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Melatonin modulates acute testicular damage induced by carbon ions in mice

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The present study was performed to obtain evidence of the radioprotective function of melatonin at different administration levels on carbon ion-induced mouse testicular damage. Outbred Kun-Ming strain mice were divided into six groups, each composed of eight animals: control group, melatonin alone group, irradiation group and three melatonin plus irradiation-treated groups. An acute study was carried out to determine alterations in DNA-single strand break, cell apoptosis, and oxidative stress parameters as well as histopathology in mouse testis 24 h after whole-body irradiation with a single dose of 4 Gy. The results showed that pre-treatment and post-treatment with high-dose melatonin (10 mg/kg) both significantly alleviated carbon ion-induced acute testicular damage, a greater radioprotective effect being observed in the pre-treatment group. On the other hand, low-dose melatonin (1 mg/kg) had a limited radioprotective effect on irradiation-induced degeneration and DNA lesions in mouse testis. Taken together, the data suggest that prophylactic treatment with a higher dose of melatonin is probably advisable to protect against the effects of heavy-ion irradiation.

1. Introduction

Human exposure to ionizing radiation, in particular heavy ions such as carbon and iron ions, is acknowledged to be a potential showstopper for long duration manned interplanetary missions (Durante et al. 2005; Blakely and Chang 2007a, b; Ballarini et al. 2007). Compared with low-linear energy transfer (low-LET) ionizing irradiations such as x-rays or gamma (γ)-rays, the track of a heavy ion is complex: energy is not only deposited by the primary interaction but also by secondary electrons which may travel considerable distances from the core (Zhang et al. 2006). These high-linear energy transfer (high-LET) heavy ions produce more irreparable DNA breaks (Ritter et al. 1977) and chromosomal aberrations (Sekine et al. 2008), and so are more cytotoxic and genotoxic to irradiated cells (Pathak et al. 2007). The testis is one of the most radiosensitive organs of the body. Our previous findings showed that heavy ions can lead to prominent morphological damage (Zhang et al. 2005), and destroy poly(ADP-ribose) polymerase (PARP) activity and its expression linked with DNA repair (Zhang et al. 2008), and increase spermatocyte chromosomal aberrations (Zhang et al. 1998) in mouse testis. With the widening of human behavior in relation to space missions and hadrontherapy, crews of manned space missions and radiotherapy patients of child-bearing age are concerned about the risk to the offspring. Thus, there is a demand for reliable estimates of the protection of the testis against heavy ion radiation.

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous neurohormone predominantly synthesized in and secreted by the pineal gland which participates in several important physiological processes (Shirazi et al. 2007). It is also found to have effects in immune reactions and be the most potent physiological free radical scavenger via donating electrons to several reactive oxygen and nitrogen species (Sener et al. 2003). Moreover, melatonin has been demonstrated to ameliorate the oxidative injuries caused by X-and γ-ray irradiation (Rim et al. 2001; Ündeğer et al. 2004; EI-Missiry et al. 2007; Leaden et al. 2007). However, compared to the well documented data on low-LET irradiation, few studies have been reported on melatonin-mediated protection against high-LET irradiation. Hence, based on ground experiments at accelerators, which is the main source of knowledge of space radiation health effects, the present study has been undertaken to investigate and compare the radioprotective effects of melatonin with different administration modes including low-dose or high-dose treatment as well as pre-treatment or post-treatment, against carbon-ion irradiation.

2. Investigations and results

2.1. Effects of melatonin on lipid peroxidation and antioxidant status induced by carbon-ion irradiation

Markers of testicular tissue lipid peroxidation (as indicated by the level of MDA) and antioxidant status of all the groups are given in the Table. Carbon-ion irradiation resulted in a significant increase in MDA level ($P < 0.001$), and also induced a marked decrease of both GSH ($P < 0.001$) and TAC ($P < 0.001$) content in comparison to the control group, indicating that

Table: MDA levels, GSH contents and TAC of mouse testis in various treatment groups

	Treatment group					
	Control	Mel	IR	1 mg/kgMel pretreatment +IR	10 mg/kgMel pretreatment +IR	10 mg/kgMel posttreatment +IR
MDA	2.927 ± 0.620	2.731 ± 0.389	$8.081 \pm 0.996^{***}$	$6.085 \pm 0.792^{***,++}$	$3.511 \pm 0.44^{+++}$	$4.214 \pm 0.490^{*,+++}$
GSH	4.256 ± 0.303	4.628 ± 0.339	$1.060 \pm 0.229^{***}$	$1.813 \pm 0.145^{***,++}$	$3.575 \pm 0.256^{**,+++}$	$3.118 \pm 0.295^{***,+++}$
TAC	5.114 ± 0.092	5.131 ± 0.150	$2.920 \pm 0.241^{***}$	$3.874 \pm 0.337^{***,++}$	$4.840 \pm 0.406^{+++}$	$4.193 \pm 0.449^{**,+++}$

Note: All values expressed as mean ± S.E.M of 8 animals. $^{*}P$ <0.05, $^{**}P$ <0.01 and $^{***}P$ <0.001 vs. control groups for the other treatment groups, ^{++}P <0.01and ^{+++}P <0.001 vs. irradiation group for melatonin + irradiation-treated groups. MDA level, GSH content and TAC measured as nmol/mg protein, μmol/mg protein and U/mg protein, respectively. C: Control group, Mel: melatonin alone group, IR: irradiation group

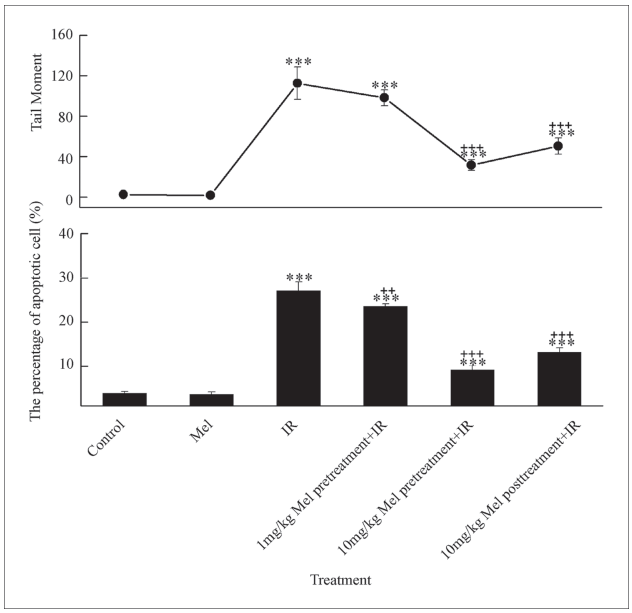

Fig. 1: Effect of melatonin on DNA damage and cell apoptosis induced by carbon ions in the testes. Mice treated with melatonin with different modes before/after whole body exposure to carbon ions, and killed 24 h after irradiation. DNA damage assayed by tail moment and cell apoptosis assayed by percentage of apoptotic cells (%). $^{**}P$ <0.01, $^{***}P$ <0.001 vs. control group for the other treated groups, ^{++}P <0.01, ^{+++}P <0.001 vs. the irradiation group for the melatonin + irradiation-treated groups. C: Control group, Mel: melatonin only-treated group, IR: irradiation group

imbalance between pro-oxidants and antioxidant leads to the overproduction of reactive oxygen species. However, administration of melatonin to irradiated mice significantly ($P < 0.001$) depressed the carbon-ion-induced rise in MDA level associated with elevated antioxidant status including GSH ($P < 0.001$) and TAC ($P < 0.001$) content in the all melatonin (10 mg/kg) plus irradiation groups. On the other hand, low-dose melatonin (1 mg/kg) also decreased MDA ($P < 0.01$), and raised GSH ($P < 0.01$) and TAC ($P < 0.01$) compared to the irradiated group. Meanwhile, melatonin treatment only did not observably affect any of the markers studied in relation to the control group.

2.2. Effects of melatonin on DNA damage and cell apoptosis produced by carbon-ion irradiation

Fig. 1 shows that, apart from the low-dose (1 mg/kg) melatonin treated group, melatonin plus carbon-ion beam treatment provided a marked reduction ($P < 0.001$) in the elevation of DNA lesions (as indicated by the DNA-SSB) induced by carbon-ion irradiation when compared to the irradiated group, in particular pretreatment with melatonin at 10 mg/kg. The effects of melatonin on carbon-ion irradiation-induced cell apoptosis are also presented in Fig. 1. The percentages of apoptotic cells in the low- and high- dose melatonin treated groups were reduced to 23.6% and 9.22% relative to the irradiation group, respectively, indicating that pretreatment with melatonin at low or high dose is able to mitigate this cell apoptosis. Posttreatment with melatonin also decreased the percentage of apoptotic cells ($P < 0.001$) in mouse testis compared with the irradiation group. Moreover, there were no adverse effects on testicular DNA-SSB and cell apoptosis in the melatonin alone-treated group versus the control group.

2.3. Histopathological findings

The photomicrographs in Fig. 2 illustrate the various histopathological changes in the testes when animals were given the different treatments used in this work. Compared to the control group, carbon-ion irradiation led to the decrease of tubule diameter, the formation of interstitial edema and coagulative necrosis of spermatozoa, as well as the reduction or disappearance of germ, Leydig or Sertoil cells and tubular degeneration. However, the administration of melatonin to irradiated mice improved nearly all the irradiation-induced severe histopathological lesions in the structure of the testis except that treatment with low-dose melatonin showed only a slight benefit. Furthermore, compared with the control group, melatonin only treatment did not result in changes to the structure of the testis.

3. Discussion

The deleterious effects of ionizing radiation in biological system are attributed to two different mechanisms. Its direct action is to break down sensitive molecules in the cells, while indirectly radiation interacts forming highly reactive free radicals, in particular the hydroxyl radical ($^{\bullet}OH$). $^{\bullet}OH$ is the most damaging free radical, and attacks all biological molecules at very fast rate exceeding $10^9\ M^{-1}\ s^{-1}$ (Yilmaz et al. 1998). Among them, high LET irradiation like carbon or iron ions has a greater effect in inducting detrimental effects such as cell killing (Pathak et al. 2007), mutation (Ritter et al. 1977), transformation and chromosome damage (Sekine et al. 2008). The testis is known to be particularly susceptible to irradiation. The results obtained in the present study together with our previous findings show that carbon ions can result in marked histopathological changes, DNA strand breaks, cell apoptosis, lipid peroxidation, imbalance of antioxidant status and chromosome aberrations as well as the inactivation of DNA repair enzymes (PARP-1) (Zhang et al. 2008) in mouse testis. In addition, the findings of Wang et al. also elucidated that carbon ion beams induced significant detrimental effects on prenatal gonads, postnatal testicular development and breeding activity in male offspring when the abdomens of pregnant rats are irradiated with accelerated carbon ions on gestation day 15.

Melatonin is well known for its functional versatility (Shirazi et al. 2007). Due to its small size and high lipophilicity, melatonin can cross biological membranes easily and reaches all compartments of the cell. It has been also found to have effects in immune reactions. Moreover, melatonin and its direct

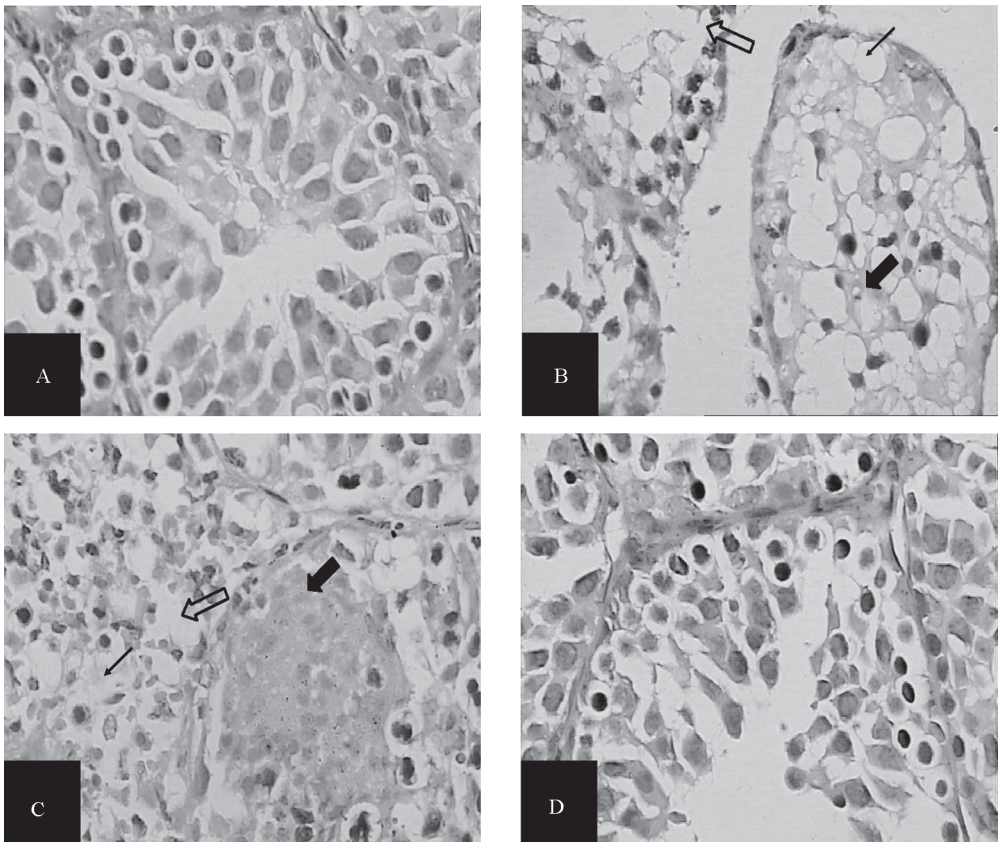

Fig. 2: Typical photomicrographs (original magnification: 400×) of seminiferous tubules of mouse testes. (A) Control group showing normal morphological features. (B) IR group showing serious destruction and deterioration of tubules in testis. (C) Administration of melatonin (1 mg/kg) before exposure to carbon ions showing slight recovery effect of melatonin on seminiferous tubules of damaged testis. (D) Administration of melatonin (10 mg/kg) before exposure to carbon ions showing complete recovery effect of melatonin on seminiferous tubules of damaged testis. Necrosis (black block arrows); tubular degeneration (white block arrows); interstitial edema (line arrows)

metabolite N^1-acetyl-N^2-formyl-5-methoxykynuramine scavenge $^{\bullet}OH$ with a very high rate constant, roughly in the order of $2.7 \times 10^{10}\,M^{-1}\,s^{-1}$ (Matuszak et al. 1997; Sliwinski et al. 2007), and about 60–70% of cellular damage produced by low-LET ionizing irradiation is thought to be caused by $^{\bullet}OH$ formed from water radiolysis (Ward et al. 1998). Both *in vivo* and *in vitro* studies have widely verified that melatonin gives effective radioprotection against X- and γ-ray irradiation. In comparison with low-LET ionizing irradiation, the effect of heavy-ion irradiation is thought to involve more direct damage to biological targets rather than indirectly generating reactive free radicals. To the best of our knowledge, Zhou et al. (2006) were the first to report radioprotection by melatonin against high-LET-radiation induced damage in V79 Chinese hamster cells *in vitro* and to put forward the idea that melatonin may bind nuclear receptors (RZR/ROR) to lessen the direct reaction of particles with DNA molecules. In our present study, the data show that various melatonin treatments (high-dose, pre-and post-treatment) of carbon-ion irradiated mice also ameliorated the harmful injury induced by irradiation in mouse testes to different degrees indicated by restoring the morphological features of seminiferous tubules, and reducing DNA-SSB, cell apoptosis and MDA level, together with increasing GSH and TAC content. Furthermore, melatonin-mediated radioprotection was more pronounced in the group with melatonin pretreatment at high dose. On the other hand, although melatonin at 1 mg/kg significantly reduced MDA level and enhanced GSH and TAC content of mouse testis, it is noteworthy that low-dose (1 mg/kg) melatonin supplement had limited effects to protect against testicular damage from carbon-ion irradiation. For example, compared with the irradiated group, the structure of the testis still retained interstitial edema, tubular degeneration and coagulative necrosis of spermatozoa, and neither was the degree of DNA-SSB significantly inhibited. Further experiments are required to disclose the underlying mechanisms. This also agrees with the observations of Badr et al. (1999) and Kim et al. (2000) who found that melatonin administration after exposure to γ-irradiation and low dose (0.1 M) respectively, did not confer protection against damage in mice. Hence, we consider that the optimum mode of melatonin administration plays a crucial role in melatonin-mediated radioprotection.

In conclusion, whole-body irradiation of mice with high-LET carbon ions significantly increased DNA single-strand breaks, cell apoptosis, lipid peroxidation and the imbalance of antioxidant status as well as causing histopathological changes in testes. However, pretreatment and posttreatment with melatonin at 10 mg/kg both markedly prevented harmful injury induced by carbon-ion irradiation, pretretment being more effective. In addition, administration of low-dose (1 mg/kg) melatonin has little effect to protect against testicular damage due to carbon-ion irradiation. Hence, the data obtained by our study suggest that prophylactic supplementation with melatonin is probably advisable to protect against heavy-ion irradiation injury.

4. Experimental

4.1. Animals

Male outbred Kun-Ming strain mice (20 ± 2 g) obtained from Lanzhou Medical College (Lanzhou, China) were used. All animal studies were performed according to the requirements of the Animal Care Committee at the Institute. Mice were kept at a constant temperature ($22 \pm 1\,^{\circ}C$) with 12-h light and dark cycles.

4.2. Irradiation procedure

A mouse was positioned in a chamber fixed to the irradiation equipment at the Heavy Ion Research Facility in Lanzhou (HIRFL, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, China). The whole

body of the mouse was irradiated with carbon ion beams at an energy of 80.55 MeV u^{-1} and LET 33.3 keV μm^{-1} in the water (the plateau region) and 137.9 keV μm^{-1} the water (the Bragg peak region and the region close to the Bragg peak) generated from the HIRFL, with a dose of 0.5 Gy min^{-1}. The collimation of the beams to the irradiated site was controlled by a microcomputer. Data acquisition (preset numbers converted by doses of irradiation) was performed automatically using a microcomputer during irradiation. Particle fluence was determined from air ionizing chamber signal according to the calibration of the detector (Type: PTW-UNIDOS, PTW-FREIBURG Co., Germany). Dose was calculated from particle fluence and LET.

4.3. Sample collection

A total of 48 animals were randomly divided into six groups each containing 8 individuals. The first group were injected intraperitoneally (i.p.) with physiological saline solution (0.85% NaCl) 1 h prior to whole-body carbon-ion irradiation as a control group (data on mice injected with physiological saline solution 1 h after irradiation are not shown as there was no significant difference between the two control groups). The second group received only melatonin (10 mg/kg) 1 h before exposure to carbon ions as the melatonin-treated group to avoid adverse effects (Sakano et al. 2004, data concerning melatonin treatment after irradiation are not shown because there was no significant difference between the two melatonin-treated groups). The third group of animals just received carbon-ion irradiation as the irradiated group. The fourth group was administer melatonin at 1 mg/kg (Sigma, USA; dissolved in alcoholic saline) 1 h before irradiation. The fifth group was pretreated with melatonin at 10 mg/kg 1 h prior to carbon-ion irradiation. The last group was irradiated with carbon ions, and 1 h later was treated with melatonin (10 mg/kg). After this, the animals were sacrificed by cervical dislocation 24 h after irradiation. Testes were quickly removed, and then fresh samples were used for analysis of DNA damage and histopathological analysis, and residual samples were immediately frozen and stored at −80 °C until other biochemical determinations were carried out.

4.4. Assays for enzyme activities

The samples were minced and homogenized in ice-cold physiological saline solution. The supernatant fluid was obtained by centrifugation at 10 000 g for 30 min at 4 °C. The level of malondialdehyde (MDA) was assayed as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa et al. (1979) The glutathione (GSH) content and total antioxidant capability (TAC) were determined by diagnostic reagent kit (Nanjing Jiancheng Bioengineering, China) according to the method specified. Protein content was determined by the method of Bradford, using bovine serum albumin (Sigma, USA) as standard.

4.5. Determination of cell apoptosis

Flow cytometric analysis of cellular DNA was performed using prepared cell suspensions. In brief, 70% ethanol-fixed cells were stored overnight at 4 °C before analysis, then washed once with ice-cold PBS treated with RNAse (1 h at 37 °C, 500 U ml^{-1}), stained with propidium iodide (50 $\mu g\ ml^{-1}$) and analyzed on a flow cytometer (USA). The percentage of apoptotic cells relative to the total cells in the lung was calculated according to FlowJo 7.1 (Tree Star, Inc., San Carlos, CA).

4.6. Alkaline comet assay

The standard procedure was used as originally described by Singh et al with modifications (Hartman et al. 2004). Briefly, the cell suspension was mixed with 0.6% low melting point agarose (kept in a 37 °C water bath). Thereafter this suspension was rapidly removed to the first agarose that (80 µl) normal melting point agarose was spread on each fully frosted microscope slide, covered with a 24 × 24 mm coverslip, the coverslip gently removed, and the slide submerged in lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH10, 1% sodium lauroyl sarcosine, 10% dimethl sulfoxid, 1% Triton X-100 added just before use, 4 °C) for least 1 h. Then the slides were placed in the electrophoresis solution (300 mM NaOH, 1 mM EDTA, pH > 13, 4 °C) for 20 min to allow DNA unwinding and electrophoresis was conducted for 20 min at 25 V and 300 mA. After electrophoresis, the slides were drained and neutralized for 3 × 5 min with neutralization buffer (0.4 M Tris-HCl, pH 7.5) and stained with ethidium-bromide (5 µg/ml). The slides were covered with a coverslip and analyzed using a fluorescence microscope (Nikon). Images of 50 randomly selected nuclei per experimental point were analyzed using Casp1.2.2 software (Institute of Theoretical Physics, University of Wroclaw, Wroclaw, Poland). Tail moment, defined as the product of the percentage of DNA in the tail distribution and the displacement between the head and the tail means (Olive et al. 1990), was used to measure the level of DNA damage.

4.7. Testicular histopathology

After sacrificing the animals by cervical dislocation, the left testis was extracted, and immediately fixed in 10% buffered formalin solution. Testis tissue samples were then embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin (H&E), and examined with an optical microscope (Olympus).

4.8. Statistical analysis

Each experiment was performed at least three times. The results were expressed as means ± standard errors (S.E.M). Multiple comparisons were performed using one-way ANOVA followed by LSD as a post-hoc test. Statistical differences between the two groups were analyzed by Student's t-test. A p-value less than 0.05 was selected as the criterion for a statistically significant difference. Correlation analyses were performed using Spass 11.5 for Windows.

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References

Ballarini F, Alloni D, Facoetti A, Mairani A, Nano R, Ottolenghi A (2007) Radiation risk estimation: Modelling approaches for "targeted" and "non-targeted" effects. Adv Space Res 40: 1392–1400.

Badr FM, EI Habit OH, Harraz MM (1999) Radioprotective effect of melatonin assessed by measuring chromosomal in mitotic and meitotic cells. Mutat Res 444: 367–372.

Blakely EA, Chang PY (2007a) A review of ground-based heavy-ion radiobiology relevant to space radiation risk assessment: cataracts and CNS. Adv Space Res 40: 1307–1319.

Blakely EA, Chang PY (2007b) A review of ground-based heavy-ion radiobiology relevant to space radiation risk assessment. Part II: cardiovascular and immunological effects. Adv Space Res 40: 461–469.

Durante M, Kronenberg A (2005) Ground-based research with heavy ions for space radiation protection. Adv Space Res 35: 180–184.

EI-Missiry MA, Fayed TA, EI-Sawy MR, EI-Sayed AA (2007) Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. Ecotoxicol Environ Saf 66: 278–286.

Hartmann A, Schumacher M, Plappert-Helbig U, Lowe P, Suter W, Mueller L (2004) Use of the alkaline in vivo comet assay for mechanistic genotoxicity investigations. Mutagenesis 19: 51–59.

Kim JK, Lee CJ (2000) Effect of exogenous melatonin on the ovarian follicles in γ–irradiated mouse. Mutat Res 449: 33–39.

Leaden PJ, Catala A (2007) Melatonin and N-acetyl serotonin inhibit selectively enzymatic and non-enzymatic lipid peroxidation of rat liver microsomes. Prostaglandins Leukot Essent Fatty Acids 77: 29–35.

Matuszak Z, Reszka KJ, Chignell CF (1997) Reaction of melatonin and related indoles with hydroxyl radicals: EPR and spin trapping investigations. Free Radic Biol Med 23: 367–372.

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358.

Olive PL, Banath JP, Durand RE (1990) Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet assay". Radiat Res122: 84–90.

Pathak P, Dey SK, Sarma A, Khuda-Bukhsh AR (2007) Cell killing, nuclear damage and apoptosis in Chinese hamster V79 cells after irradiation with heavy-ion beams of ^{16}O, ^{12}C and ^{7}Li. Mutat Res 632: 58–68.

Rim BC, Sham BS, Ryoo YW, Kim SP, Lee KS (2001) Melatonin reduces X-ray irradiation-induced oxidative damages in cultured human skin fibroblasts. J Dermatol Sci 26: 194–200.

Ritter MA, Cleaver JE, Tobias CA (1997) High-LET radiations induce a large proportion of non-rejoining DNA breaks. Nature 266: 653–655.

Sakano K, Oikawa S, Hiraku Y, Kawanishi S (2004) Oxidative DNA damage induced by a melatonin metabolite, 6-hydroxymelatonin, via a unique non-o-quinone type of redox cycle. Biochem Pharmacol 68: 1869–1878.

Sekine E, Okada M, Matsufuji N, Yu D, Furusawa Y, Okayasu R (2008) High LET heavy ion radiation induces lower numbers of initial chromosome breaks with minimal repair than low LET radiation in normal human cells. Mutat Res 652: 95–101.

Sener G, Jahovic N, Tosun O, Atasoy BM, Yegen BC (2003) Melatonin ameliorates ionizing radiation-induced oxidative organ damage in rats. Life Sci 74: 563–572.

Shirazi A, Ghobadi G, Ghazi-Khansari M (2007) A radiobiological review on melatonin: a novel radioprotector. J Radiat Res 48: 263–272.

Singh NP, McCoy MT, Tice RR, Schneider EL (1998) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175: 184–191.

Sliwinski T, Rozej W, Morawiec-Bajda A, Morawiec I, Reiter R, Blasiak J (2007) Protective action of melatonin against oxidative DNA-damage-Chemical inactivation versus base-excision repair. Mutat Res 634: 220–227.

Ündeğer Ü, Giray B, Zorlu AF, Öge K, Başaran N (2004) Protective effects of melatonin on the ionizing radiation induced DNA damage in the rat brain. Exp Toxic Pathol 55: 379–384.

Wang B, Murakami M, Eguchi-Kasai K, Nojima K, Shang Y, Tanaka K, Watanabe K, Fujita K, Moreno SG, Coffigny H, Hayata Z (2007) Effects of prenatal irradiation with an accelerated heavy-ion beam on postnatal development in rats. Adv Space Res 40: 550–562.

Ward JF (1998) DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acid Res Mol Biol 35: 95–125.

Yilmaz S, Yilmaz E (2006) Effect of melatonin and vitamin E on oxidative-antioxidative status in rats exposed to irradiation. Toxicology 222: 1–7.

Zhang H, Zheng RL, Wei ZQ, Li WJ, Gao QX, Chen WQ, Wang ZH, He J, Liang JP, Han GW, Huang T, Li Q, Xie HM (1998) Effects of pre-exposure of mouse testis with low-dose $^{16}O^{8+}$ ions or ^{60}Co gamma-rays on sperm shape abnormalities, lipid peroxidation and superoxide dismutase (SOD) activity induced by subsequent high-dose irradiation. Int J Radiat Biol 73: 163–167.

Zhang H, Zheng RL, Wang RY, Wei ZQ, Li WJ, Gao QX, Chen WQ, Wang ZX, Han GW, Liang JP (1998) Chromosomal aberrations induced by $^{12}C^{6+}$ heavy ion irradiation in spermatogonia and spermatocytes of mice. Mutat Res 398: 27–31.

Zhang H, Li WJ, Zheng RL (2005) Effects of low-dose heavy ion irradiation on male germ cell adaptation and genetics. Nucl Sci Technol 16: 209–213.

Zhang H, Zhang X, Yuan ZG, Li XD, Li WJ, Zhou QM, Min FL, Xie Y, Liu B, Duan X (2006) Germ cell loss induced by$^{12}C^{6+}$ irradiation in young female mice. J Radiat Res 47: 131–134.

Zhang H, Zhao WP, Wan YL, Li N, Wu ZH, Liu Y, Chen JD, Cai YJ (2008) Induction of cytogenetic adaptive response in spermatogonia and spermatocytes by pre-exposure of mouse testis to low-dose $^{12}C^{6+}$ ions. Mutat Res 653: 109–112.

Zhou G, Kawata T, Furusawa Y, Aoki M, Hirayama R, Ando K, Ito H (2006) Protective effects of melatonin against low-and high-LET irradiation. Radiat Res 47: 175–181.