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Melatonin Protection against Ionizing Radiation in Space

Mu-Tai Liu[1,2]* and RJ Reiter[3]

[1]*Department of Radiation Oncology, Changhua Christian Hospital, Changhua 500, Taiwan*
[2]*Department of Radiology, Yuanpei University of Science and Technology, Hsinchu 300, Taiwan*
[3]*Department of Cell Systems and Anatomy, The University of Texas Health Science Centre, San Antonio, USA*

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 defcits through co-activation of NRF2 and PINK1 signaling pathway'.

Conclusion:

***Corresponding author:** Mu-Tai Liu, Department of Radiation Oncology, Changhua Christian Hospital, Changhua 500, Taiwan, Tel: +886-4-7238595; Fax: +886-4-7232942; E-mail: tomgiessen@gmail.com

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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 in uence on immune reactions [10] and is an e ective 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].

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

Carbon ion irradiation induced a signi cant elevation in protein

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 broblasts were irradiated with Fe ion beam with or without 2 mM melatonin. Transformation experiments on broblast cells utilizing so 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 e ects [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]. e reduction in the transformation rate mediated by melatonin identi ed 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 di cult 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.

e enhancement in clonogenic survival and conspicuous reduction in cell transformation mediated by melatonin con rm 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.

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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]. e 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). e 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. e 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. e study of Manda and colleagues investigated the e ect of a melatonin metabolite (AFMK) against high-LET ^{56}Fe radiation-induced neurobehavioral alterations in mice particularly in association with hippocampal neurogenesis [32].

e initial manifestation of radiation-induced memory impairment was detected on day 24 a er irradiation. is memory impairment continued to exist until day 60 a er irradiation. AFMK pretreatment exhibited a notable protection against radiation-induced memory impairment. e protection was statistically signi cant from days 42 to 60 a er irradiation. Motor activities of mice were not in uenced by irradiation [32].

Radiation-induced alterations in the population of immature and proliferating neurons in the dentate gyrus were identi ed 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 a er irradiation, there was a reduction of 81% in Dcx positive cells and 86% in Ki-67 positive cells as compared to the control. e AFMK-pretreated, irradiated mice demonstrated a notably higher count of Dcx and ki-67 positive cells. e 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). e level of 4-hydroxyalkenal+malondialdehy de (HAE+MDA) in brain homogenates of irradiated mice was 169% higher than that of the control group. e protein carbonyl content increased 173% compared to the control group. AFMK pre-treatment of irradiated mice exhibited a signi cantly lower value of the protein carbonyl and lipid peroxidation products. e 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 sul ydryl (NP-SH) content in the brain. e 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.

s s : e 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]. ese 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].

e 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 o spring. Consequently, there is a need for thorough evaluation of the protection of the testis against heavy ion radiation.

E ects of melatonin on lipid peroxidation and antioxidant status induced by carbon-ion irradiation: Carbon-ion irradiation gave rise to a signi cant 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 signi cantly 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].

E ects 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. e 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. is 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 ndings: 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 e ect was detected in the pre-treatment group. On the contrary, low-dose melatonin (1 mg/kg) had a minimal radio protective e ect on carbon ion-induced degeneration and DNA lesions in mouse testis [13]. Brie y, 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.

A s s s - s - 2 ls : 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 a er exposure to high-LET carbon ion irradiation [36].

Cognitive de cits were induced by high-LET carbon ion irradiation: Morris water maze is the most typical experimental procedure utilized to evaluate cognitive function [36]. e carbon ion-irradiated mice exhibited a marked increase of the escape latency compared to the control group on day 2 to day 6 a er 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. e 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. e 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. ese ndings indicate that the high-LET carbon ion irradiation is likely related to the hippocampus cognitive de cits, 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

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 therea er [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 a er 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]. e 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 e caciously recovered by melatonin through preserving the mitochondrial functions and in addition eliminating the oxidative damage [36].

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Pre-treatment with melatonin successfully inhibited the oxidative damage induced by high energy charged particle irradiation. e 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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