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Melatonin and roentgen irradiation-induced acute radiation enteritis in Albino rats: An animal model

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Abstract

Background: Roentgen irradiation can affect normal cells, especially the rapidly growing ones such as the mucosal epithelial cells of the small intestine. The small intestine is the most radiosensitive gastrointestinal organ and patients receiving radiotherapy directed to the abdomen or pelvis may develop radiation enteritis. Although roentgen rays are widely used for both imaging and therapeutic purposes, our knowledge about the morphological changes associated with radiation enteritis is lacking.
Hypothesis: This study tries to tests the hypothesis that ''the intake of melatonin can minimize the morphological features of cell damage associated with radiation enteritis''.
Objectives and methods: We performed this investigation to test our hypothesis and to examine the possible radioprotective effects of melatonin in acute radiation enteritis. To achieve these goals, an animal model consisting of 60 Albino rats was established. The animals were divided into five groups: Group 1, non-irradiated; Group 2, X-ray irradiated (X-ray irradiation, 8 Grays); Group 3, X-ray irradiated-pretreated with solvent (ethanol and phosphate buffered saline); Group 4, non-irradiated-group treated with melatonin, and Group 5, X-ray irradiated-pretreated with melatonin. The small intestines were evaluated for gross (macroscopic), histological, morphometric (light microscopy), and ultrastructural changes (transmission electron microscopy).
Results: We found morphological variations among the non-irradiated-group, X-ray irradiated-group and X-ray irradiated-intestines of the animals pretreated with melatonin. The development of acute radiation enteritis in X-ray irradiated-group (Groups 2 and 3) was associated with symptoms of enteritis (diarrhea and abdominal distention) and histological features of mucosal injury (mucosal ulceration, necrosis of the epithelial cells). There was a significant reduction of the morphometric parameters (villous count, villous height, crypt height and villous/crypt height ratio). Moreover, the ultrastructural features of cell damage were evident including: apoptosis, lack of parallel arrangement of the microvilli, loss of the covering glycocalyx, desquamation of the microvilli, vacuolation of the apical parts of the cells, dilatation of the rough endoplasmic reticulum, and damage of the mitochondrial cristae. In the non-irradiated-group and in X-ray irradiated-intestines of the animals pretreated with melatonin (Group 5), these changes were absent and the intestinal mucosal structure was preserved.
Conclusion: Administration of melatonin prior to irradiation can protect the intestine against X-rays destructive effects, i.e. radiation enteritis. The clinical applications of these observations await further studies.

Keywords: Intestine; X-ray irradiation; Melatonin

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1. Introduction

X-rays (electromagnetic ionizing radiation) are composed of mass-less particles of energy (photons) that disrupt the electrons of the atoms within the cells and therefore affect many cellular functions. X-ray irradiation can affect normal cells especially the rapidly growing ones such as the epithelial cells of the small intestine. Although radiation is aimed to be directed at the malignant tissue, the adjacent healthy tissue is also affected as well. The small intestine is considered as one of the most sensitive gastrointestinal organs to radiation therapy (Giris et al., 2006; Hussein et al., 2006b; Hussein et al., 2007a; Somosy et al., 2002). Radiation enteritis is a challenging clinical problem in patients receiving ionizing radiation (Becciolini et al., 1997; Erbil et al., 2005; Giris et al., 2006). Direct X-ray irradiation can induce a series of poorly understood events in the rapidly renewing intestinal mucosal cells. Clinically, X-ray irradiation can result in several deleterious intestinal symptoms such as bleeding, anorexia, nausea, vomiting and diarrhea, i.e. gastrointestinal radiation syndrome (Hwang et al., 2003; Somosy et al., 2002). Although, X-ray is widely used for both imaging and therapeutic purposes, our knowledge about the morphological changes associated with their early and acute injurious effects on the small intestine is limited.

Melatonin is a secretory product of the pineal gland that regulates several physiological and cellular functions. It is a potent antioxidant that can scavenge many harmful free radicals including hydroxyl groups, peroxyl radicals and peroxynitrite anions (Deger et al., 2003). Melatonin is one of few antioxidants that can penetrate the mitochondrial membrane and enter the mitochondria. Also, it accumulates more in the nucleus than in the cytosol of the cells and thus has potent antioxidant effects. Melatonin can minimize the extent of DNA damage and the frequency of chromosomal abrasions in radiosensitive organs following exposure to the electromagnetic ionizing radiation (el-Aziz et al., 2005; Hussein et al., 2005; Hussein et al., 2006b). We previously examined the radioprotective effects of melatonin in Albino rats. We found that the administration of this agent prior to irradiation protects the germ cells (testis) and keratinocytes (skin) against the destructive effects of X-ray irradiation. In the testis, the intake of melatonin prior to X-ray irradiation was associated with amelioration of germ cell depletion. Also the morphological features indicative of cell damage following X-ray irradiation was minimal (Hussein et al., 2006a, b). In the skin, X-ray irradiation was associated with features of both cell injury (keratinocytes with condensation of the nuclei, vacuolization of the cytoplasm, dilatation of the rough endoplasmic reticulum, swelling of the mitochondria with cristolysis, destruction of the ribosomes and intermediate filaments, fragmentation of the keratohyaline granules and loss of the irregularity of the basal cell borders) and increased metabolic activity (keratinocytes with increased euchromatin, irregularity of the nuclear membrane and increased branching of the melanocytes). These changes were mild or absent in the skin of X-ray irradiated-animals pretreated with melatonin (Hussein et al., 2005). Mornjakovic and his colleagues examined the seminiferous epithelium and testis interstitium in sham pinealectomized adult Wistar rats after melatonin treatment and whole body irradiation with 8 Grays (Gy) of gamma rays. They found that melatonin can reduce the destructive effects on the seminiferous epithelium and interstitial cells of Leydig originally produced by irradiation (Mornjakovic et al., 1998, 1991).

Unfolding studies have examined the radioprotective effects of melatonin in different organs (Hussein et al., 2006a, b; Kim et al., 2001). However to date, available reports that bears directly on radioprotective effects of melatonin against X-ray induced acute radiation enteritis are lacking. Nor have the histological and ultrastructural changes associated with the intake of melatonin before X-ray irradiation directed to the intestine been investigated. This study tries to address these issues and to test the hypothesis that ''the intake of melatonin can minimize the morphological features of cell damage associated with radiation enteritis''. We carried out this investigation to test our hypothesis. To accomplish our goals, we established an animal model consisting of five different groups of Albino rats: non-X-ray irradiated; X-ray irradiated; X-ray irradiated-pretreated with solvent; non-X-ray irradiated-pretreated with melatonin and X-ray irradiated-pretreated with melatonin. We addressed two questions: what are the histological and ultrastructural changes in X-ray irradiated-intestine? and what are the effects of melatonin on these morphological changes ?

2. Materials and methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Sohage University, School of Medicine, Sohag, Egypt. The experiments were executed at the Pathology and Histology departments of Sohage and Assuit Universities Faculties of Medicine.

2.1. Rats and maintenance

Three-month old Albino rats were obtained from Assuit University Animal Facility, Faculty of Medicine, Assuit University, Assuit, Egypt. The animals were housed in Animal Facility at the Faculty of Medicine, Sohage University, Sohag, Egypt, with room temperature maintained at 65–75°F, relative humidity of 50–70% and an airflow rate of 15 exchange/h. Also, a time controlled system provided 07:00–21:00 h light and 21:00–07:00 h dark cycles. All rats were given ad libitum access to Taklad rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes. These conditions were adopted following other groups (Hussein et al., 2005, 2006a, b).

2.2. X-ray irradiation

X-ray irradiation was carried out at The Department of Radiology and Oncology, Sohage University Hospitals using a linear accelerator (Philips SL75.5) adjusted to provide X-ray