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Melatonin Protection against Ionizing Radiation in Space

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Abstract

Aim: During space travel, astronauts are exposed to high-LET galactic cosmic rays at higher doses than humans experience on Earth. This is one of the recognized *obstacles* for long-duration, manned interplanetary missions. Estimation of the track structure of high-LET particles indicates that these particles could give rise to detrimental biological damage at the cellular level. Melatonin mitigates oxidative damage induced by ionizing radiation. Only a small number of *investigations* have been reported on melatonin-mediated protection against high-LET irradiation. A comprehensive understanding of the mechanisms and molecular pathways associated with protection against high-LET irradiation is essential in developing novel countermeasures for interplanetary travel by crewed spacecraft.

A systematic review of the existing literature was conducted using the following search terms: 'melatonin', 'space radiation', 'charged particle irradiation', 'free radicals', 'oxidative stress' and 'antioxidant'. The search used PubMed and spanned the period from January 2000 to December 2018.

The collected data included 'Melatonin mitigates oxidative damage and apoptosis in mouse cerebellum induced by high-LET ^{56}Fe particle irradiation', 'Exogenous melatonin modulates apoptosis in the mouse brain induced by high-LET carbon ion irradiation', 'Protective effects of melatonin against high-LET radiation', 'Melatonin protects human cells from clustered DNA damages, killing and acquisition of soft agar growth induced by 970 MeV/n Fe ions', 'Space radiation-induced inhibition of neurogenesis in the hippocampal dentate gyrus and memory impairment in mice: ameliorative potential of the melatonin metabolite, AFMK', 'Melatonin modulates acute testicular damage induced by carbon ion irradiation in mice' and 'Ameliorating mitochondrial dysfunction restores carbon ion-induced cognitive deficits through co-activation of NRF2 and PINK1 signaling pathway'.

Conclusion: Pre-treatment with melatonin **successfully** inhibited the oxidative damage induced by high energy charged particle irradiation. The above-mentioned results provide a prospective application for protective strategies with respect to the space radiation hazards. Further investigation to elucidate the seriousness and outcomes of organ damage associated with space radiation is warranted.

Keywords: Melatonin; Space radiation; Charged particle irradiation; Free radicals; Oxidative stress; Antioxidant

Introduction

Outer space beyond low Earth orbit (LEO) contains various types of ionizing radiation [1,2]. In space travel, astronauts are exposed to galactic cosmic rays (GCR) consisting of high-energy protons and high charge (Z) and energy (E) (HZE) nuclei and solar particle events (SPE) *containing numerous* low to medium energy protons [1]. GCR nuclei are high-LET particles, holding abundant energies to penetrate any shielding technology employed on contemporary mission vehicles [3]. The GCR spectrum *is composed of* about 87% hydrogen ions (protons) and 12% helium ions (alpha particles), with the *residual* 1-2% of particles being HZE nuclei with charges ranging from Z=3 (lithium) to Z=28 (nickel) [4]. Ionized transition metals, such as iron (Z=26), are biologically detrimental, since no appropriate amount of spacecraft material is able to shield them [5]. The intense ionization power of GCR ions is a crucial health hazard to astronauts and *comprises* one of the essential *obstacles interfering with* plans for interplanetary travel by crewed spacecraft. GCR particle energies are adequate to pass through a number of centimetres of biological tissue or other organic and inorganic materials. Shielding only incompletely decreases the doses experienced inside a spacecraft [5].

During conveyance outside of LEO, every cell within an astronaut would be impacted, in general, by a hydrogen ion every few days and by heavier HZE nuclei (e.g., ^{16}O, ^{28}Si, ^{56}Fe) every few months [5]. Accordingly, in spite of their low flux, HZE ions *comprise* a detrimental biological hazard and provide a considerable amount of energy to the cumulative GCR dose that astronauts will experience outside of LEO [5]. Hazardous and inconstant SPEs generate *substantial amounts* of energetic protons with fluences in excess of 109 protons/cm2 [5]. *There are concerns that* high density fluxes of protons with energies greater than 30 MeV can give rise to a detrimental biological hazard to astronauts in thinly-shielded spacecraft [5].

There is a threefold increase in GCR exposure rate during the time that spacecrafts travel out of Earth orbit into deep space where protection of the Earth's magnetosphere and solid body are lost. *The radiation standard of NASA* restricts astronaut exposures to a 3% risk of exposure induced death (REID) at the upper 95% confidence interval (CI) of the risk estimate. Utilizing models of risk and uncertainties of NASA, Cucinotta and colleagues *anticipated* that central estimates for radiation induced mortality and morbidity could be more than 5% and 10% with upper 95% CI near 10% and 20%, respectively, for a Mars mission [1].

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Melatonin (N-acetyl-5-methoxytryptamine), an indole compound synthesized by the pineal gland and many other tissues [6,7], participates in numerous important physiological processes [8,9]. It exerts an influence on immune reactions [10] and is an effective physiological free radical scavenger by contributing electrons to several reactive oxygen and nitrogen species [11]. Additionally, melatonin mitigates the oxidative damage induced by radiation [12]. Only a small number of *investigations* have been reported on melatonin-mediated protection against high-LET irradiation [13].

A comprehensive understanding of the mechanisms and molecular pathways associated with protection against high-LET irradiation is essential in developing novel countermeasures for interplanetary travel by crewed spacecraft.

Literature Search Strategy

A systematic review of the existing literature was conducted using the following search terms: 'melatonin', 'space radiation', 'charged particle irradiation', 'free radicals', 'oxidative stress' and 'antioxidant'. The search used PubMed and spanned the period from January 2000 to December 2018.

Inclusion and exclusion criteria

We identified reports documenting melatonin protection against ionizing radiation in space for inclusion. Reports which were published in languages other than English, only published in abstract form, not related to ionizing radiation in space, duplicate articles and those containing insufficient detail were excluded. All titles and abstracts were screened to assess whether they were eligible for inclusion. Then abstracts and full texts of all eligible studies were examined and data was evaluated.

Results

Literature search results

The search identified 132 potentially eligible articles. After application of the exclusion criteria, only 7 met the criteria and were therefore evaluated. The collected data included 'Melatonin mitigates oxidative damage and apoptosis in mouse cerebellum induced by high-LET ^{56}Fe particle irradiation', 'Exogenous melatonin modulates apoptosis in the mouse brain induced by high-LET carbon ion irradiation', 'Protective effects of melatonin against high-LET radiation', 'Melatonin protects human cells from clustered DNA damages, killing and acquisition of soft agar growth induced by 970 MeV/n Fe ions', 'Space radiation-induced inhibition of neurogenesis in the hippocampal dentate gyrus and memory impairment in mice: ameliorative potential of the melatonin metabolite, AFMK', 'Melatonin modulates acute testicular damage induced by carbon ion irradiation in mice' and 'Ameliorating mitochondrial dysfunction restores carbon ion-induced cognitive deficits through co-activation of NRF2 and PINK1 signalling pathway'.

Melatonin mitigates oxidative damage and apoptosis in mouse cerebellum induced by high-LET ^{56}Fe particle irradiation: *Outer space* contains cosmic rays made up of ionized atomic nuclei of various elements, which include hydrogen, helium, carbon, oxygen, and iron. It is probable that the neurochemical and behavioral deficits resulting from exposure to ^{56}Fe irradiation involve damage from elevated oxidative stress and inflammation of the brain [14]. An elevated oxidative stress level was detected in the frontal cortex of rats irradiated with 1.5 Gy of ^{56}Fe particles [15]. Enhancement of antioxidative activity mitigated the impairment of learning ability in rats caused by the infusion of amyloid-β peptide into the cerebral ventricle [16]. A variety of reports demonstrate the broad spectrum antioxidant function of melatonin [17,18]. The study of Manda and colleagues revealed that melatonin provided protection against oxidative stress caused by ionizing radiation [19]. This group examined the effect of melatonin pretreatment against high energy ^{56}Fe-induced oxidative alteration of biomolecules and in addition histopathological changes in cerebellum [20].

Whole-body irradiation of mice was carried out employing high-LET ^{56}Fe beams (500 MeV/nucleon) generated at the HIMAC, NIRS (Chiba, Japan). All mice received a dose of 2 Gy at a dose rate of 0.88Gy/min. High-LET ^{56}Fe irradiation enhanced necrosis of Purkinje cells and apoptosis of granule cells. Melatonin pretreatment significantly decreased the number of necrotic Purkinje cells and apoptotic granule cells [20].

DNA migration in the comet tail, a sign of primary DNA damage, was detected in ^{56}Fe irradiated animals. Melatonin-pre-treatment in irradiated mice demonstrated a significant reduction comet tail length and the percent DNA in the comet tail. ^{56}Fe radiation-induced enhancement of 8-OhdG level, an additional sign of DNA damage, was also reduced markedly by melatonin [20].

Lipid peroxidation is a crucial step in the pathogenesis of a variety of diseases and implicated in various types of neurodegenerative disorders [21]. The products of lipid peroxidation, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), are virulent to cells [22]. ^{56}Fe radiation-induced elevation of MDA + 4-HAE and protein carbonyl content in the cerebellum of mice was also reduced significantly by melatonin pretreatment [20].

^{56}Fe radiation exposure significantly reduced the levels of NP-SH content, an indicator of the antioxidant status, in the cerebellum. Melatonin pre-treatment significantly overcame the reduction of NP-SH content after ^{56}Fe irradiation. Melatonin pre-treatment also significantly alleviated the reduction of FRAP value, an indicator of total antioxidant capacity (TAC), caused by ^{56}Fe irradiation [20].

Briefly, the high energy charged particle radiation-induced cerebellar oxidative damage was suppressed efficiently by melatonin pre-treatment. The result provides an encouraging outlook for neuroprotective strategies in the matter of the space radiation hazards.

Exogenous melatonin modulates apoptosis in the mouse brain induced by high-LET carbon ion irradiation: There is the necessity for a trustworthy *assessment* with regard to the protection of the brain against high-LET heavy-ion radiation. Apoptosis can be caused by ionizing radiation. Apoptosis is identified as an essential event in the nervous system development and disease [23]. Oxidative stress is a mediator of apoptosis by diminishing the subtle balance between intracellular oxidants and the defense systems, resulting in high levels of reactive oxygen species (ROS) [24].

Melatonin plays various protective roles through the broad spectrum antioxidant functions [17,18]. Various studies indicate that the cellular antioxidant response to ROS is modulated by the nuclear factor E2-related factor 2 (Nrf2), a member of NF-E2 family of nuclear basic leucine zipper transcription factors which is employed to abolish oxidative stressors by binding to the antioxidant responsive element (ARE) or the electrophile-responsive element (EpRE) and where they encode for antioxidant and detoxifying enzymes [25]. A study by Liu and colleagues evaluated the neuroprotective mechanism of melatonin

against carbon ion-induced cell apoptosis at the level of signal transduction pathway in mouse brain [26].

Carbon ion irradiation induced a significant elevation in protein carbonyl and MDA content 12 hours after irradiation in comparison with the control group, implying that irradiation likely caused the generation of ROS and promoted oxidative stress. The level of protein carbonyl or MDA is commonly employed as trustworthy criteria of oxidative modification of proteins and lipids, respectively. Administration of melatonin at the concentrations of 1, 5 or 10 mg/kg to irradiated mice notably inhibited the carbon ion-induced enhancement of protein carbonyl and MDA contents dose-dependently. These results demonstrated that melatonin markedly mitigates the protein and lipid oxidation induced by carbon ion irradiation [26].

Carbon ion irradiation caused a noticeable reduction in superoxide dismutase (SOD) and catalase (CAT) activities and in addition the total antioxidant capability (TAC) level in comparison with the control group. Administration of melatonin to irradiated mice notably improved the antioxidant status which included elevated SOD and CAT activities and the TAC level in the brain of mice [26].

Brain tissue of mice treated with melatonin before carbon ion irradiation demonstrated marked increase of Nrf2 protein. Nrf2 expression in irradiated mice was significantly lower than those in the groups treated with various doses of melatonin [26].

A large number of TUNEL-positive apoptotic nuclei were detected in the brain tissue obtained 12 hours after carbon ion irradiation. Several doses of melatonin pre-treatment caused a marked reduction of apoptotic brain cells induced by carbon ion irradiation. There is a marked dose-dependent reduction in carbon ion irradiation-induced apoptotic cells with increasing doses of melatonin.

The ratio of Bax to Bcl-2 is a crucial determinant of cell death/survival through apoptosis. Carbon ion irradiation induced an abrupt rise in the protein level of Bax, and a minimal variation in the Bcl-2 level. On the contrary, melatonin pre-treatment reduced the Bax/Bcl-2 ratio of irradiated mice [26].

Mice irradiated with carbon ion beams obviously reduced Rh123 staining, denoting a decline in $\Delta\Psi m$ related to mitochondrial dysfunction and subsequent cell death. Pre-treatment with melatonin markedly inhibited the radiation-induced reduction of $\Delta\Psi m$ [26].

The cytosolic cytochrome c content was enhanced noticeably 12 hours after carbon ion irradiation. Melatonin pre-treatment inhibited the elevation of the cytosolic cytochrome c content dose-dependently. Activation of caspase-3 was detected in the mouse brain 12 hours after 4 Gy of carbon ion irradiation *with* manifestation of 35-kDa product and its cleaved form of 17 kDa. Melatonin-pre-treated mice demonstrated a significantly decreased expression of the corresponding caspase-3 and its cleavage [26].

These results demonstrate that melatonin exerts advantageous neuroprotective properties against carbon ion-induced mouse brain damage through inhibiting the mitochondrial apoptotic signalling cascade. These findings present a promising outlook for melatonin as a prospective neuroprotective agent against the space radiation hazards.

Protective effects of melatonin against high-LET radiation: Exposure of cells to ionizing radiation leads to instant and extensive oxidative damage to DNA by both direct and indirect actions. Direct action brings about disruption of chemical bonds in the molecular structure of DNA whereas indirect effects arise out of highly reactive free radicals generated by reaction of ionizing radiation with surrounding water molecules; this is followed by subsequent DNA destruction caused by free radicals [27].

The study of Zhou and colleagues revealed the protective capability of melatonin against high-LET radiation-induced damage *characterized by* cell killing, *hprt* gene mutation and cell cycle blockade [27]. Both X-ray (low-LET radiation) and carbon ion irradiation (high-LET radiation) gave rise to a dose-dependent cell killing of V79 cells (Chinese hamster lung fibroblast cells) [27]. Significant increase of survival fraction was observed in cells pre-treated with melatonin (0.43 mM) before irradiation with 8 Gy of X-rays. Melatonin (0.43 mM) had no protective effect on V79 cells irradiated with $<$6.8 Gy of X-rays. The shoulder of survival curves was altered by pre-treatment with melatonin with the shoulder of the survival curve of carbon ion irradiated V79 cells being much narrower than that of X- rays. The alterations in the exponential portion were a consequence of the direct action of particles. Pre-treatment with melatonin raised the survival fraction of high-dose carbon ion-irradiated cells. This finding indicates that melatonin presumably enhances the protective action against the direct effects induced by ionizing radiation [27].

Melatonin treatment altered the shape of the *hprt* gene mutation-induction curve caused by carbon ion irradiation. V79 cells pre-treated with melatonin exhibited lower mutation frequency than those without melatonin treatment [22]. Both X-rays and carbon ion irradiation impeded V79 cell cycle progress in the G2 phase. Carbon ion beam brought about G2-blockade much more powerfully than did X-rays. The G2 blockade in melatonin-treated cells was notably lower than that without melatonin treatment in V79 cells irradiated with carbon ion beams. The *hprt* gene mutation and cell cycle blockade caused by carbon ion beams were markedly decreased by melatonin. 100 keV/mm carbon ion beams are classified as densely ionizing radiation, which are more effective in generating biological damage because the greater part of its energy is released in clusters of ionizing events, resulting in more severe local damage. Direct action is the principal process of the interaction of high-LET particles with biological materials. Melatonin exhibits no effects on the gene mutation and cell cycle blockade caused by X-rays. Notwithstanding, melatonin manifests protective effects against carbon ion radiation induced gene mutation and cell cycle blockade. These findings denote that melatonin alters the direct interaction of high-LET particles with biomolecules [27].

The results indicate that melatonin reduces the direct interaction of high-LET heavy particle radiation with cells rather than via an indirect interaction. Additional investigations are warranted to identify the underlying mechanisms.

Melatonin protects human cells from clustered DNA damages, killing and acquisition of soft agar growth induced by 970 MeV/n Fe ions: Information regarding the biological consequences caused by high atomic number, high energy (HZE) particle irradiation is essential. Estimation of the track structure of HZE particles indicates that these particles could give rise to detrimental biological damage at the cellular level. Such damage in non-replicating, poorly-repairing cells could be disastrous for the function of critical organs which include the brain [28]. Das and colleagues investigated the capability of melatonin to protect DNA in solution and in human cells against biological damage caused by low or high LET radiation (100 kVp X-rays, 970 MeV/nucleon Fe ions) [28]. Supercoiled plasmid DNA in solution was treated with increasing concentrations of melatonin (0.0-3.5 mM). These samples were irradiated with 0, 10 or 25 Gy of X-rays (100 kVp; LET, 2 keV/mm). Human cells (28 SC monocytes)

were irradiated with 100 kVp X-rays or Fe ions (970 MeV/nucleon; LET, 151.3 keV/mm) with or without 2 mM melatonin. Agarose plugs containing genomic DNA were treated with Contour Clamped Homogeneous Electrophoretic Field (CHEF) followed by imaging. Clustered DNA damage was assessed with Number Average Length Analysis. Human primary fibroblasts were irradiated with Fe ion beam with or without 2 mM melatonin. Transformation experiments on fibroblast cells utilizing soft agar colony assay were performed [28].

In plasmid DNA in solution, melatonin inhibited the induction of single- and double-strand breaks. Treatment of human 28SC cells using 2 mM melatonin for 24 hours before irradiation decreased the level of X-ray induced double-strand breaks by 50% and that of abasic clustered damage by 40%. Melatonin diminished the level of Fe ion-induced double-strand breaks by 41% and that of abasic clustered damage by 34%. Melatonin also limited the transformation of human primary cells by a factor of 10 and it reduced Fe ion-induced cell killing by 20 - 40% [28].

Melatonin clearly protects against DNA damage caused by photons or charged particles (970 MeV/n Fe ions). It does not invariably protect against all classes of complex damage [28]. In the study of Das and colleagues safeguarding against double-strand breaks was superior as compared to abasic sites [28]. Pre-treatment of cells with melatonin before high energy Fe ion irradiation enhanced clonogenic survival, documenting that melatonin pre-treatment is crucial for its protective effects [28]. Kim and colleagues observed that melatonin-treated cells displayed increased viability compared with cells irradiated with 8 Gy of X-rays without melatonin treatment [29]. Zhou and co-workers revealed that melatonin enhanced survival of V-79 cells and reduced mutation levels induced by high doses of X-rays or charged carbon ion beams [27]. The reduction in the transformation rate mediated by melatonin identified changes in DNA damage. HZE particles, for instance, 970 MeV/n Fe ions cause extremely complex DNA damage [28]. Clusters of high lesion complexity are considered to be more difficult for cells to repair, or to repair accurately [30]. Consequently melatonin could reduce the frequencies of clusters of all levels or it could reduce the complexity of the damage.

The enhancement in clonogenic survival and conspicuous reduction in cell transformation mediated by melatonin confirm its powerful protective activity against damage caused by both high and low LET radiations. Melatonin's potent reduction of radiation-induced crucial DNA damage, cell killing, and conspicuous reduction of transformation indicate that it has remarkable potential as a countermeasure against radiation exposure to astronauts during space travel.

Space radiation-induced inhibition of neurogenesis in the hippocampal dentate gyrus and memory impairment in mice: ameliorative potential of the melatonin metabolite, AFMK: Neuronal exposure to space radiation may bring about a variety of dysfunctional *outcomes,* which include cognitive impairment. Current evidence points out that neuronal precursor cells in the hippocampus may be involved [31]. The dentate gyrus of the hippocampus persists in generating new neurons in the adult mammalian brain. Memory functions are related to the pyramidal and granule cells of the dentate gyrus [32]. New granule cells are generated from neural precursor/stem cells in the sub granular zone (SGZ). The generation of new cells takes place in all adult mammals, including humans. New cells then move to the granular cell layer (GCL) [28].

N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), a melatonin metabolite, is an infrequently explored biogenic amine. The fundamental principle of kynuric pathway of AFMK formation is that melatonin interacts with $^1O_2$ and H_2O_2 to generate AFMK, which is converted to N^1-acetyl-5-methoxykynuramine (AMK) by catalase. AFMK is a principal metabolite of melatonin oxidation. The study of Manda and colleagues investigated the effect of a melatonin metabolite (AFMK) against high-LET ^{56}Fe radiation-induced neurobehavioral alterations in mice particularly in association with hippocampal neurogenesis [32].

The initial manifestation of radiation-induced memory impairment was detected on day 24 after irradiation. This memory impairment continued to exist until day 60 after irradiation. AFMK pretreatment exhibited a notable protection against radiation-induced memory impairment. The protection was statistically significant from days 42 to 60 after irradiation. Motor activities of mice were not influenced by irradiation [32].

Radiation-induced alterations in the population of immature and proliferating neurons in the dentate gyrus were identified utilizing anti-Doublecortin (Dcx) and anti-Ki-67 expression. Immature neurons (Dcx positive) and proliferating Ki-67-positive cells were evaluated in the dentate gyrus. Sixty days after irradiation, there was a reduction of 81% in Dcx positive cells and 86% in Ki-67 positive cells as compared to the control. The AFMK-pretreated, irradiated mice demonstrated a notably higher count of Dcx and ki-67 positive cells. The protection provided by AFMK pretreatment for immature neurons (Dcx positive) was approximately 45% and 52% for proliferating Ki-67-positive cells [32].

Oxidative stress in the brain was assessed by assessing lipid peroxidation (HAE + MDA) and protein oxidation (protein carbonyl content). The level of 4-hydroxyalkenal+malondialdehy de (HAE+MDA) in brain homogenates of irradiated mice was 169% higher than that of the control group. The protein carbonyl content increased 173% compared to the control group. AFMK pre-treatment of irradiated mice exhibited a significantly lower value of the protein carbonyl and lipid peroxidation products. The extent of protection provided by AFMK pre-treatment for HAE + MDA was 113% and 107% for protein carbonyl content [32].

Antioxidant status of the plasma was assessed using TAC through the ferric reducing ability of plasma and nonprotein sulfhydryl (NP-SH) content in the brain. The extent of protection provided by AFMK pre-treatment for radiation-induced decline in TAC was 29% and 48% for NP-SH content [32].

AFMK pre-treatment obviously suppressed the reduction of Dcx and Ki-67 positive cells induced by high-LET ^{56}Fe radiation. In addition, AFMK pre-treatment mitigated high-LET ^{56}Fe radiation-induced enhancement of protein carbonyl and HAE + MDA in the brain and preserved the total antioxidant capacity of plasma and NP-SH content in brain.

Melatonin modulates acute testicular damage induced by carbon ion irradiation in mice: The trajectory of heavy ions is very complicated. Energy is not only deposited by the primary interaction but also by secondary electrons which may proceed substantial distances from the core [33]. These high-LET heavy ions generate additional irreparable DNA breaks and chromosomal aberrations [34]. Accordingly, heavy ion irradiation is highly cytotoxic and genotoxic to mammalian cells [35].

The testis is a radiosensitive organ. Heavy ion irradiation induces notable morphological damage, eliminates poly (ADP-ribose)

polymerase (PARP) activity and its expression associated with DNA repair, and enhances spermatocyte chromosomal aberrations in mouse testis [13]. Considering the development of human activity during space missions, crews of manned space missions with child-bearing capability may be concerned about the risk to their subsequent offspring. Consequently, there is a need for thorough evaluation of the protection of the testis against heavy ion radiation.

Effects of melatonin on lipid peroxidation and antioxidant status induced by carbon-ion irradiation: Carbon-ion irradiation gave rise to a significant increase in malondialdehyde (MDA) level, and also induced a marked reduction of both glutathione (GSH) and total antioxidant capability (TAC) status in comparison to the control group, suggesting that imbalance between pro-oxidants and antioxidants resulted in the overproduction of reactive oxygen species. Application of melatonin to irradiated mice significantly inhibited the carbon ion-induced increase in MDA level related to elevated antioxidant status including GSH and TAC content in all of the melatonin plus irradiation groups [13].

Effects of melatonin on DNA damage and cell apoptosis generated by carbon ion irradiation: Melatonin together with carbon ion beam therapy promoted a noticeable reduction in DNA damage induced by carbon ion irradiation when compared to the irradiated group. The proportion of apoptotic cells in the low-dose (1 mg/kg) and high-dose (10 mg/kg) melatonin treated groups decreased to 23.6% and 9.22% of the irradiation group, respectively. This implies that pre-treatment with melatonin at low or high dose reduces cell apoptosis. Post treatment with melatonin also lowered the proportion of apoptotic cells in mouse testis compared with the irradiation group [13].

Histopathological findings: Carbon ion irradiation caused a reduction in testicular tubule diameter, formation of interstitial edema, coagulative necrosis of spermatozoa, tubular degeneration, and a reduction of germ, Leydig or Sertoil cells. Application of melatonin to irradiated mice ameliorated carbon ion-induced histopathological lesions in the testis [13].

Both pre-treatment and post treatment with high-dose melatonin (10 mg/kg) noticeably mitigated carbon ion-induced acute testicular damage, but a greater radio protective effect was detected in the pre-treatment group. On the contrary, low-dose melatonin (1 mg/kg) had a minimal radio protective effect on carbon ion-induced degeneration and DNA lesions in mouse testis [13]. Briefly, the data support a recommendation that prophylactic treatment with a higher dose of melatonin is a prospective strategy to protect against heavy ion irradiation-induced testicular damage.

Ameliorating mitochondrial dysfunction restores carbon ion-induced cognitive deficits through co-activation of NRF2 and PINK1 signalling pathway: Liu and colleagues *studied* the mechanism of deterioration and amelioration of cognitive functions through the regulation of the crucial molecules of the signal transduction pathway in the hippocampus of the mouse brain after exposure to high-LET carbon ion irradiation [36].

Cognitive deficits were induced by high-LET carbon ion irradiation: Morris water maze is the most typical experimental procedure utilized to evaluate cognitive function [36]. The carbon ion-irradiated mice exhibited a marked increase of the escape latency compared to the control group on day 2 to day 6 after irradiation. On day 6, the mean escape latency of the control group was *approximately* 18 seconds. However, it was about 55 seconds in the irradiated group. The irradiated group also revealed a noticeable reduction of the residence time in the target quadrant compared to the control group [36].

Consecutive sections with H&E staining of the hippocampus documented that blurred karyotheca and pyknotic nuclei were found in the hippocampus of the irradiated animals. The carbon ion irradiated group manifested an obvious reduction in the density of Nissl-stained cells in the hippocampal CA1 pyramidal neurons and granule cells of the dentate gyrus. These findings indicate that the high-LET carbon ion irradiation is likely related to the hippocampus cognitive deficits, coexisting with neurodegeneration and neuronal cell damage [36].

Mitochondrial damage was induced by carbon ion irradiation in the hippocampus region: Electron microscopy of irradiated cells displayed swollen mitochondria, broken cristae and the fragmented internal membranes in the hippocampal neurons of the irradiated mice. Following carbon ion irradiation, the activities of mitochondrial respiratory chain complex I, IV and V in the hippocampus declined by 47.0%, 36.1% and 31.3%, respectively. Pyruvate dehydrogenase (PDH), citrate synthase (CS), succinate dehydrogenase (SDH) and alpha-ketoglutarate dehydrogenase (α-KGDH) in the hippocampus of the irradiated mice decreased markedly in contrast to the sham-irradiated mice. The ATP content of the irradiated hippocampal mitochondria dropped by 29.1% compared with the control animals.

These findings indicated that high-LET carbon ion beam induced incessant damage to mitochondrial structure and function as observed 1 month after irradiation [36].

Carbon ion irradiation regulated the mitochondrial homeostasis in the hippocampus with or without melatonin: Carbon ion irradiation notably enhanced Drp-1 (an essential executor of mitochondrial fission) and inhibited Mfn-2 (a crucial factor of mitochondrial fusion) in comparison with the control mice. Administration of melatonin (10 mg/kg daily for 7 days i.p.) markedly enhanced the expression of Mfn-2 1.6-fold and inhibited Drp-1 by 35.6% compared with the irradiated only animals. There was a noticeable reduction in the mito-LC3II / LC3I ratio in the irradiation group. Confocal microscopy documented that reduced co-localization of COX IV with LC3B-positive signal, evidence of mitophagosome, was detected in the carbon ion irradiated cells. Administration of melatonin markedly restored the mitophagic process in the carbon-ion irradiated cells [36].

Carbon ion irradiation modulated the redox status in the hippocampus with or without melatonin: Carbon ion irradiation *brought about* a marked increase in malondialdehyde (MDA) level or 8-hydroxy-2′-deoxyguanosine (8-OHdG) content compared to the control group, denoting that carbon ions induced a long-lasting elevation of oxidative damage in the hippocampus. Administration of melatonin before carbon ion irradiation markedly decreased the content of MDA and 8-OHdG in the irradiated animals. The TAC activity declined by 37.3% in the irradiated mice compared with that in the control group. Western blot analysis showed that a decreased expression of SOD2 was detected in the irradiated group. Carbon ion irradiation induced a marked reduction in the GSH/GSSG ratio compared with the control group. Administration of melatonin before carbon ion irradiation enhanced the TAC activity and the levels of SOD2 or GSH/GSSG [36].

Carbon ion irradiation regulated the PINK1 signalling pathway in the hippocampus with or without melatonin: Confocal microscopy demonstrated an indistinct PINK1 fluorescence in the dentate gyrus region of the irradiated mice compared to the control animals. In cells exposed to carbon ion irradiation, there was a marked reduction in the PINK1 and TOMM20 colocalization, denoting that carbon ions diminish the PINK1 accumulation in the outer mitochondrial

membrane. The level of PINK1 was markedly reduced in the irradiated mice compared to that in the sham irradiation group. On the contrary, administration of melatonin markedly enhanced the level of PINK1. The level of Parkin protein expression was notably reduced by carbon ion irradiation, but melatonin pre-treatment also overcame that reduction. These results denote that carbon ion irradiation inhibited the PINK1/Parkin activation. On the contrary, administration of melatonin enhanced PINK1/Parkin activation in hippocampal cells [36].

Carbon ion irradiation modulated the NRF2 redox signalling in the hippocampus with or without melatonin: Administration of melatonin before carbon ion irradiation markedly increased the level of NRF2 expression in the combined melatonin-radiation group. NRF2 is a transcription factor that reacts to oxidative stress by binding to the antioxidant response element (ARE) [36]. Imaging Flow Cytometry demonstrated that the merged color regions with high density documented NRF2 translocation into the nucleus. There were a large number of the merged color regions with low density in the carbon ion-irradiated cells. Simultaneous treatment with melatonin enhanced the NRF2 nuclear translocation in the carbon ion-irradiated cells. Co-immunoprecipitation (Co-IP) and surface plasmon resonance (SPR) assays documented that noticeable co-immunoprecipitation of NFR2 with PINK1 and amplification of their binding signal were detected in the cells pretreated with melatonin. These findings disclose that treatment with melatonin leads to the positive interaction between NRF2 and PINK1 signalling [36].

Amelioration of cognitive deficits induced by carbon ion irradiation through regulating mitochondrial function: Pretreatment with melatonin neutralized the deteriorated activities of pyruvate dehydrogenase (PDH), citrate synthase (CS), succinate dehydrogenase (SDH) and alpha-ketoglutarate dehydrogenase (α-KGDH) induced by carbon ion irradiation. Administration of melatonin prior to carbon ion irradiation markedly increased the activity of mitochondrial respiratory chain complex I and IV in the hippocampus. The combined melatonin and carbon ion irradiation mice demonstrated a notable reduction of the escape latency compared to the radiation-alone group [36].

Neuroprotection against the injury induced by carbon ion irradiation through NRF2 and PINK1 signaling pathway: Carbon ion irradiation significantly inhibited the growth of HT22 cells for up to 72 hours, and raised the proportion of apoptotic cells 48 hours after exposure to irradiation compared to the sham-irradiated cells. This finding shows that the suppression of proliferation might be an apoptosis-dependent induction in the irradiated hippocampal cells. Overexpression of NRF2 and PINK1 conspicuously recovered cell growth, and markedly reduced the number of apoptotic cells in the hippocampal cells treated with carbon ion irradiation [36]. In brief, these findings indicate that melatonin is a promising agent for alleviation of the persistent mitochondrial dysfunction and oxidative stress through co-modulation of NRF2 and PINK1 signaling resulting in a restoration of the cognitive impairment induced by high-LET carbon ion irradiation.

Discussion

High LET irradiation *such as* carbon or iron ions have a greater impact on the induction of biologically deleterious effects [13].

The testis is extremely vulnerable to irradiation. Carbon ion irradiation brings about notable deleterious effects on prenatal gonads, postnatal testicular development and breeding activity in male offspring when pregnant rats are irradiated with accelerated carbon ions on gestation day 15 [13]. Because of its small size and high lipophilicity, melatonin traverses biological membranes *freely* and *disperses into* all compartments of the cell. Melatonin and its direct metabolite N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) scavenge hydroxyl radical (•OH) with a very high rate of the order of $2.7 \times 10^{10}\ M^{-1}\ s^{-1}$ [37]. Manda and colleagues indicated that the neuroprotection provided by AFMK pretreatment is likely a consequence of its free-radical scavenging function [32].

The action of heavy ion irradiation is considered to be associated with more direct effects on biological targets rather than indirectly produced reactive free radicals. Melatonin probably has a protective action against direct effects caused by ionizing radiation [13,27]. One proposal is that melatonin is in the immediate vicinity of DNA molecules during irradiation [27]. The receptor RZR/ROR is an essential member of the nuclear receptor superfamily which may be a nuclear binding site for melatonin. With melatonin binding to DNA molecules by means of the receptor RZR/ROR, the proposition that melatonin reduces the direct reaction of ionizing radiation with DNA molecules becomes possible [27].

The brain contains high quantities of mitochondria, which are one of the principal sources of reactive oxygen species (ROS), owing to the fact that mitochondria employ oxygen for energy production [38]. Moreover, recent evidence shows that mitochondria both synthesize [39,40] and avidly take up melatonin when it is administered [41,42].

Because of its high oxygen consumption and its high content peroxidizable fatty acids of the brain, neuronal tissues are vulnerable to ionizing radiation, eventually resulting in radiation encephalopathy [36]. The extensive cognitive impairment induced by high-LET carbon ion irradiation is demonstrated by increased latency to detect the hidden platform in the Morris water maze test. This phenomenon points out that carbon ion irradiation interferes with spatial learning and memory capabilities. Similar outcomes of cognition assessment are detected in the animals exposed to high-LET ^{56}Fe, ^{28}Si and ^{16}O particle irradiation [36].

Carbon ions decrease the level of mitofusin-2 (Mfn-2), a mitochondrial membrane protein that takes part in mitochondrial fusion. On the contrary, carbon ions increase the level of dynamin-related protein 1 (Drp1), which enhances mitochondrial fission. Administration of melatonin significantly elevates Mfn-2 level and reduces Drp1 level. Marked inhibition of mitophagy is detected in mice exposed to carbon ion irradiation. Pretreatment with melatonin successfully overcomes the inhibition of mitophagy. Melatonin, a mitochondria-targeted antioxidant, is able to regulate mitochondrial dynamics and mitophagy to maintain the mitochondrial homeostasis in the carbon ion-irradiated mice [7].

Oxidative stress is *associated with* mitochondrial dysfunction and cognitive impairment [36]. Carbon ion irradiation brought about marked oxidative damage to lipids and DNA, and in addition inhibited total antioxidant capacity and mitochondrial antioxidant SOD2 [36,43]. But melatonin eradicated the persistent oxidative damage induced by high-LET carbon ions.

PINK1 and Parkin are crucially implicated in cell survival and neurological disorders [44]. The study of Liu and colleagues indicated that PINK1 expression and stabilization on the outer mitochondrial membranes were markedly declined after carbon ion irradiation, particularly in the dentate gyrus region of the hippocampus; these alterations coexisted with the down-regulation of PINK1 and Parkin

expression [36]. Melatonin reversed down-regulation of PINK1 and Parkin expression together with enhanced stabilization of PINK1 on the outer mitochondrial membranes, and preserved mitochondria homeostasis thereafter [36].

NRF2, a modulator of oxidative stress, also provides neuroprotective action and prevention of cognitive impairment. A marked reduction of NRF2 expression with translocation to the nucleus in mice was detected after carbon ion irradiation. On the contrary, melatonin pretreatment notably enhanced NRF2 expression along with translocation to the nucleus [36]. Co-IP and SPR assays documented that melatonin treatment promoted the favorable interaction between NRF2 and PINK1 signaling [36]. The study of Liu and colleagues [36] indicated that 4 Gy of carbon ion irradiation brought about apparent spatial cognitive impairment, interfered with the mitochondrial homeostasis and caused a redox imbalance, and was closely related to inactivation of NRF2 and PINK1 signaling. Melatonin treatment enhanced the NRF2-PINK1 signaling and increased the crosstalk between NRF2 and PINK1.

Consequently, a carbon ion-induced cognitive impairment was efficaciously recovered by melatonin through preserving the mitochondrial functions and in addition eliminating the oxidative damage [36].

Conclusion

Pre-treatment with melatonin successfully inhibited the oxidative damage induced by high energy charged particle irradiation. The above-mentioned results provide a prospective aspiration for protective strategies with respect to the space radiation hazards. Further investigation to elucidate the seriousness and outcomes of organ damage associated with space radiation is warranted.

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