Blood Cells, Molecules and Diseases 77 (2019) 82–87

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Protective effect of the antioxidative peptide SS31 on ionizing radiation-induced hematopoietic system damage in mice

Xiaoliang Han[a,*], Ping Gao[a], Ying Zhang[a], Jinyan Wang[b], Fengtao Sun[a], Qingguo Liu[b], Shubo Zhang[a]

[a] Affiliated Hospital North China University of Science and Technology, Tangshan, Hebei, China, 063000
[b] Tangshan Gongren Hospital, Tangshan, Hebei 063000, China

ARTICLE INFO

Editor: Mohandas Narla

Keywords:
SS31
Ionizing radiation
Reactive oxygen species
Hematopoietic system

ABSTRACT

Ionizing radiation (IR) causes severe damage to the hematopoietic system; thus, it is necessary to explore agents or compounds that can reduce this damage. SS31 is a mitochondria-targeted peptide that can scavenge cellular reactive oxygen species (ROS) and inhibit the production of mitochondrial ROS. Therefore, in this study, we discuss the protective effect of SS31 on IR-induced hematopoietic system damage. Our results showed that treatment with 6 mg/kg SS31 elevated the survival rate of lethally irradiated mice and increased the numbers of white blood cells, red blood cells, hemoglobin and platelets in mice exposed to 4 Gy whole-body irradiation. In addition, SS31 administration improved the number of hematopoietic stem/progenitor cells (HSPCs) and the self-renewal and reconstitution abilities of these cells in irradiated mice. The elevation of ROS levels is the main cause of IR-induced hematopoietic system damage, and SS31 can effectively reduce the ROS level in HSPCs. The above results suggest that SS31 can protect the hematopoietic system from radiation-induced damage by reducing cellular ROS levels.

1. Introduction

Ionizing radiation (IR)-induced body damage can be divided into direct and indirect effects. Through direct effects, IR induces ionization, excitation, and chemical bond rupture in biologically active macromolecules, such as proteins and nucleic acids, resulting in changes in molecular structure and properties, which ultimately leads to abnormal functional and metabolic disorders [1,2]. Indirect effects refer to the action of radiolysis in water molecules to generate free radicals. The hematopoietic system is sensitive to IR. It is well known that increased reactive oxygen species (ROS) levels in hematopoietic stem/progenitor cells (HSPCs) are the primary cause of hematopoietic system damage. Therefore, ROS scavenging compounds can effectively alleviate hematopoietic system injury induced by ionizing radiation [3,4].

SS31 is a new type of small molecular antioxidant peptide that can target mitochondria and aggregate in the mitochondrial inner membrane, which is the site of ROS production. Therefore, SS31 can scavenge free radicals, reduce ROS production in the mitochondria, and inhibit translocation of mitochondrial membrane and the release of cytochrome C [5–9]. Previous studies have shown that SS31 can protect mitochondrial function by scavenging free radicals and alleviate kidney injury and limb ischemia-reperfusion injury [9,10], improve mitochondrial function [11] and prevent atherosclerosis progression in mice after traumatic brain injury [12]. However, it is unclear whether SS31 has a protective effect on hematopoietic system damage caused by IR.

In this study, an IR-induced damage mouse model was used to explore the protective effect of SS31 on hematopoietic system injury. The results showed that SS31 can effectively protect against whole-body irradiation (WBI)-induced injury and enhance cell self-renewal and differentiation abilities in irradiated mice. Furthermore, SS31 can reduce H_2O_2-derived ROS, mitochondrial superoxide radical and superoxide radical levels in HSPCs. Our research provides the experimental basis for further application of SS31, which merits further exploration.

2. Materials and methods

2.1. Experimental animals

Male ICR mice were purchased from Beijing Vital River Experimental Animal Co, Ltd. Male C57BL/6 (CD45.1) mice were purchased from the College of Medicine, Peking University, and male

* Corresponding author.
E-mail address: mayastarfx2008@163.com (X. Han).

https://doi.org/10.1016/j.bcmd.2019.04.001
Received 15 January 2019; Received in revised form 5 April 2019; Accepted 5 April 2019
Available online 07 April 2019

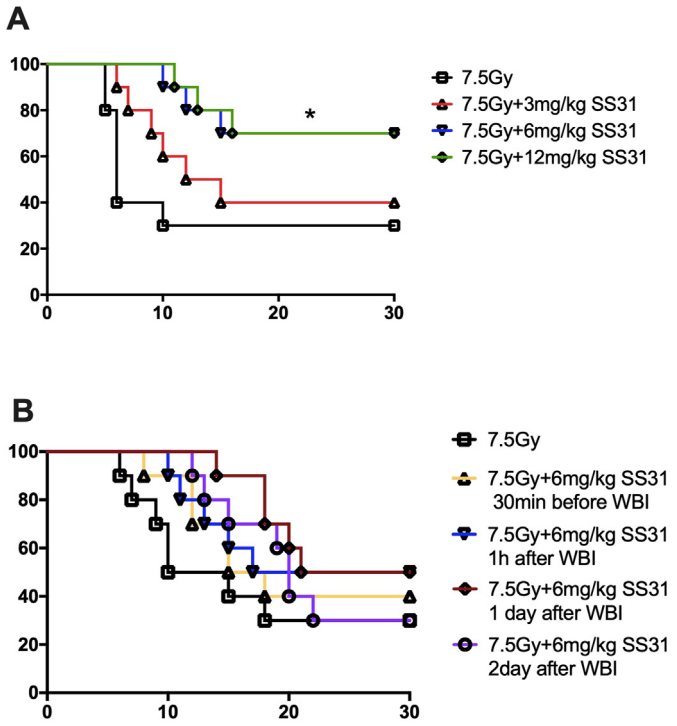

Fig. 1. SS31 increases 30-day survival in mice exposed to lethal irradiation. Mice were injected with normal saline or SS31 starting 30 min before WBI and/or up to 7 days after WBI, as described in the Materials and Methods. (A) Survival rate of lethal irradiated mice treated with SS31 before and after exposure; (B) survival rate of lethal irradiated mice treated with SS31 before or after irradiation exposure. Survival rates were analyzed by using a *log-rank* test (n = 10). *$p < 0.05$ compared with the 7.5 Gy group.

C57BL/6 (CD45.1/CD45.2) mice were bred in the animal facility of North China University of Science and Technology. The Animal Care and Ethics Committee of North China University of Science and Technology approved the use of animals for these experiments, which complied with the Guide for the Care and Use of Laboratory Animals and the National Institutes of Health guide for the Care and Use of Laboratory Animals.

2.2. Antibodies

Biotin anti-mouse CD4, biotin anti-mouse CD8, biotin anti-mouse CD45R/B220, biotin anti-mouse CD11b, biotin anti-mouse Ly-6G/Ly-6C (Gr1), biotin anti-mouse Ter119, anti-mouse CD117 (c-kit) APC, anti-mouse Ly-6 A/E (Sca1) PE, anti-mouse CD34 FITC, anti-mouse CD16/32 percp, anti-mouse CD48 PE-Cy7, anti-mouse CD150 PB and streptavidin APC-Cy7 were purchased from Biolegend (San Diego, CA, USA).

2.3. WBI and SS31 administration

For the 30-day survival experiment, mice were divided into 4 groups, as follows: 7.5 Gy WBI, 7.5 Gy WBI + 3 mg/kg SS31, 7.5 Gy WBI + 6 mg/kg SS31, and 7.5 Gy WBI + 12 mg/kg SS31. For the remaining experiments, mice were divided into 4 groups, as follows: control, SS31, 4 Gy WBI, and 4 Gy WBI + 6 mg/kg SS31. Mice were administered SS31 or normal saline by intraperitoneal injection at a volume of 200 μL, and irradiated mice received SS31 30 min before WBI and once a day for up to 7 days after WBI.

2.4. Peripheral blood cell and bone marrow cell counts

Heparin sodium (30 μL) was placed into a 1.5 mL EP tube; then, blood collected from murine hearts was added to the tube. In total,

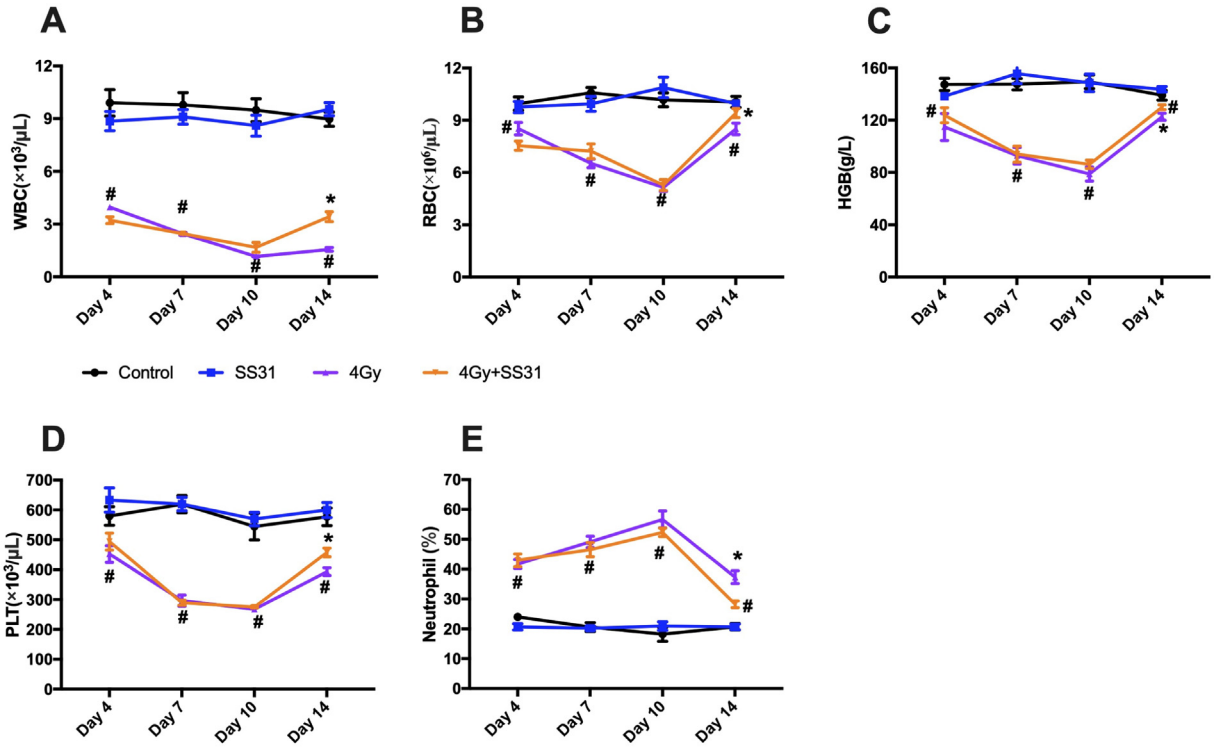

Fig. 2. SS31 reduces myelosuppression caused by WBI. Mice were injected with normal saline or SS31 starting 30 min before WBI and up to 7 days after WBI, as described in Materials and Methods. The control mice were sham irradiated. (A) WBC count, (B) RBC count, (C) HGB concentration, (D) PLT count and (E) neutrophil percentage in peripheral blood. The data are presented as the mean ± SEM (n = 5 in the day 4, 7, and 10 groups and 10 in the day 14 group). #$p < 0.05$ vs Control; *$p < 0.05$ vs 4 Gy.

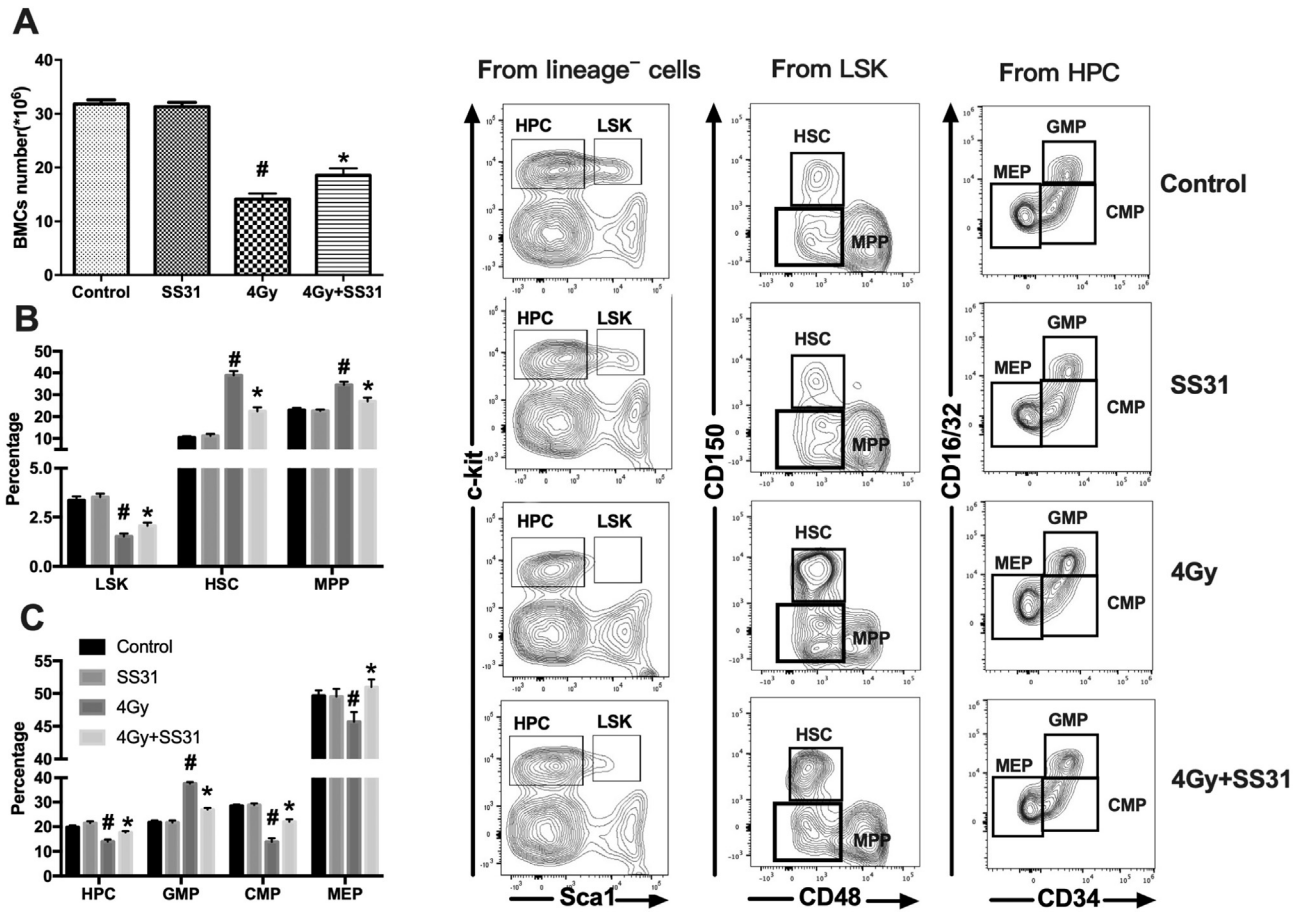

Fig. 3. SS31 promotes recovery of BMCs number after WBI. Mice were injected with normal saline or SS31 starting 30 min before WBI and up to 7 days after WBI, as described in Materials and Methods. The control mice were sham irradiated. (A) BMC number; (B) LSK cell, HSC, and MPP percentage; (C) HPC, GMP, CMP and MEP percentage; (D) representative flow scatter plots showing the identification of HSPCs. The data are presented as the means ± SEM (n = 10). #$p < 0.05$ vs Control; *$p < 0.05$ vs 4 Gy.

100 μL of blood was used to determine the cell number count using a hemocytometer (Pulang Biotechnology Co., Ltd., Nanjing, China). Red blood cells from the obtained bone marrow cells were removed first; then, the number of bone marrow mononuclear cells was determined.

2.5. Flow cytometry analysis

Briefly, 5×10^6 bone marrow cells were first stained with a mixture of biotin-conjugated antibodies targeting Grl, Ter119, CD11b, B220, CD4, and CD8; the cells were then were stained with streptavidin-APC-Cy7, Scal-PE, c-kit-APC, CD34-FITC and CD16/32 percp antibodies for the analysis of LSK cells, hematopoietic progenitor cells (HPCs), common myeloid progenitors (CMPs), granulocyte/monocyte progenitors (GMPs) and megakaryocyte/erythroid progenitors (MEPs) or stained with streptavidin-APC-Cy7, Scal-PE, and c-kit-APC. CD48 FITC and CD150 percp were utilized for the analysis of hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs). The above cells were detected using a BD FACSCalibur flow cytometer (BD Bioscience, San Jose, CA, USA), and the obtained data were analyzed using FlowJo software.

2.6. Competitive bone marrow transplantation

First, 1×10^6 donor-derived BMCs (CD45.2) were mixed with 2.5×10^5 competitor-derived BMCs (CD45.1/45.2); then, the cells were transplanted into lethally irradiated (9 Gy) mice (CD45.1). Four months after transplantation, peripheral blood was collected and stained with antibodies against CD45.1 and CD45.2, and the percentage of CD45.2-positive cells was detected with the BD FACSCalibur flow cytometer.

2.7. Colony-forming units of granulocytes and macrophages (CFU-GM) and burst-forming unit-erythroid (BFU-E) experiments

For the control, 2 Gy and 4 Gy groups, 1×10^4, 4×10^4, and 1×10^5 BMCs were utilized, respectively, and the BMCs were added into M3434 methylcellulose medium (Stem Cell Technologies). CFU-GM was evaluated after 5 days of culture, and BFU-E was evaluated after 12 days of culture; then, the colonies were counted, and all the results are presented as the number of colonies formed per femur.

2.8. Intracellular reactive oxygen species detection

Briefly, 5×10^6 BMCs were first stained with markers for LSK cells, HPCs, HSCs and MPPs; then, BMCs were incubated with 2,7-dichlorodihydrofluorescein diacetate (Thermo Fisher, Waltham, MA, USA, 10 μM) and MitoSox (Thermo Fisher, Waltham, MA, USA, 10 μM) for 30 min or with dihydroethidium (DHE; Beyotime Biotechnology, Nanjing, China; 5 μM) for 10 min in a water bath. The mean fluorescence intensity of ROS in LSK cells and HPCs was measured using BD FACSCalibur and Aria II flow cytometers.

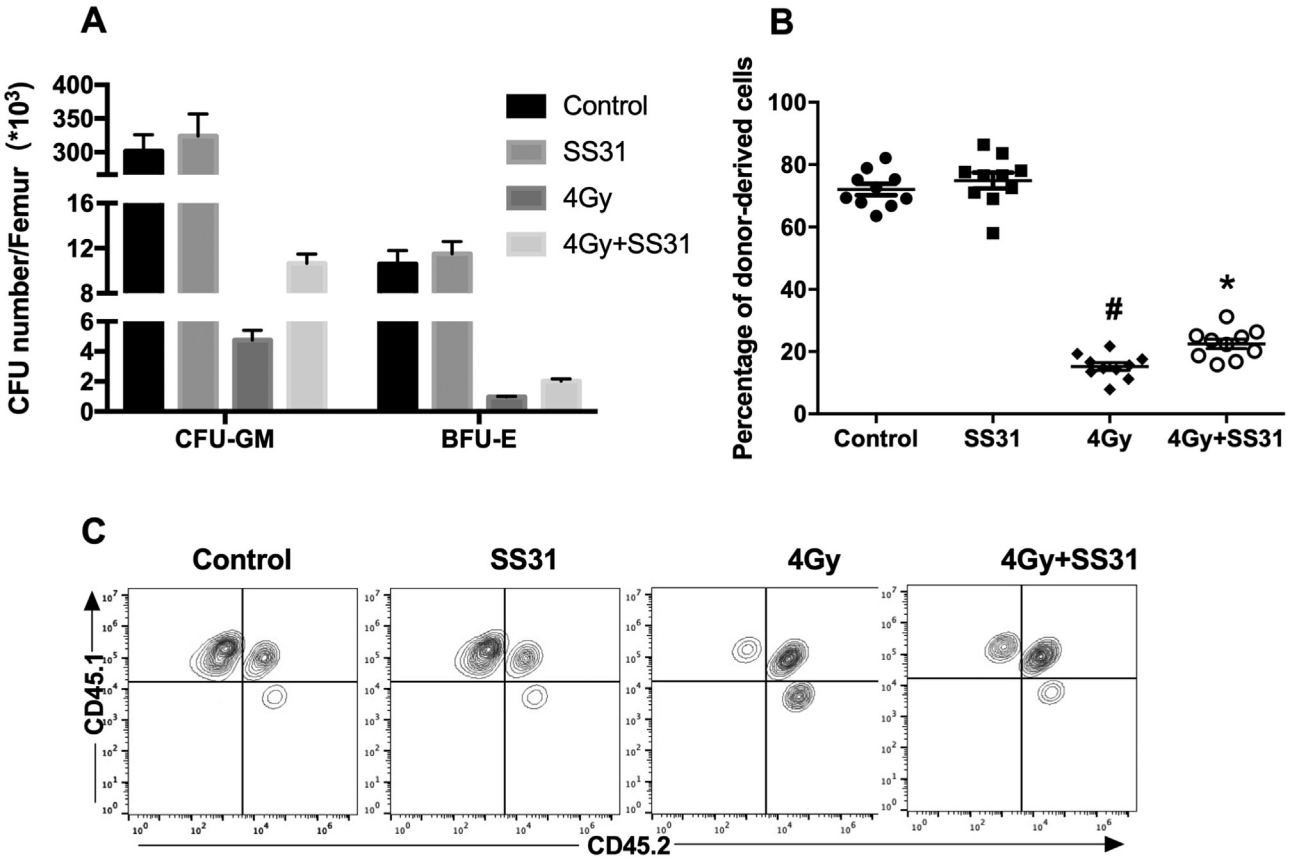

Fig. 4. SS31 improves the proliferative capacity of HSPCs in irradiated mice. Mice were injected with normal saline or SS31 starting 30 min before WBI and up to 7 days after WBI, as described in Materials and Methods. The control mice were sham irradiated. (A) The CFU-GM and BFU-E numbers; (B) the percentage of donor-derived cells in peripheral blood cells; (C) representative flow scatter plots showing donor-derived cell chimerism. The data are presented as the means ± SEM (n = 6 in panel A; n = 10 in panel B). #$p < 0.05$ vs Control; *$p < 0.05$ vs 4 Gy.

2.9. Statistical analysis

The results were analyzed using GraphPad Prism 5.0 software. The survival rate experiment was assessed with a *log-rank* test. The rest of the experiments were analyzed using a *Mann-Whitney* test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. SS31 elevated the 30-day survival rate of irradiated mice

Our previous experiments showed that when mice were exposed to 7 Gy WBI, the 30-day survival rate was > 90%, and when the dose was increased to 8 Gy, < 10% of mice survived. Therefore, we used a 7.5 Gy radiation dose to explore the 30-day survival rate of mice. As shown in Fig. 1, when mice were treated with SS31 both before and after WBI, the survival rate in the 7.5 Gy irradiated group was 30%, and the survival rate in the 7.5 Gy + 3 mg/kg SS31 group was 40%, and the 30-day survival rate of irradiated mice increased to 70% after treatment with 6 mg/kg and 12 mg/kg SS31. These results suggest that both the 6 mg/kg and 12 mg/kg SS31 dose has protective effects in IR-induced mouse injury; thus, the dose of SS31 used in the remaining experiments was 6 mg/kg. Next, we sought to determine whether SS31 can act as a radiomitigation or radio-protection agent. The mice were treated once with SS31 30 min before WBI or treated after WBI at different time points for up to 7 days. As shown in Fig. 1B, when mice were treated before WBI, there was only a 10% increase in survival rate; when mice were treated 1 h and 1 day after WBI, the survival rate was 20% more than that in the irradiation only group, but this difference was not significant. Therefore, combined application of SS31 both before and after WBI is the best way to protect against radiation-induced injury in mice.

3.2. SS31 reduces myelosuppression and myeloid skewing in irradiated mice

The main manifestation of hematopoietic system damage in peripheral blood caused by WBI is myelosuppression, including leukocyte, erythrocyte and platelet reduction [13,14]. WBI also induces myeloid skewing, which is defined as an increased percentage of neutrophils in peripheral blood. To explore the underlying protective effect of SS31 on WBI-induced myelosuppression and myeloid skewing, we detected the number of white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs); the concentration of hemoglobin (HGB); and the percentage of neutrophils in peripheral blood at 4, 7, 10, and 15 days after mice were exposed to 4 Gy IR. As shown in Fig. 2A–E, the number of WBCs, RBCs, and PLTs and the concentration of HGB decreased, and the percentage of neutrophils gradually increased with the increase in irradiation time. Most of the indexes reached their nadir or peak at day 10 after WBI and then recovered. Although there was an increase in the WBC number and the HGB concentration and a decrease in the percentage of neutrophils when mice were administered SS31 at day 10, all the blood cell numbers were significantly recovered at day 14. Our data indicate that SS31 can alleviate myelosuppression and myeloid skewing in mice caused by irradiation.

3.3. SS31 improves BMC number and HSPC frequency recovery after WBI

The hematopoietic system is sensitive to radiation, and WBI can significantly reduce the BMC number. To explore the effect of SS31 on

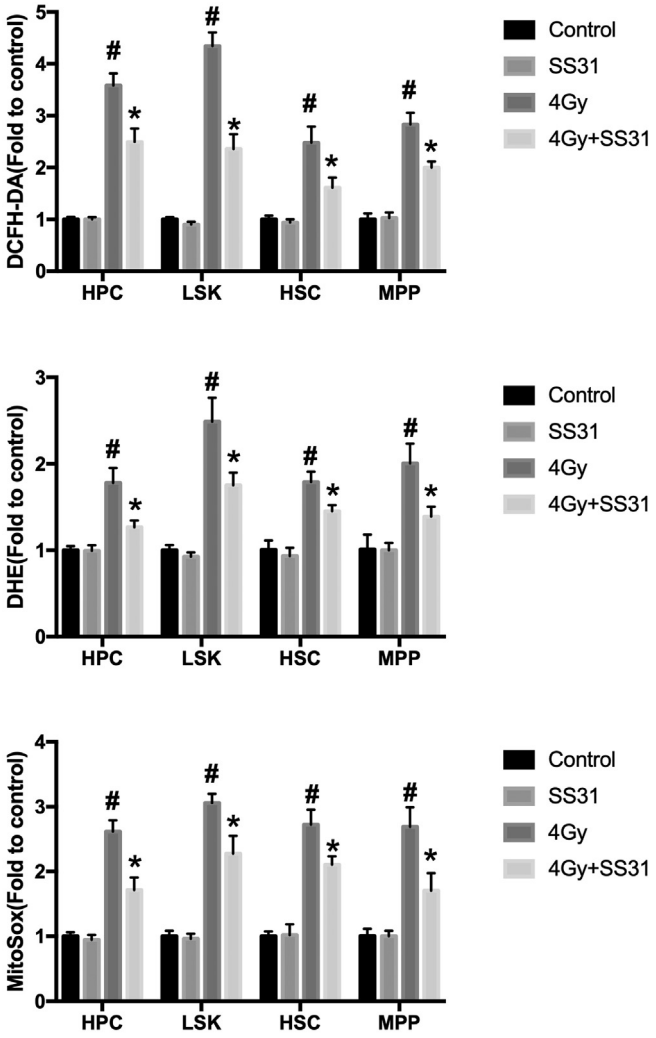

Fig. 5. SS31 reduces ROS levels in HSPCs. Mice were injected with normal saline or SS31 starting 30 min before WBI and up to 7 days after WBI, as described in Materials and Methods. The control mice were sham irradiated. (A) H_2O_2-derived ROS level in LSK cells, HPCs, HSCs and MPPs; (B) superoxide anion radical levels in LSK cells, HPCs, HSCs and MPPs; (C) mitochondrial superoxide radical levels in LSK cells, HPCs, HSCs and MPPs. The data are presented as the means ± SEM (n = 5). #$p < 0.05$ vs Control; *$p < 0.05$ vs 4 Gy.

BMC number after irradiation, we detected the number of BMCs and the proportions of LSK cells, HPCs, HSCs, MPPs, CMPs, GMPs, and MEPs 15 days after 4 Gy WBI. As shown in Fig. 3, after mice were exposed to 4 Gy IR, the BMC number and the LSK cell, HPC, CMP and MEP percentages decreased and the HSC, MPP and GMP percentages increased significantly. Thus, SS31 treatment effectively increased the BMC number and the LSK cell, HPC, CMP and MEP percentages and decreased the HSC, MPP, and GMP percentages. Therefore, our results suggest that SS31 can promote recovery of the BMC number after irradiation.

3.4. SS31 improves the colony formation and reconstitution ability of irradiated mice

To explore the effect of SS31 on the proliferative and reconstitution ability of HSPCs, CFU-GM, BFU-E and bone marrow transplantation experiments were performed. As shown in Fig. 4A, WBI induced a decrease in the CFU-GM and BFU-E numbers after mice were exposed to 4 Gy IR, and SS31 treatment improved the colony formation ability in irradiated mice. To further explore the reconstitution ability of HSCs, we conducted a bone marrow cell transplantation experiment and detected the donor-derived cells 4 months after transplantation. After 4 Gy irradiation, the percentage of donor-derived cells in mouse peripheral blood was significantly decreased, and SS31 treatment increased the donor-derived cell chimerism in irradiated mice (Fig. 4B–C). Our study showed that SS31 can improve the proliferative and reconstitution ability of irradiated mouse HSPCs.

3.5. SS31 reduces ROS levels in irradiated mouse HSPCs

ROS contribute to IR-induced hematopoietic system injury; therefore, we detected H_2O_2-derived ROS, mitochondrial superoxide radicals and superoxide radicals using DCFH-DA, MitoSox, and DHE, respectively, in HPCs, LSK cells, HSCs and MPPs. As shown in Fig. 5, increased H_2O_2-derived ROS, mitochondrial superoxide radical and superoxide radical levels were observed in irradiated HPCs, LSK cells, HSCs and MPPs after mice were exposed to 4 Gy IR, and SS31 decreased the above ROS levels in these cells. Our results indicate that SS31 may protect against WBI-induced hematopoietic system injury through ROS scavenging in irradiated HSPCs.

4. Discussion

The hematopoietic system is one of the organs most sensitive to ionizing radiation, and a dose higher than 0.5 Gy can damage this system. The potential damage to the hematopoietic system includes acute and chronic damage, which ultimately leads to hematopoietic system senescence and leukemia [1]. Therefore, application of drugs that protect against radiation is an effective method to reduce IR-induced hematopoietic system damage.

Our results showed that SS31, a mitochondria-targeted peptide, can effectively alleviate IR-induced hematopoietic system damage, elevate the 30-day survival rate of mice exposed to lethal radiation, alleviate myelosuppression, and improve the proliferative and reconstitution ability of irradiated HSCs. SS31 is a mitochondria-targeted peptide, and our study suggested that alleviating radiation-induced mitochondrial damage may protect against IR-induced hematopoietic system injury. In fact, it has been reported that a mitochondria-targeted iron chelator protected skin against the deleterious effects of the UVA component of sunlight [15], mitochondria-targeted SkQR1 (plastoquinone conjugated through a hydrocarbon linker with cationic rhodamine 19) protected against nuclear DNA damage induced by gamma radiation in K562 erythroleukemia cells [16], and isofraxidin protected U937 leukemia cells from radiation-induced apoptosis via the ROS/mitochondria pathway [17]. Therefore, it seems that mitochondria-targeting compounds could be a promising strategy to protect against IR-induced damage.

We next detected ROS levels in HSPCs to explore the underlying radio-protective mechanisms of SS31. SS31 has been reported to scavenge various types of ROS in an injured brain [11]; in our study, SS31 not only scavenged mitochondrial superoxide radicals but also H_2O_2-derived ROS and superoxide radicals in LSK cells, HPCs, HSCs and MPPs. Thus, our experiments indicate that SS31 can provide protection against IR-induced hematopoietic system injury by scavenging several types of ROS.

IR instantaneously induces ROS production through water radiolysis; then, the ROS generate secondary injury in cells, tissues and organs. Yamamori and colleagues reported that IR upregulated mitochondrial ETC function and mitochondrial content, resulting in mitochondrial ROS production; IR was also shown to induce G2/M arrest, further increasing the mitochondrial ROS level by accumulating cells in the G2/M phase [18]. Because SS31 can scavenge free radicals, reduce ROS production in mitochondria, and inhibit translocation of the mitochondrial membrane and the release of cytochrome C [5–9], the mechanisms by which SS31 affects mitochondria merit further

exploration.

Abbreviations

IR	ionizing radiation
ROS	reactive oxygen species
WBI	whole-body irradiation
HSPCs	hematopoietic stem/progenitor cells
HSCs	hematopoietic stem cells
HPCs	hematopoietic progenitor cells
MPPs	multipotent progenitors
CMPs	common myeloid progenitors
MEPs	megakaryocyte/erythroid progenitors
CFU-GM	colony-forming units of granulocytes and macrophages
BFU-E	burst-forming unit-erythroid
WBCs	white blood cells
RBCs	red blood cells
PLTs	platelets
HGB	hemoglobin

Acknowledgments

This study was supported by the Key Project Plan of Medical Science Research in Hebei Province (20180774).

References

[1] L. Shao, Y. Luo, D. Zhou, Hematopoietic stem cell injury induced by ionizing radiation, Antioxid. Redox Signal. 20 (2014) 1447–1462.
[2] J. Zhang, X. Xue, X. Han, et al., Hydrogen-rich water ameliorates total body irradiation-induced hematopoietic stem cell injury by reducing hydroxyl radical, Oxidative Med. Cell. Longev. 2017 (2017) 8241678.
[3] G. Xu, H. Wu, J. Zhang, et al., Metformin ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice, Free Radic. Biol. Med. 87 (2015) 15–25.
[4] H. Zhang, Z. Zhai, Y. Wang, et al., Resveratrol ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice, Free Radic. Biol. Med. 54 (2013) 40–50.
[5] P.H. Reddy, M. Manczak, R. Kandimalla, Mitochondria-targeted small molecule SS31: a potential candidate for the treatment of Alzheimer's disease, Hum. Mol. Genet. 26 (2017) 1483–1496.
[6] S. Cho, H.H. Szeto, E. Kim, et al., A novel cell-permeable antioxidant peptide, SS31, attenuates ischemic brain injury by down-regulating CD36, J. Biol. Chem. 282 (2007) 4634–4642.
[7] D.A. Thomas, C. Stauffer, K. Zhao, et al., Mitochondrial targeting with antioxidant peptide SS-31 prevents mitochondrial depolarization, reduces islet cell apoptosis, increases islet cell yield, and improves posttransplantation function, J. Am. Soc. Nephrol. 18 (2007) 213–222.
[8] J. Huang, X. Li, M. Li, et al., Mitochondria-targeted antioxidant peptide SS31 protects the retinas of diabetic rats, Curr. Mol. Med. 13 (2013) 935–945.
[9] Y. Hou, S. Li, M. Wu, et al., Mitochondria-targeted peptide SS-31 attenuates renal injury via an antioxidant effect in diabetic nephropathy, Am. J. Physiol. Ren. Physiol. 310 (2016) F547–F559.
[10] J. Cai, Y. Jiang, M. Zhang, et al., Protective effects of mitochondrion-targeted peptide SS-31 against hind limb ischemia-reperfusion injury, J. Physiol. Biochem. 74 (2018) 335–343.
[11] Y. Zhu, H. Wang, J. Fang, et al., SS-31 provides neuroprotection by reversing mitochondrial dysfunction after traumatic brain injury, Oxidative Med. Cell. Longev. 2018 (2018) 4783602.
[12] M. Zhang, H. Zhao, J. Cai, et al., Chronic administration of mitochondrion-targeted peptide SS-31 prevents atherosclerotic development in ApoE knockout mice fed Western diet, PLoS One 12 (2017) e0185688.
[13] X.L. Xue, X.D. Han, Y. Li, et al., Astaxanthin attenuates total body irradiation-induced hematopoietic system injury in mice via inhibition of oxidative stress and apoptosis, Stem Cell Res Ther 8 (2017) 7.
[14] X. Han, X. Xue, Y. Zhao, et al., Rutin-enriched extract from Coriandrum sativum L. ameliorates ionizing radiation-induced hematopoietic injury, Int. J. Mol. Sci. 18 (2017).
[15] O. Reelfs, V. Abbate, R.C. Hider, C. Pourzand, A powerful mitochondria-targeted iron chelator affords high photoprotection against solar ultraviolet a radiation, J. Invest. Dermatol. 136 (2016) 1692–1700.
[16] E.K. Fetisova, M.M. Antoschina, V.D. Cherepanynets, et al., Radioprotective effects of mitochondria-targeted antioxidant SkQR1, Radiat. Res. 183 (2015) 64–71.
[17] P. Li, Q.L. Zhao, L.H. Wu, et al., Isofraxidin, a potent reactive oxygen species (ROS) scavenger, protects human leukemia cells from radiation-induced apoptosis via ROS/mitochondria pathway in p53-independent manner, Apoptosis 19 (2014) 1043–1053.
[18] T. Yamamori, H. Yasui, M. Yamazumi, et al., Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint, Free Radic. Biol. Med. 53 (2012) 260–270.