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

Mahmoud R. Hussein [a,b,*,1], Eman E. Abu-Dief [c,d], Esam Kamel [e], Amal T. Abou El-Ghait [c,d], Saad Rezk Abdulwahed [f], Mohamed H. Ahmad [c,d]

[a] *Department of Pathology, Faculty of Medicine, Assuit University, Assuit, Egypt*
[b] *Assir Central Hospital, Saudi Arabia*
[c] *Department of Histology, Faculty of Medicine, Sohag University, Egypt*
[d] *Department of Histology, Faculty of Medicine, Assuit University, Egypt*
[e] *Department of Anatomy, Faculty of Medicine, Sohag University, Egypt*
[f] *Department of Radiology, Faculty of Medicine, AL-Azhar University, Assuit, Egypt*

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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

* Corresponding author. Tel.: +20 0966 05672 21519; fax: +20 0966 0722 46673.
E-mail addresses: mrh17@gawab.com, mrcpath17@gmail.com (M.R. Hussein).
[1] Former address: University of Wisconsin Medical School and William S. Middleton Memorial Veteran Hospital, Madison, WI 53705, USA. Tel.: +1 608 256 1901x17818.

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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

irradiation. Each animal was placed in a special small box with adjustable width that can fairly accommodate the animal without allowing any movements. Each animal was exposed to a whole body X-ray irradiation dose of 8 Gy. The dose was delivered at a rate of 400 motor unit/min. The X-ray irradiation dose for the intestine was measured using special equation and it was 8 Gy/intestine.

2.3. Melatonin and X-ray irradiation

After a 7-day acclimatization period, a randomized block design based on the animal body weights was used to divide rats into five different groups. Five separate experiments were executed using a total of 60 rats. Each experiment had 12 rats in each of the following five subgroups: subgroup A, non-X-ray irradiated; subgroup B, intraperitoneal injection of melatonin (100 mg/kg body weight); subgroup C, X-ray irradiated (8 Gy whole body); subgroup D, X-ray irradiated-pretreated with solvent (5% ethanol in phosphate buffer saline 1 h before irradiation); and subgroup E, X-ray irradiated-pretreated with melatonin (100 mg/kg body weight melatonin 1 h before irradiation). Non-irradiated subgroups (A and B) were initially evaluated as separate ones, and as no differences were found between them, they were summed together as one group (Group 1, non-X-ray irradiated-intestine). Similarly, no differences were found between animals in subgroups C and D and thus considered as one group (Group 2, X-ray irradiated-intestine). Animals in subgroup E, which received X-ray irradiation and melatonin pretreatment, were considered as a separate group (Group 3, X-ray irradiation + melatonin pretreatment intestine). Therefore animals in subgroups A and B served as controls for experimental animals in subgroups C, D and E.

Irradiation was carried out using a linear accelerator (Philips SL75.5). Animals in subgroups C, D and E were exposed to a whole body X-ray irradiation dose of 8 Gy. Animals in subgroups B and D were given an intraperitoneal injection of freshly prepared melatonin (Sigma, St. Louis, MO) in 1000 μL of 5% ethanol (made with phosphate buffer saline). Following other groups, we selected this X-ray irradiation-specific dose as it can generate reactive oxygen radicles, induce apoptosis, and alter cell cycle protein expression in rapidly proliferating cells such as germ cells and basal cell keratinocytes (Hussein et al., 2005, 2006a, b). Our previous studies indicated that X-ray irradiation at the selected dose (8 Gy) can induce severe damage to the radiosensitive organs (Hussein et al., 2005, 2006a, b). The small intestine is considered as the most radiosensitive organ in the gastrointestinal tract. Therefore a very high dose of melatonin was used to combat the severe damage (acute radiation enteritis) induced by the selected high dose of X-ray irradiation.

2.4. Abdominal laparotomy and morphological examination of the small intestine

The animals were scarified at 48 h after X-ray irradiation and laparotomy was performed. Immediately after laparotomy, the small intestine (ileum and jejunum) was examined and removed. Two-centimeter segments of jejunum, ileum, and macroscopically pathologic segments of intestine showing edema and change in color (purple) were resected, luminal contents were washed with 0.9% NaCl by injecting saline through the lumen of the small bowel segments. Tissue samples were pinned out on paraffin block, and floated upside-down in 10% buffered formalin overnight, dehydrated, and embedded in paraffin. Sections of 4 μm were cut with a Leica sliding microtome (SM 2000R, Nussbach, Germany), and slides (at least 10 sections) were stained with hematoxylin and

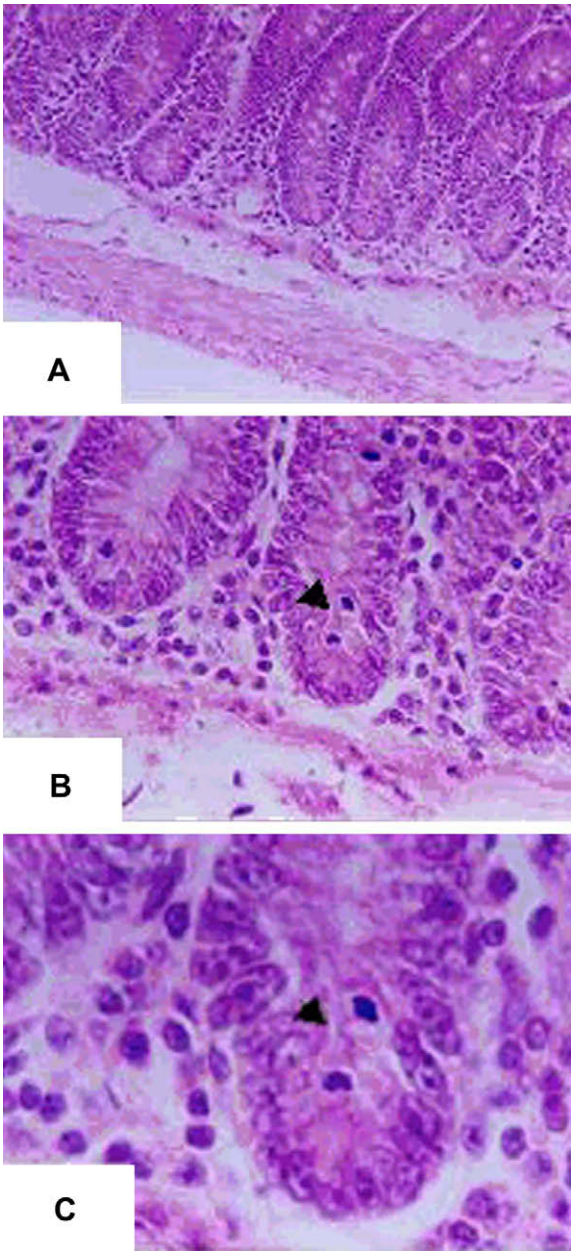


Fig. 1. (A–C) Histological features of the non-irradiated-intestine featuring: (A) the basal portion of intestinal crypts, submucosa and muscle layers (×200); (B) modest mitotic activity (arrow) indicative of regenerative changes (×400); and (C) absorptive cells of the intestinal crypts with oval vesicular nuclei and prominent nucleoli. The goblet cells (arrow) have supranuclear vacuole (×1000).

eosin and periodic acid Schiff stains. Some representative sections (at least three sections) of the intestine were processed for ultrastructural studies. Sections were evaluated in blinded fashion. The analyses included both quantitative and qualitative ones (Hussein et al., 2005, 2006a, b).

2.5. Histological evaluation of the small intestine

For histological assessment, villous height (from the base to the tip of the villous), crypt height (from the base to the tip of each crypt), the villous:crypt ratio, and the number of villi per square millimeter were determined on individual intestinal segments at five separate microscopic fields for each animal and recorded as the mean values by using ocular micrometer-adapted light microscopy at a magnification of ×100.

2.5.1. Ultrastructural evaluation of the small intestine using transmission electron microscopy

Some tissue fragments were fixed in 2.5% glutradehyde in 0.1 M sodium cacodylate buffer at 4 °C and pH 7.2 for 24 h, washed in 0.1 M buffer, post fixed in osmium tetroxide in 0.2 M buffer for 1 h. The specimens were dehydrated in 70%, 90% and 100% ethanol and then embedded in labeled capsules with freshly prepared resin and left to polymerize at 60 °C for

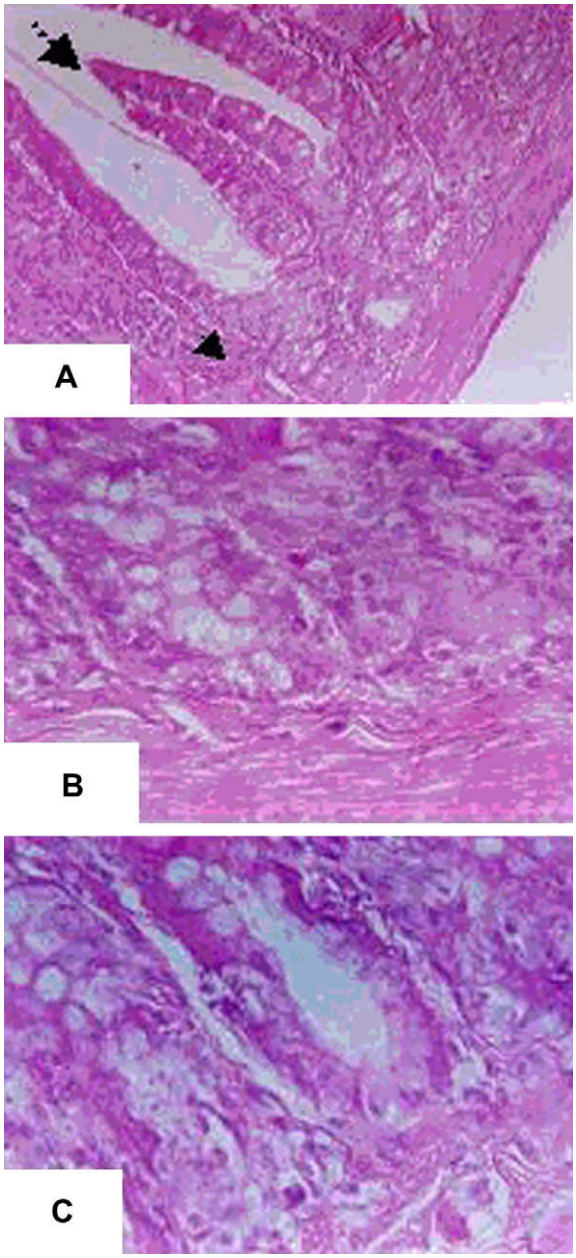


Fig. 2. (A–C) Histological features of X-ray irradiated-intestine featuring: (A) shortened intestinal villi (dashed arrow) and congested blood vessels (arrow) (×200); (B) loss of normal architecture and cellular arrangements (×400); and (C) degenerative and necrotic cells (×1000).

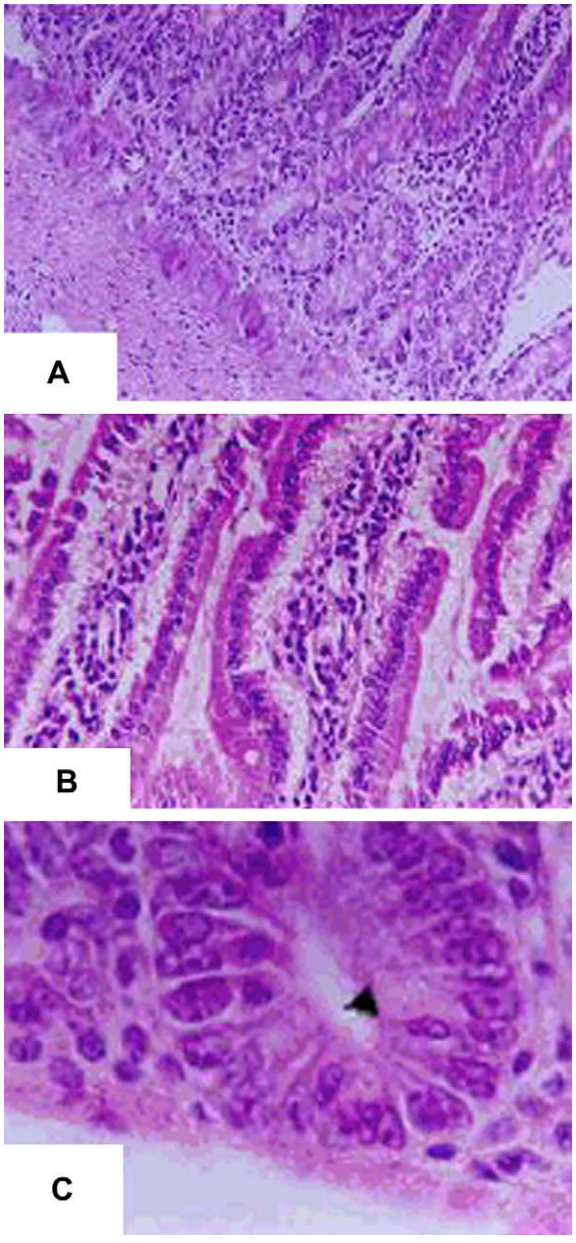


Fig. 3. (A–C) Histological features of X-ray irradiated-intestine from animals treated with melatonin featuring: (A) intestinal crypts with normal architecture (×200); (B) recovering intestinal villi, with relatively normal lining of enterocytes with few goblet cells (×400); and (C) enterocytes and goblet cells with normal cytology and increased mitotic activity (arrow) (×1000).

Table 1
Clinicopathological and morphometric changes in radiation enteritis

Animal findings	Control (non-X-ray irradiated)	X-ray irradiated-group	X-ray irradiation- and melatonin pretreatment
Clinical			
Diarrhea	−	+	−
Abdominal distention	−	+	−
Gross			
Intestinal dilatation	−	+	−
Mucosal ulceration	−	+	−
Edema	−	+	−
Histologic	−	+	−
Shortening of villi	−	+	−
Architectural disarray	−	+	−
Necrosis of mucosal cell	−	+	−
Degeneration of mucosal cells	−	+	−
Apoptosis of mucosal cells	−	+	−
Mitotic activity in mucosal cells	+	+	+++
Congested blood vessels	−	++	−
Morphometric			
Villous number/mm	65.1 ± 2.3	35.8 ± 1.8[a]	55.7 ± 2.1
Villous height (mm)	556.0 ± 6.6	435.6 ± 3.6[a]	531.9 ± 3.5
Crypt height (mm)	221.4 ± 2.5	186.1 ± 2.6[a]	204.9 ± 1.7
Villous/crypt height	720 ± 9.3	589.5 ± 13.7[a]	691.0 ± 6.8
Ultrastructural changes			
Features of cell damage	−	Lack of normal alignment of microvilli Loss of glycocalyx covering desquamation of microvilli Damage of mitochondrial cristae	−
Features of apoptosis	−	Cytoplasmic vacuolization Condensation of the nuclear chromatin Apoptotic bodies	−
Features of increased metabolic activity	−	Increased mitotic activity Prominent endoplasmic reticulum Prominence of the nucleoli	−

(−): absent; (+): present.
[a] $p < 0.05$, X-ray irradiated-group compared to the control group.

48 h. Several resin semithin sections were cut at approximately 1 micron using glass knives and ultramicrotome. The sections were stained with 1% toluidine blue in 1% borax solution for 1 min at 80 °C. The stain was rinsed off with distilled water and sections were dried and examined. Selective areas from trimmed blocks were cut by using a diamond knife, with the ultramicrotome set to cut at around 50–70 nm using heat advances. The sections were picked up onto 300 mesh copper grids, stained with methanolic uranyl acetate and examined by transmission electron microscopy (TEM). Some of the examined fields were photographed (Hussein et al., 2005, 2006a, b, 2007b).

2.6. Statistical analysis

Analysis of variance (ANOVA) with a statistical significance of $p < 0.05$, was used. Data were subjected to analysis of variance (ANOVA test) of a completely randomized designed according to other groups (Simpson et al., 1960; Petersen, 1985). Examination of the statistical level of significance was performed with Student's *t*-test resulting from the ANOVA tests. Level of significance (p) was considered as follows: (i) $p > 0.05$, non-significant; (ii) $p \leq 0.05$, significant; and (iii) $p \leq 0.01$, highly significant. Computations were performed with SAS version 8.1 Software (SAS Institute Inc, Cary, NC). All the analyses were performed in a blinded fashion by the authors.

3. Results

All rats tolerated the experiments and survived throughout the duration of the study (48 h). All X-ray irradiated-animals developed diarrhea, abdominal distention and laparotomy revealed intestinal dilation (lumens distended with gases and fluids) and edema. These findings were absent in the control and melatonin pretreated groups. The morphological findings were examined by the authors, in agreement, and categorized into histological, morphometric and ultrastructural changes.

3.1. Histological and morphometric changes

Non-irradiated-intestinal mucosal glands showed a preserved architecture. They were lined by absorptive (enterocytes) and mucous secreting cells (goblet cells) (Fig. 1A). The enterocytes appeared as tall columnar cells with oval nuclei in the basal part of the cells and multiple mitotic figures (Fig. 1B). Goblet cells appeared as rounded cells with basal nuclei and vacuolation in their apical regions (Fig. 1C). The most obvious changes in

intestinal histology (induced by radiation, radiation enteritis) were architectural disorganization and shortening of villi (Figs. 2A,B). The number of the villi and the villous height markedly decreased after irradiation ($p < 0.05$). Also, radiation enteritis was associated with degeneration, necrosis of the epithelial cells, congestion of blood vessels in the submucosa and increased inflammatory cell infiltrate. Cytologically, the enterocytes and the goblet cells had dense irregular nuclei and acidophilic vacuolated cytoplasm (Fig. 2C). There was a significant decrease in the number of goblet cells (Fig. 2C). The X-ray irradiated-intestine from animals pretreated with melatonin had mucosal glands with a relatively normal structure but with prominent mitotic activity. Melatonin pretreatment significantly protected the integrity of the villi and preserved the villous height ($p < 0.05$). It also resulted in a marked amelioration of the degenerative and necrotic changes in the mucosa (Figs. 3A–C). A summary of the morphometric changes is presented in Table 1.

3.2. Ultrastructural features

In non-irradiated-intestine, the enterocytes had numerous parallel cylindrical microvilli, which had thick prominent covering of glycocalyx (Fig. 4A). Their cytoplasmic cores showed fine filaments, which extended basaly into the cytoplasm forming the terminal web (Figs. 4B,C). The irradiated mucosa showed loss of normal alignment of microvilli, loss of glycocalyx covering, desquamation of microvilli, abnormal vacuolation of the apical parts of the cells, nuclear pyknosis with deep indentation and irregularity of the nuclear membrane (Fig. 5A). The rough endoplasmic reticulum was dilated, and mitochondria were small with destructed cristae (Figs. 5A,B). Alternatively, intestine from animals pretreated with melatonin prior to X-ray irradiation had normal architecture, regular microvilli with beaded appearance and preservation of terminal webs (Figs. 6A–C).

4. Discussion

Melatonin is a hormone with multiple functions, produced by the pineal gland and stimulated by beta-adrenergic receptors (Kim et al., 2001). It is a neural hormone that acts as a free radical scavenger and general antioxidant. When compared with other antioxidant melatonin has greater efficacy in protecting against cellular oxidative stress. It can preserve macromolecules including DNA, protein and lipid from oxidative damage following exposure to ionizing radiation. Considering its high non-toxic nature as well as its ability to readily cross many biological membranes, melatonin may prove to be an effective and important molecule in protecting mucosal integrity following X-ray irradiation. Radiation therapy is a widely used adjuvant treatment for various abdominal and pelvic cancers. Although radiation enteritis is a significant health problem in cancer patients receiving irradiation, our knowledge about the radioprotective effects of melatonin against X-ray irradiation-induced enteric damage is limited. In this investigation, we hypothesized that ‘‘the intake

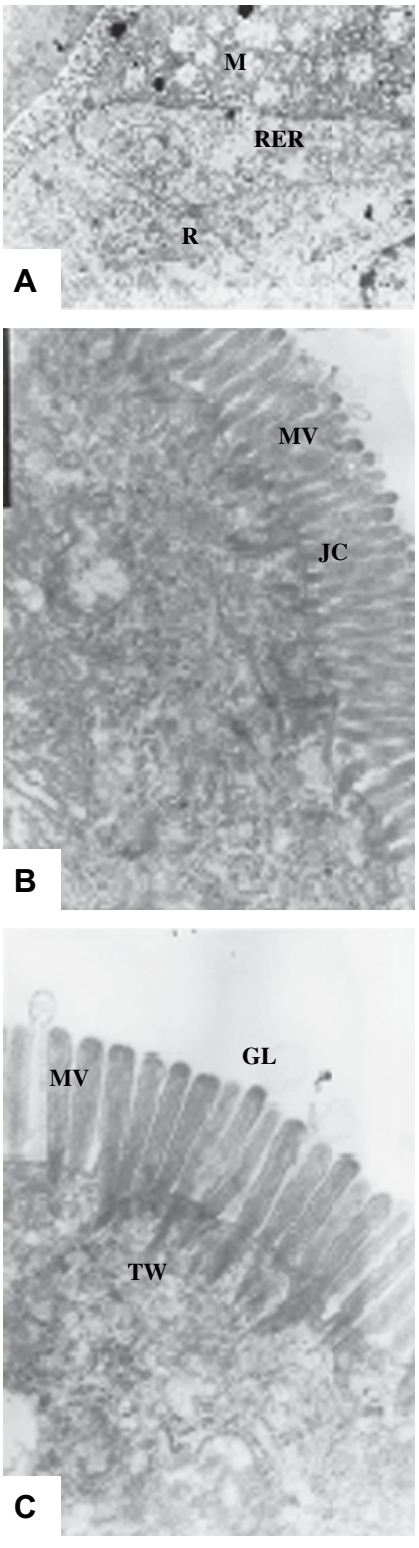
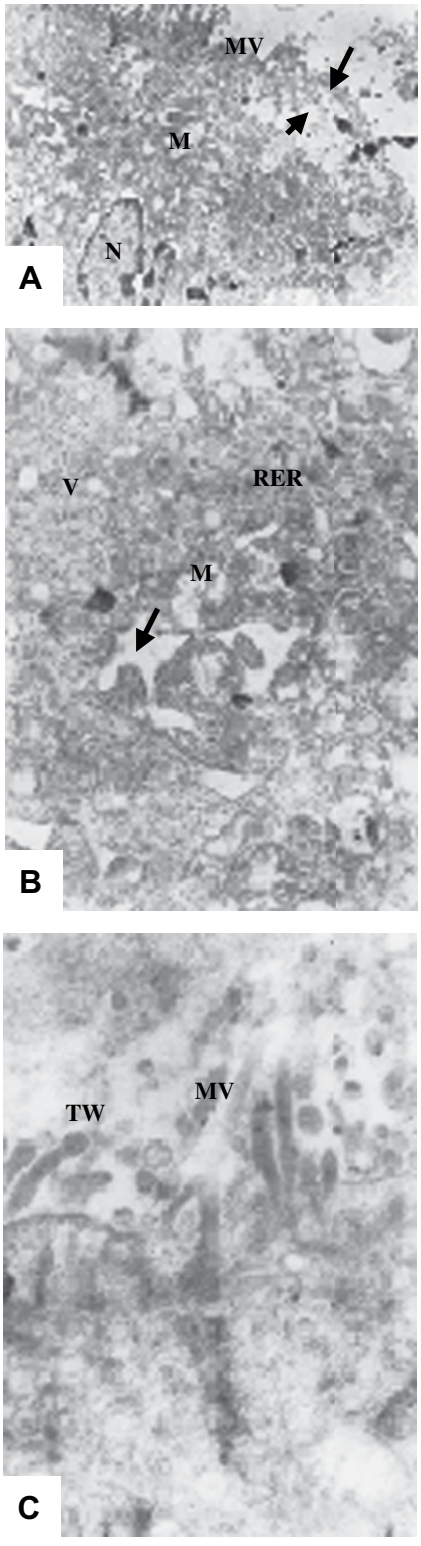


Fig. 4. (A–C) Ultrastructural micrographs of the non-irradiated-intestine featuring: (A) upper part of absorptive cells, stuffed with mitochondria (M), rough endoplasmic reticulum (RER) and ribosomes (R) (×4000); (B) numerous parallel cylindrical microvilli (MV) with the adjacent cells being interdigitated, tightly bound near their luminal surface by junctional complex (JC) (×14,000); and (C) parallel cylindrical microvilli (MV) with thick prominent covering glycocalyx (GL) and their cytoplasmic core showing fine filaments which extends basely into the cytoplasm forming the terminal web (TW) (×20,000).



XRI group

of melatonin can minimize the morphological features of cell damage associated with radiation enteritis''. To test our hypothesis and to fill this existing gap in literature, we carried out this investigation. To accomplish our goals, we established an animal model consisting of non-irradiated-animals, X-ray irradiated-animals and X-ray irradiated-animals, pretreated with melatonin. Our study clearly demonstrates that X-ray irradiation of the small intestine is associated with significant ultrastructural changes indicative of cell damage. It also indicates that the administration of melatonin is able to minimize these changes. Further kinetic studies will certainly provide more insights into the observations revealed by this study.

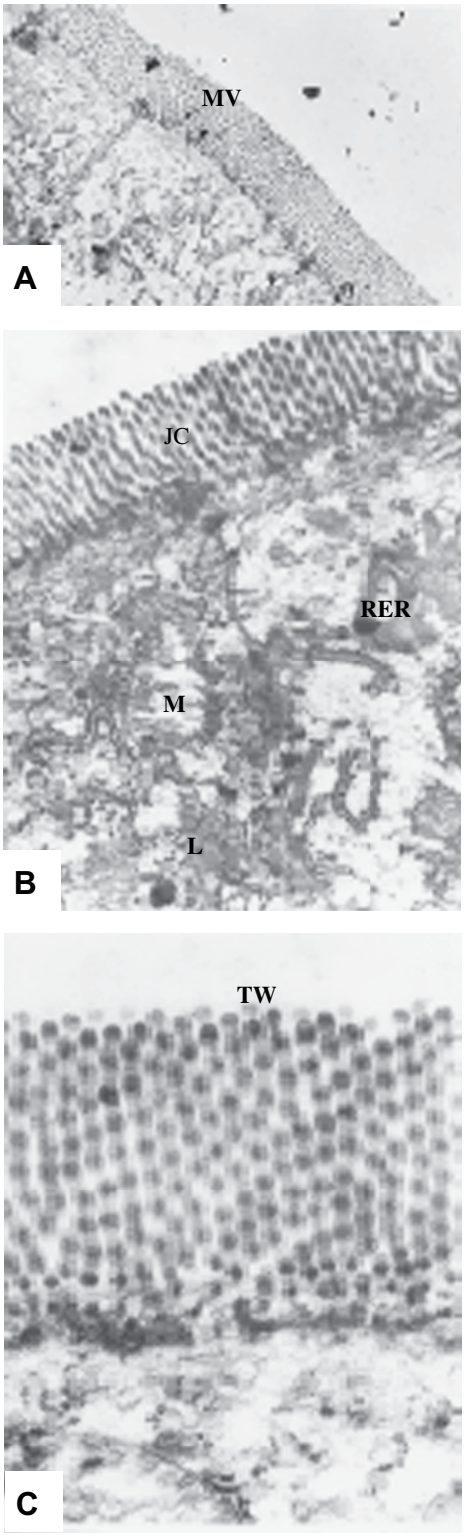
4.1. X-ray irradiation of the small intestine was associated with a marked cellular damage

In X-ray irradiated-intestine, the presence of morphological features of cellular damage (cellular degeneration and necrosis) agrees with previous studies (Becciolini et al., 1997; Erbil et al., 1998; Hwang et al., 2003). Hwang et al examined the effects of irradiation on the intestine in rats. Animals subjected to a single dose of 1100 cGy irradiation directed to the abdomen developed severe diarrhea on Day 4 post-irradiation. On histological examination, radiation caused serious damage to the various segments of the intestine (Hwang et al., 2003).

Erbil and his colleagues examined the intestinal mucosal structure in Wistar-albino rats after irradiation of the abdomen and found disruption of the mucosal integrity with marked changes in the villous height and numbers as opposed to non-irradiated-animals (Erbil et al., 1998).

Irradiation with 3 Gy produced a 2–3-fold increase within 36 h in jejunal goblet cells relative to the controls, followed by a reduction to very low levels (Becciolini et al., 1997). The ultrastructural features reported in our investigation concur with previous reports in the skin and testes (Hussein et al., 2005, 2006a, b; Oguchi et al., 1978). Oguchi et al. examined the fine structural changes in the keratinocytes following X-ray irradiation (6000 r over 28 days) in normal skin area around a lesion of Bowen's disease. They reported several findings indicative of cell damage including a decreased number of desmosomes and microvilli, formation of cytoplasmic vacuoles with or without membranes, perinuclear

Fig. 5. (A–C) Ultrastructural characteristics of X-ray irradiated-intestine featuring: (A) upper part of two adjacent enterocytes, luminal surface of the cells showing loss of regular alignment of the microvilli (MV) and desquamation of the villi (arrow), absence of terminal web, abnormal vacuolation in the apical part of the cell (arrowhead), abnormal junctional complex between cells with lost continuity of the upper border of cell membrane, small sized nucleus with deep indentation and irregular outline (N), dilated rough endoplasmic reticulum and small sized mitochondria with destructed cristae (M) (×5000); (B) apical part of an enterocyte having multiple empty areas (arrow), small sized mitochondria with destructed cristae (M), dilated rough endoplasmic reticulum (RER) and some membranous vesicles (V) (×10,000); and (C) damaged, sparse, short irregularly arranged microvilli (MV), thick abnormal terminal web (TW), Some microvilli appeared swollen without glycocalyx and separated from the surface (×20,000).



XRI+ Melatonin group

Fig. 6. (A–C) Ultrastructural features of X-ray irradiated-intestine from animals treated with melatonin featuring: (A) upper part of three adjacent enterocytes, regular continuation of the luminal surface of the cells with normal and regular microvilli (×5000); (B) upper part of two adjacent enterocytes with normally appearing junctional complex (JC), a relatively vacuolated cytoplasm containing mitochondria (M), rough endoplasmic reticulum (RER) and lysosomes (L) (×10,000); and (C) numerous long microvilli with beaded-shaped appearance, and preserved terminal web (TW) (×20,000).

aggregation of tonofibrils, intracytoplasmic desmosomes and gap junctions. Besides these, cytoplasmic occurrence of dense bodies, lipid droplet and glycogen particles, changes in mitochondria, endoplasmic reticulum and Golgi complex, and deep invagination of the nuclear membrane were also observed (Oguchi et al., 1978). In our investigation, the marked destruction of the intestinal epithelial cells following X-ray irradiation may be due to their rapid proliferation and therefore enhanced intake of more radiants. These destructive effects of X-ray irradiation may be due to the induction of several oxidative stress mechanisms with generation of reactive oxygen radicles (Hussein et al., 2005, 2006a, b). These molecules can induce oxidative damage to many vital cellular molecules and structures including DNA, lipids, proteins and biological membranes. Also, X-ray irradiation can cause inflammatory response (enteritis) in the small intestine with recruitment of activated inflammatory cells (Uchida et al., 1989; Vorbrodt et al., 1972). These immune cells synthesize and release several different cytokines, inflammatory mediators and reactive oxygen metabolites (Agrawal et al., 2001). Uchida et al. examined the in vitro effect of X-ray irradiation on the human natural killer cells. When K562 cells were irradiated with X-rays and cultured for 18 h, they showed increased sensitivity to lysis by blood lymphocytes and purified large granular lymphocytes. Irradiation with X-rays of natural killer cells at 5–15 Gy resulted in a transient increase in natural killer cells activity at 1 h, and then the activity declined and was completely lost after 24 h. However, when large granular lymphocytes were cultured with interferon immediately after irradiation, they maintained elevated natural killer cells activity (Uchida et al., 1989). It is still possible that X-ray irradiation-induced cellular damage may be due to rapid modification of intestinal motility and to the structural alteration of the intestinal mucosa (cell loss and altered crypt integrity) (Somosy et al., 2002). Several types of irradiations (X-ray, neutron, cobalt gamma) can induce a series of events in the rapidly renewing tissue of the small intestine resulting in the well-known symptoms of the gastrointestinal radiation syndrome, such as gastrointestinal hemorrhage, endotoxemia, bacterial infection, anorexia, nausea, vomiting, diarrhea, and loss of electrolytes and fluids. Growing evidence suggests that radiation-induced dysfunctions and structural changes of the intestine (changes in subcellular, cellular and histological structure) are mediated by alterations of various extracellular mediators and their intracellular messengers (Giris et al., 2006; Somosy et al., 2002).

4.2. Administration of melatonin was able to minimize X-ray induced cellular damage

In our series, the administration of melatonin was associated with amelioration of X-ray induced cellular damage. These observations concur with recent findings in the skin and testis (Hussein et al., 2005, 2006a, b; Kim et al., 2001). Observations support the notion that melatonin has a radioprotective effects against X-ray irradiation. Kim et al. investigated the effects of X-ray irradiation and melatonin on the

cytotoxicity and lipid peroxidation in the cultured skin fibroblast. An 8 Gy dose of X-ray irradiation resulted in cell death in 63% of irradiated fibroblasts (up to 37% of cells were survived) and this cellular damage was associated with lipid peroxidation of the cell membrane. In contrast, pre-incubation with melatonin was associated with significant preventive effects and an increase in the absolute numbers of the surviving cells (up to 68% of cells were survived), and decrease in lipid peroxidation of the cell membrane. DNA flow-cytometry analysis revealed that X-ray irradiation increased pre-G1 apoptotic population by 7.6% compared to a low level (4.5%) in the presence of the pre-incubation with melatonin (Kim et al., 2001). Several observations support this notion. Melatonin administration prior to irradiation prevented radiation damage in the peripheral blood cells (Hussein et al., 2005, 2006a, b). X-ray irradiation of rats with 6 and 8 Gy was associated with increased levels of malondialdehyde, myeloperoxidase, nitric oxide and decreased glutathione levels. All these indices were reduced after the pretreatment with melatonin. Therefore, melatonin by its free radical scavenging and antioxidant properties, seems to ameliorate irradiation-induced cell damage (Hussein et al., 2005, 2006a, b). We propose that melatonin can ameliorate cell damage by its ability to easily enter not only cells but also their subcellular compartments; a feature not shared by most antioxidants (Reiter et al., 2001). Melatonin can specifically enter the nucleus where it protects DNA from oxidative damage. Also it can improve cellular communication between normal and proliferating cells and alter the intracellular redox state (Reiter et al., 2001). Moreover, melatonin can induce alterations in the membrane fluidity and lipid peroxidation in the microsomal membranes (Hussein et al., 2005, 2006a, b; Kim et al., 2001).

To conclude, our understanding of the acute ionizing radiation exposure damage to the intestine is of great significance, as is management of radiation enteritis. Here we report the histological and ultrastructural changes following the administration of melatonin in X-ray irradiated-intestine. The presence of morphological changes indicative of cell damage following X-ray irradiation supports the detrimental effects of these rays. Our data strongly suggest the ability of melatonin to protect the mucosal integrity of the intestine following X-ray irradiation. These radioprotective effects of melatonin manifested on the histological level as preservation of architecture of the villi and cellular associations of the epithelial cells. Ultrastructurally, these effects manifested as preservation of the nuclear and cytoplasmic features of the epithelial cells. The underlying mechanisms and the possible clinical applications of these results are open for further investigations.

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