the plasma nestin levels after one and seven days (Rad-1 and Rad-7 groups) post irradiation. Pre-irradiation treatment with MLT significantly ameliorated the increase in nestin levels in MLT+Rad-1 and MLT+Rad-7 groups.

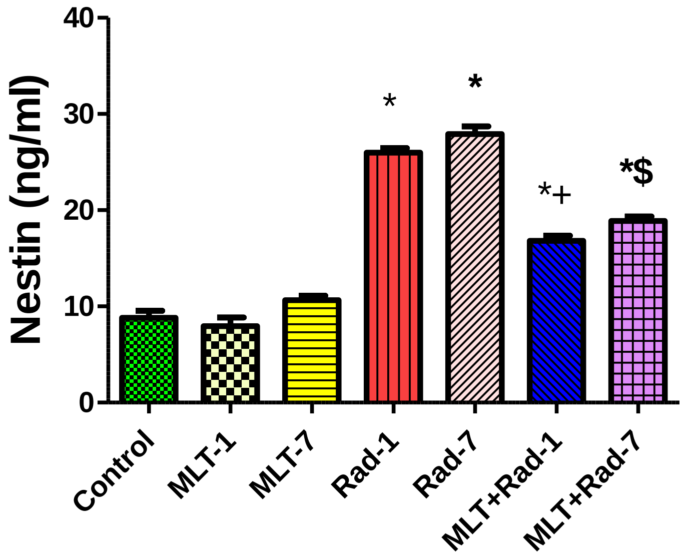

Figure 2. Effect of gamma radiation and melatonin on the plasma levels of nestin (pg/ ml) in the studied groups.

Histology of hippocampus in the studied groups

Control animal showed normal histological architecture of hippocampus and displayed normal granular and molecular (M) layers of Cornu Ammonis (CA) (Figure 3). Similarly, animals that treated with melatonin for one and 7 days showed similar histological pattern of hippocampus of the control rats. Rats that subjected to 4 Gy gamma radiation showed marked ischemic neuronal degeneration within the granular layer of CA, with pyknosis and loss of the nucleus and condensation of the cytoplasm. The Hippocampal of rats after seven days of irradiation displayed severe degree of ischemic neuronal degeneration (arrowhead) within the granular layer of CA associated with neuronophagia (arrow). Hippocampal section of rats irradiated and treated with melatonin for one day and seven days showed mild vacuolar degeneration (arrow) and mild ischemic injury (arrowhead) of granular layers of CA Figure 3).

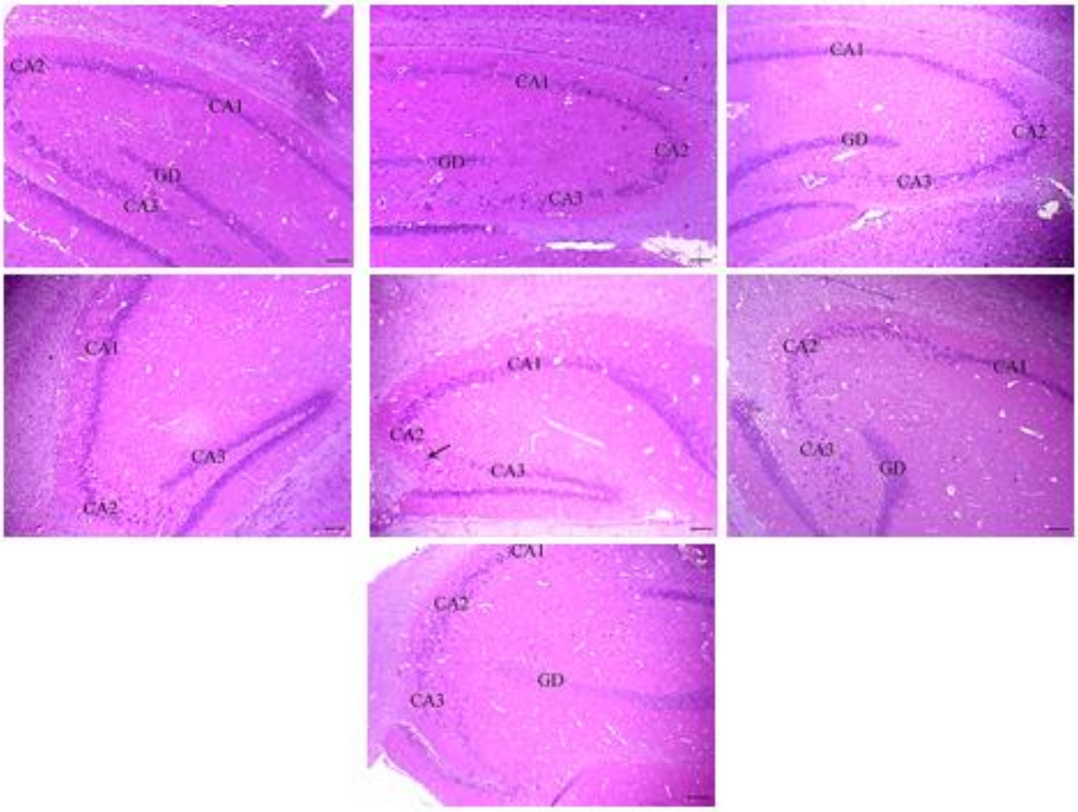

Figure 3. Hippocampal sections of the studied groups showing granular and molecular (M) layers of Cornu Ammonis (CA), H&E, bar= 50 µm.

Discussion

Rats that treated with 10 mg/Kg melatonin for one and seven days did not produce significant change in body weights compared to control group indicating it wide safety margin. On the other hand, exposure to γ-radiation caused a significant decrease in body weight one and seven day post irradiation compared to control group. The data showed pronounced improvement in the body weight as compared to irradiated rat groups, but it is still lower than control group. These results indicate that melatonin can protect against body weight loss due to irradiation.

Nestin is a protein in a form of intermediate filament expressed in many cells in different tissues and organs [6]. Among neural cells in the developing and adult central nervous system, nestin expression is thought to occur exclusively in uncommitted neural progenitor cells [7]. Nestin is considered sensitive marker of astroglial cells in brain. The current study showed that radiation exposure increased nestin levels in serum and the treatment with melatonin ameliorated the enhanced nestin levels. In previous studies, enhanced expression of nestin was observed in traumatic brain injury and ischemic cerebral lesions [8] and in experimental Parkinson model after administration of neurotoxicant to mice [9]. Another study showed ionizing radiation up-regulated nestin expression in ependymal cells of the brain , suggesting that these reactive ependymal cells may be involved in remodeling and repairing processes of brain irradiation injury [10]. In the current investigation melatonin did not produce additional enhancement of nestin levels in plasma of irradiated rats. Although, in a recent study melatonin exerted neuroprotection by up-regulation of nestin expression in subventricular brain [11].

Animals that treated with 10 mg/kg melatonin for one and 7 days showed similar histological pattern of hippocampus of the control rats. On the other hand, rats that exposed to 4 Gy gamma radiation showed marked ischemic neuronal degeneration within the granular layer of Cornu Ammonis, with pyknosis and loss of the nucleus and condensation of the cytoplasm. Hippocampal section of rats irradiated and treated with melatonin for one day and seven days showed mild vacuolar degeneration (arrow) and mild ischemic injury (arrowhead) of granular layers of Cornu Ammonis (CA). These results indicate that melatonin is able to protect the histological structure of hippocampus.

In conclusion, in this study we investigated the radio-protective effect of melatonin on brain of rats exposed to 4 Gy radiation from cesium-137 source. Melatonin can improve acute influence of radiation exposure during radiotherapy, in highly radiosensitive organs such as brain, gonads and bone marrow. Melatonin has several favorable properties and can be beneficial in clinical settings after support and confirmation of further investigation on human.

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