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Arabinoxylan rice bran (MGN-3/Biobran) alleviates radiation-induced intestinal barrier dysfunction of mice in a mitochondrion-dependent manner

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ABSTRACT

MGN-3 is an arabinoxylan from rice bran that has been shown to be an excellent antioxidant and radioprotector. This study examined the protective effects of MGN-3 on radiation-induced intestinal injury. Mice were treated with MGN-3 prior to irradiation, then continued to receive MGN-3 for 4 weeks thereafter. MGN-3 increased the activity of mitochondrial respiratory chain complexes I, III, IV and V, the intercellular ATP content, the mitochondria-encoded gene expression and mitochondrial copy numbers in the jejunal and colonic mucosa. MGN-3 reduced the oxidative stress levels and inflammatory response indicators in the serum and jejunal and colonic mucosa. Antioxidant indicators such as superoxide dismutase, glutathione peroxidase, catalase and total antioxidant capacity were significantly increased in the serum and jejunal and colonic mucosa in the MGN-3 group. Moreover, MGN-3 decreased the gene abundances and enzymatic activities of caspase-3, 8, 9 and 10 in the jejunal and colonic mucosa. The endotoxin, diamine peroxidase, D-lactate and zonulin levels were significantly reduced in the serum and jejunal and colonic mucosa in the MGN-3 group. MGN-3 also markedly upregulated the gene abundances of ZO-1, occludin, claudin-1 and mucin 2. MGN-3 effectively attenuated radiation-induced changes in the intestinal epithelial mitochondrial function, oxidative stress, inflammatory response, apoptosis, intestinal permeability and barrier function in mice. These findings add to our understanding of the potential mechanisms by which MGN-3 alleviates radioactive intestinal injury.

1. Introduction

Radiation therapy is widely used to treat various cancers. Despite advances in radiation therapy technology, the effective use of ionizing radiation (IR) is limited because of radiation damage to normal tissues [1]. IR causes water to decompose and promotes the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by nitrogen oxide synthase. Radiation can also lead to mitochondrial electron leakage, resulting in excessive ROS and superoxide [2]. The toxic effects of these molecules include DNA/RNA damage, amino acid oxidation, and lipid peroxidation, leading to intracellular nucleic acid damage, mutation, and protein and lipid damage [3,4]. Studies have shown that IR can cause adverse effects, including oxidative stress [5] and oxidative damage to macromolecular substances in cells [6–8], resulting in damage and functional loss of tissues and organs.

The intestine is one of the most sensitive organs to IR. Abdominal radiation therapy can cause acute and chronic intestinal injury, which manifests as radiation-induced intestinal injury, clinically known as radiation-induced intestinal disease [9,10], with the main symptoms including anorexia, vomiting, diarrhea, dehydration, systemic infection, septic shock and death [11]. Experimentally, radiation can increase intestinal epithelial permeability, induce oxidative stress in intestinal epithelial cells, and cause crypt and villous epithelial cell death [12,13]. High IR doses can even cause acute injury to intestinal epithelial cells and death within 10 days, reflecting the toxicity of IR to the intestinal tract [14]. Although the mortality and prevalence associated with radiation-induced intestinal damage have been studied, no recognized methods exist to prevent or treat radiation-induced intestinal disease, despite the available methods to prevent radiation-induced bone marrow injury [15]. Radiation-induced intestinal injury

Abbreviations: CAT, catalase; DAO, diamine peroxidase; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; IL, interleukin; IR, ionizing radiation; MDA, malondialdehyde; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TJ, tight junction; TNF-α, tumor necrosis factor-α

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negatively affects the therapeutic efficacy of patients and reduces their quality of life. Therefore, drugs that can prevent and treat radiation-induced intestinal damage are urgently needed.

The safety of many radioprotectors remains a major issue. Most existing synthetic radiation-protective compounds are potentially toxic, including aminothiols, zinc aspartate, cysteamine, and their derivatives [16–18]. In contrast to synthetic compounds, MGN-3 is an arabinoxylan in rice bran, a polysaccharide containing beta-1,4-xylosyl hemicellulose [19]. Subchronic toxicity studies, antigenic studies and genotoxicity tests in rats have shown that MGN-3 is a safe, nontoxic agent [20,21]. Because MGN-3 is an effective antioxidant in mice, it protects the hematopoietic system in mice by preventing free radical formation, regulating lipid peroxidation, enhancing the antioxidant defense system, and preventing oxidative stress [22]. However, whether MGN-3 has an excellent therapeutic effect on radiation-induced intestinal injury is unclear. In the present study, we hypothesized that MGN-3 uses distinct antioxidant properties to relieve radiation-induced intestinal diseases. To test this hypothesis, we systematically investigated the effects of MGN-3 pretreatment on intestinal mitochondrial function, ATP synthesis, oxidative-antioxidative status, inflammatory response status, intestinal epithelial cell apoptosis, intestinal permeability and barrier function in irradiated mice.

2. Material and methods

2.1. Animals and MGN-3/Biobran

Forty male C57BL/6 mice aged 8–10 weeks were purchased from the Institute of Model Animals of Nanjing University. All experimental mice were housed in a specific-pathogen-free environment, maintained at constant temperature and humidity, on a 12-h light/dark cycle, with free access to food and water, and were fed adaptively for at least 1 week before the experiment. The mice were maintained in accordance with the “Guidelines for the Protection and Application of Laboratory Animals” issued by the National Institutes of Health (NIH Publication No. 85-23, 1996 version) and the corresponding regulations of the Animal Management Committee of Jinling Hospital.

2.2. MGN-3/Biobran

MGN-3 is a modified rice bran extract treated enzymatically with an extract from shiitake mushrooms. It contains polysaccharide β1 and 4-xylopyronase hemicellulose. The main chemical structure of MGN-3 is arabinoxylan, with a xylose on its main chain and an arabinose polymer on its side chain [19]. MGN-3 was freshly prepared in a 0.9 % saline solution, and 0.1 mL of the solution was intraperitoneally injected into the mice at 40 mg/kg body weight/day every other day [23]. Treatment started on day 0 and continued throughout the experimental period. MGN-3 was provided by Daiwa Pharmaceuticals Co. Ltd., Tokyo, Japan.

2.3. Irradiation

C57BL/6 mice were anesthetized with 35 mg/kg of 1 % pentobarbital, then fixed on cardboard and subjected to local high-dose abdominal precision radiation (a 225 Kv/17 mA Cs137 linear accelerator at 2 Gy/min for 5 min and a single dose of 10 Gy). The radiation range was concentrated at the two-leg connection level to 2 cm above this area; the rest of the body was shielded with a 5-cm lead.

2.4. Experimental design and sample collection

The 40 mice were divided into four groups (CON, IR, IR + MGN-3 and MGN-3). The CON group served as the untreated vehicle saline control group (receiving neither irradiation nor MGN-3). The MGN-3 group received only MGN-3 every other day. The IR group received only irradiation at 2 weeks after the beginning of the experiment. The IR + MGN-3 group was pretreated with MGN-3 for 2 weeks then exposed to irradiation and continued to receive MGN-3 every other day.

2.5. Sample collection

Animals from all groups were euthanized 4 weeks after radiation. To reduce sample variability, the jejunal and colonic segments were collected approximately from the middle of the organ, then rinsed with phosphate-buffered saline to remove the intestinal contents. The intestinal epithelium was separated from the muscular layers by blunt dissection and stored at −80℃ prior to further analysis. Blood samples were collected to obtain the serum, which was stored at -80℃ for further analysis.

2.6. Pretreatment of jejunal and colonic samples

One hundred milligrams of the frozen jejunal and colonic mucosal specimens were minced and homogenized in 1 mL of ice-cold cytoplasm RIPA containing complete EDTA-free protease inhibitor cocktail (Roche, Penzberg, Germany). The homogenates were centrifuged at 12,000*g* for 15 min at 4℃, then the supernatant was collected. Protein concentration was determined using a BCA Protein Assay kit (Pierce, Rockford, IL, USA), then the protein was diluted to the same concentration for subsequent analysis.

2.7. Determination of mitochondrial respiratory chain complex activity and ATP content

The activities of intracellular mitochondrial respiratory chain complexes I–V were evaluated with mitochondrial respiratory chain complex assay kits (Suzhou Comin Biotechnology Ltd., China), following the manufacturer's instructions. Intracellular ATP content was measured with the firefly luciferase ATP assay kit (S0026, Beyotime Biotechnology, China) as described previously [24].

2.8. Determination of mitochondrial copy numbers

Total DNA was isolated from 200 mg of each colonic mucosal and liver specimen, and the mtDNA copy number was determined using real-time polymerase chain reaction (PCR) as previously described [25]. For DNA extraction, colonic mucosal and liver specimens were incubated in a lysis solution containing 0.5 mol/L of EDTA at pH 8.0 and 2 mg/mL of proteinase K (Amresco, Solon, OH, USA) at 37 ˚C for 50 min. Specific primers of the mtDNA-encoded genes (Table 1) were amplified. The mtDNA copy numbers were calculated using the $2^{-\triangle\triangle Ct}$ method relative to the nuclear-encoded reference gene, GAPDH.

2.9. Determination of oxidative status

ROS, RNS, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents were determined using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai, China) per the manufacturer’s instructions.

2.10. Determination of antioxidative status

The superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, catalase (CAT) activity and total antioxidant capacity (T-AOC) were determined using ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai, China) per the manufacturer’s instructions.

2.11. Determination of inflammatory status

Interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor-α (TNF-α)

Table 1
Primer sequences used for the mitochondria-encoded genes.

Target genes	Primer forward/reverse	Primer sequence (5′→3′)
COX1	Forward	ACTGTGGGACCCAGATGAAG
	Reverse	AAGGTGGATGATCCAGGGTT
COX2	Forward	CTTGGTGGCAAGCCGTGA
	Reverse	CTTGACAGTCGAAGTGTCAC
COX3	Forward	GCCTATCCTGCTCATCCAC
	Reverse	TCTCACCTGCTAATCGGGAC
ND1	Forward	AGCTTGTTAAGCAGGGCAGC
	Reverse	ACTTGGCGCAGCAAGTGGTA
ND2	Forward	GTGAACCGTGGACAGGAAAA
	Reverse	ACAGATTTCCTCCCGCTCAGATT
ND3	Forward	GTGGACAGGAAAAGAACCGT
	Reverse	GAACCGTTAAGCAGGCTAAA
ND4	Forward	GTAAAGAACCGTGGACAGGA
	Reverse	GAACCGAGGCTAAATTAAGC
ND5	Forward	ACCCAGATGAAGTGTGGGAC
	Reverse	AAGGTGGGTTATGATCCAGG
ND6	Forward	CTGTGAAGCCACACTCGCTA
	Reverse	AGGGCCCAAAGCAAAGGTAT
ND4L	Forward	ACGCTGAAGTGGAATGGTCC
	Reverse	GGGAATCCGGCATCAAGTCA
CYTB	Forward	CTGGCAACAGCTTCTTCCCT
	Reverse	CAAGCGGGGAAGCCCATC
ATP6	Forward	ACTTGGGCCCTCTTCTTTGG
	Reverse	TAAGACTCCAGAAGACCTGGAT
ATP8	Forward	AGGCCACCTTGGCATCTTTT
	Reverse	TTCAATGGCCGAGGCAGAAT

Table 2
Primer sequences used for the nuclear-encoded genes.

Target genes	Primer forward/reverse	Primer sequence (5′→3′)
GAPDH	Forward	GAAGACTGTGGGACCCAGAT
	Reverse	AAGGTGGATGATGTTCCAGG
Caspase-3	Forward	AGCACGCCTCCCATTCTCAAT
	Reverse	TGCTAGGCTTGCTGCTAGTAGG
Caspase-8	Forward	CTGAGGAGCTACGGTCATCACA
	Reverse	GCTGCGAGGGCGGTAATGAT
Caspase-9	Forward	ATCGGAGGGTGAGGAGGGCTAA
	Reverse	GTTGTGGTTGCTGAGCTGTGGA
Caspase-10	Forward	GATCGCCCTTGCAGGGTTACTT
	Reverse	CTAGTGCAGCTTCGCAGGCT
ZO-1	Forward	GCCCACCTATCCTGCTCAT
	Reverse	CCTGCTCTCATAATCGGGAC
Occludin	Forward	GGCAGCAGCTTGTTAAGCAG
	Reverse	ACTTGGCGCAGTGGTAAGCA
Claudin-1	Forward	GTGGGAAAAAACCGTGGACA
	Reverse	CTCCCACAGATTTCGCTCAGATT
Mucin 1	Forward	CAGATTCTCCCCTCAATCGGATT
	Reverse	CCCATAAGGACTGCTCTTCG
Mucin 2	Forward	AGCCCTCATACCCCAATGTCT
	Reverse	GGCCTATGTTGCTGTACTAGGG
Mucin 4	Forward	CTGGCTAGGGTCGCACACATAA
	Reverse	GAGGGCTGCGTAATGATGGC

levels were determined using ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai, China) per the manufacturer's instructions.

2.12. Determination of caspase activity

Caspase-3, 8, 9 and 10 activities were determined using ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai, China) per the manufacturer's instructions.

2.13. Determination of intestinal permeability

Endotoxin, diamine peroxidase (DAO), D-lactate and zonulin contents were determined using porcine ELISA kits (Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai, China) per the manufacturer's instructions.

2.14. RNA isolation, cDNA synthesis and real-time quantitative PCR

Total RNA was extracted from 100 mg of each jejunal and colonic mucosal and liver specimen using TRIzol Reagent. RNA concentration and quality were measured using a NanoDrop ND-1000 spectrophotometer (Thermo, USA). Next, 2 mg of total RNA was treated with RNase-Free DNase and reverse transcribed per the manufacturer's instructions. Two microliters of diluted cDNA (1:20, vol/vol) was used for real-time PCR, which was performed in an Mx3000P (Stratagene, USA). GAPDH, which is unaffected by the experimental factors, was chosen as the reference gene. All primers used in this study (Table 2) were synthesized by Generay Company (Shanghai, China). The $2^{-\triangle\triangle Ct}$ method was used to analyze the real-time PCR results, and the gene mRNA levels were expressed as the fold-change relative to the mean value of the control group.

2.15. Statistical analysis

Data are presented as the means ± SEM. Statistical significance was assessed via independent sample *t*-tests using SPSS (SPSS v. 20.0, SPSS Inc., Chicago, IL, USA). Data were considered statistically significant at $P < 0.05$. Numbers of replicates used for the statistics are noted in the figures.

3. Results

3.1. Effect of MGN-3 on mitochondrial respiratory chain complex activity and ATP content after irradiation

To investigate the effect of MGN-3 on mitochondrial function in the intestinal epithelium after radiation, we examined mitochondrial respiratory chain complex activity and intercellular ATP production in the jejunal and colonic mucosa of mice. Radiation significantly reduced the activity of the jejunal and colonic mitochondrial respiratory chain complexes I, III, IV and V, while MGN-3 restored the normal activity of these complexes ($P < 0.05$; Fig. 1A, C, D and E). However, radiation and MGN-3 did not affect the activity of the mitochondrial respiratory chain complex II ($P > 0.05$; Fig. 1B). In addition, radiation significantly reduced the intercellular ATP content in the jejunal and colonic mucosa, but this reduction was rescued by MGN-3 ($P < 0.05$; Fig. 1F).

3.2. Effects of MGN-3 on mitochondrial function after irradiation

To demonstrate the effect of MGN-3 on mitochondrial function in the intestinal epithelium after radiation, we determined mitochondrial-encoded genes and mitochondrial copy numbers in the jejunal and colonic mucosa of mice. Thirteen mitochondria-encoded genes and mitochondrial copy numbers in the jejunal and colonic mucosa of mice were significantly reduced after irradiation ($P < 0.05$; Fig. 2A–D). MGN-3 increased the abundances of mitochondria-encoded genes and mitochondrial copy numbers in the jejunal and colonic mucosa of mice in the MGN-3 + IR group compared with those of the IR group ($P < 0.05$; Fig. 2A–D).

3.3. Effect of MGN-3 on oxidative status after irradiation

To evaluate the effect of MGN-3 on oxidative status in the intestinal epithelium after radiation, we examined levels of the chemical markers, ROS, RNS, MDA and H_2O_2, in the serum and jejunal and colonic mucosa of mice. Radiation markedly increased the serum ROS, RNS, MDA and H_2O_2 levels ($P < 0.05$; Fig. 3A–D). MGN-3 decreased the serum ROS, RNS, MDA and H_2O_2 contents compared with those of the IR group

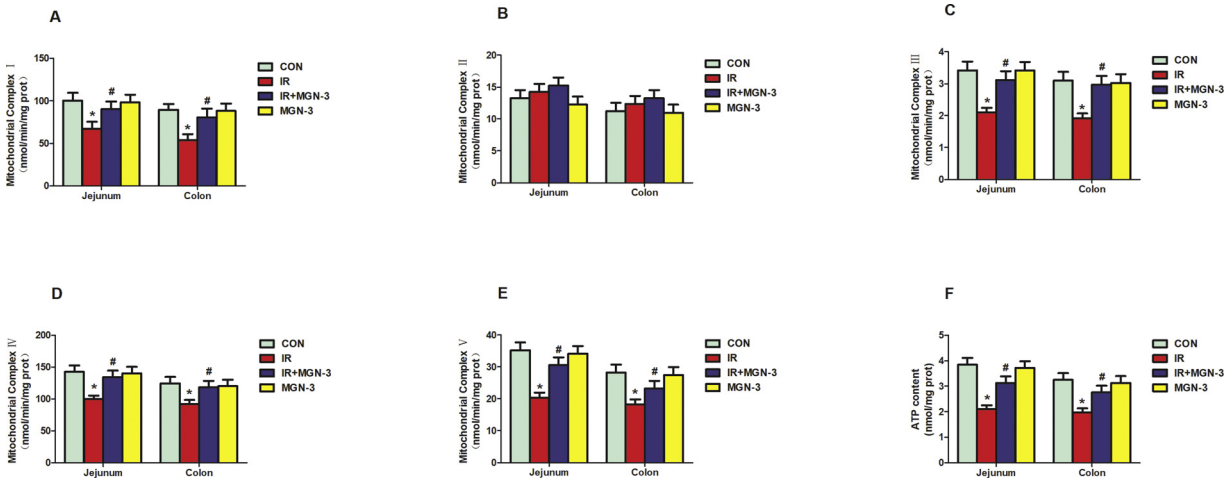

Fig. 1. Effect of MGN-3 on mitochondrial respiratory chain complex activity and ATP content after irradiation. Activities of the mitochondrial respiratory chain complexes I–V (A–E) and intracellular ATP production (F). Values are the mean ± standard error (n = 10). *$P < 0.05$ vs. CON group. #$P < 0.05$ vs. IR group.

($P < 0.05$; Fig. 3A–D). Consistently, the ROS, RNS, MDA and H_2O_2 contents in the jejunal and colonic mucosa of the IR group were significantly higher than those of the CON group, while MGN-3 restored these levels in the IR + MGN-3 group to those of the CON group ($P < 0.05$; Fig. 3E–H).

3.4. Effect of MGN-3 on antioxidative status after irradiation

To explore the effect of MGN-3 on the antioxidative status in the intestinal epithelium after radiation, we examined the activities of the chemical markers, SOD, GPx, CAT, and T-AOC, in the serum and jejunal and colonic mucosa of mice. Radiation markedly reduced the serum SOD, GPx, CAT, and T-AOC activities ($P < 0.05$; Fig. 4A–D). MGN-3 increased the serum SOD, GPx, CAT, and T-AOC activities compared with those of the IR group ($P < 0.05$; Fig. 4A–D). Similarly, the SOD, GPx, CAT, and T-AOC activities in the jejunal and colonic mucosa of the IR group was significantly lower than those of the CON group, while MGN-3 restored the activity levels in the IR + MGN-3 group to those of the CON group ($P < 0.05$; Fig. 4E–H).

3.5. Effect of MGN-3 on proinflammatory cytokines after irradiation

To demonstrate the effect of MGN-3 on inflammatory responses in the intestinal epithelium after radiation, we determined the levels of proinflammatory cytokines in the serum and jejunal and colonic mucosa of mice. Radiation markedly increased the serum IL-1β, IL-6, IL-8 and TNF-α levels ($P < 0.05$; Fig. 5A–D). MGN-3 reduced the serum IL-1β, IL-6, IL-8 and TNF-α levels compared with those of the IR group ($P < 0.05$; Fig. 5A–D). Similarly, the IL-1β, IL-6, IL-8 and TNF-α contents in the jejunal and colonic mucosa of the IR group were significantly higher than those of the CON group, while MGN-3 restored the contents of the IR + MGN-3 group to those of the CON group ($P < 0.05$; Fig. 5E–H).

3.6. Effect of MGN-3 on apoptotic status after irradiation

To investigate the effect of MGN-3 on apoptotic status in the intestinal epithelium after radiation, we examined the gene expression and activity of caspase-3, 8, 9 and 10 in the jejunal and colonic mucosa of mice. The gene abundances of caspase-3, 8, 9 and 10 in the jejunal and colonic mucosa of the IR group were significantly upregulated

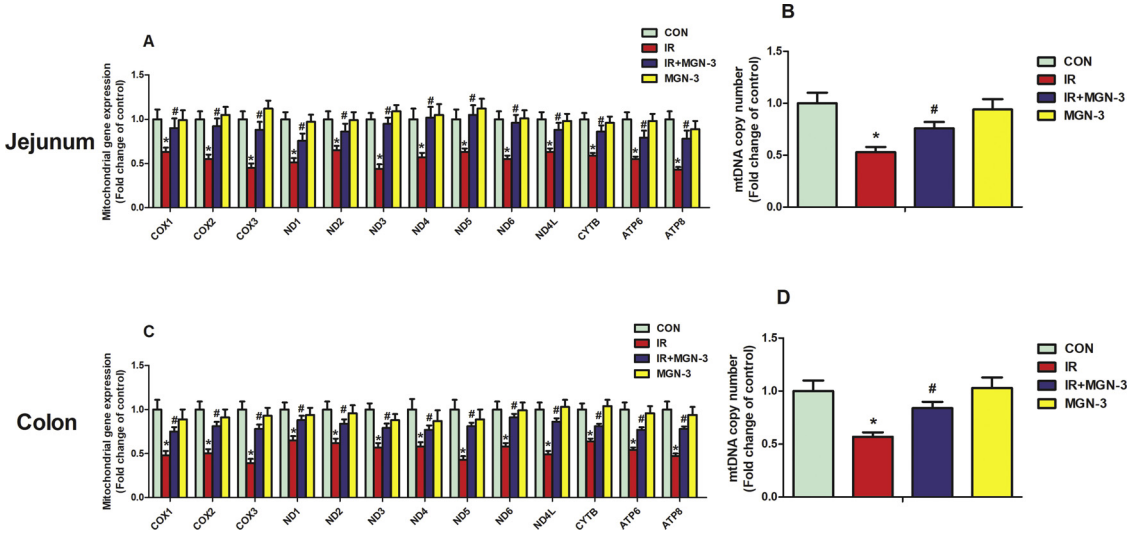

Fig. 2. Effects of MGN-3 on mitochondrial function after irradiation. Jejunal mitochondrial-encoded gene expression (A) and mitochondrial copy numbers (B). Colonic mitochondrial-encoded gene expression (C) and mitochondrial copy numbers (D). Values are the mean ± standard error (n = 10). *$P < 0.05$ vs. CON group. #$P < 0.05$ vs. IR group.

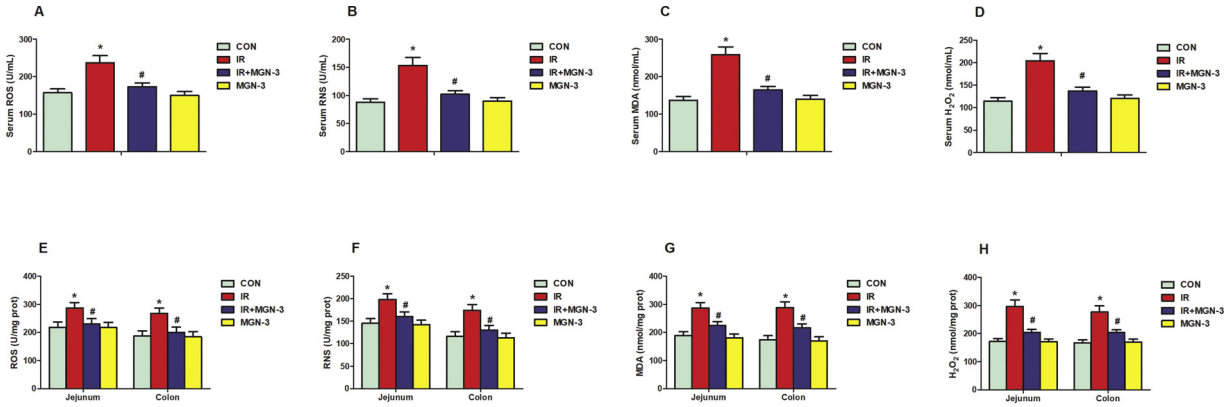

Fig. 3. Effect of MGN-3 on oxidative status after irradiation. Serum levels of ROS (A), RNA (B), MDA (C) and H_2O_2 (D). Jejunal and colonic ROS (E), RNA (F), MDA (G) and H_2O_2 (H) levels. Values are the mean ± standard error (n = 10). $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. IR group.

compared with those of the CON group ($P < 0.05$; Fig. 6A–D). MGN-3 reduced the caspase-3, 8, 9 and 10 gene expression compared with that of the IR group ($P < 0.05$; Fig. 6A–D). Consistent with the gene expression results, caspase-3, 8, 9 and 10 activity in the jejunal and colonic mucosa of the IR group was significantly higher than that in the CON group, and MGN-3 rescued the caspase activity in the IR + MGN-3 group to the level of the CON group ($P < 0.05$; Fig. 6E–H).

3.7. Effect of MGN-3 on intestinal permeability after irradiation

To evaluate the effect of MGN-3 on intestinal permeability after radiation, we examined the levels of the chemical markers, endotoxin, DAO, D-lactate and zonulin, in the serum and jejunal and colonic mucosa of mice. Radiation markedly increased the serum levels of these markers ($P < 0.05$; Fig. 7A–D), while MGN-3 decreased them compared with the IR group ($P < 0.05$; Fig. 7A–D). Similarly, the endotoxin, DAO, D-lactate and zonulin contents in the jejunal and colonic mucosa of the IR group were significantly higher than those of the CON group, while MGN-3 restored these contents compared with those of the CON group ($P < 0.05$; Fig. 7E–H).

3.8. Effect of MGN-3 on intestinal barrier function after irradiation

To demonstrate the effect of MGN-3 on intestinal barrier function after radiation, we determined the expressions of tight junction proteins (ZO-1, occludin and claudin-1) and mucins (mucin 1, 2 and 4) in the jejunal and colonic mucosa of mice. Radiation significantly reduced the gene expressions of ZO-1, occludin and claudin-1 in the jejunal and colonic mucosa, while MGN-3 restored the expressions of these tight junction proteins ($P < 0.05$; Fig. 8A–C). In addition, radiation significantly reduced the mucin 2 mRNA abundance in the jejunal and colonic mucosa, while MGN-3 rescued the mucin 2 mRNA abundance ($P < 0.05$; Fig. 8E). However, neither radiation nor MGN-3 affected the mucin 1 and 4 expressions ($P > 0.05$; Fig. 8D and F).

4. Discussion

IR produces many ROS. Excessive ROS attack cell macromolecules, such as DNA, lipids, and proteins, and induce a series of harmful biological reactions, such as apoptosis, inflammation, and autoimmune reactions, ultimately impairing several organs and systems and even causing death [26]. The intestinal epithelium is fast-renewing, and IR-induced ROS production also attacks intestinal stem cells and damages the intestinal epithelium, subsequently causing intestinal and systemic diseases [27]. Thus, effective methods and drugs that can mitigate radiation-induced intestinal injuries are urgently needed in cancer therapy. Many studies have reported that MGN-3 is a potential radioprotector because of its powerful antioxidant capacity [28,29]. In the present study, we evaluated the protective effects of MGN-3 on radiation-induced intestinal injuries. IR exposure induced mitochondrial dysfunction, impaired ATP synthesis, promoted oxidative stress, inhibited antioxidant capacity, enhanced apoptosis and increased intestinal permeability. However, MGN-3 treatment adequately protected all areas of the intestine from radiation damage and restored the intestinal permeability to normal physiological levels.

Mitochondria are the main sites of intracellular oxidative

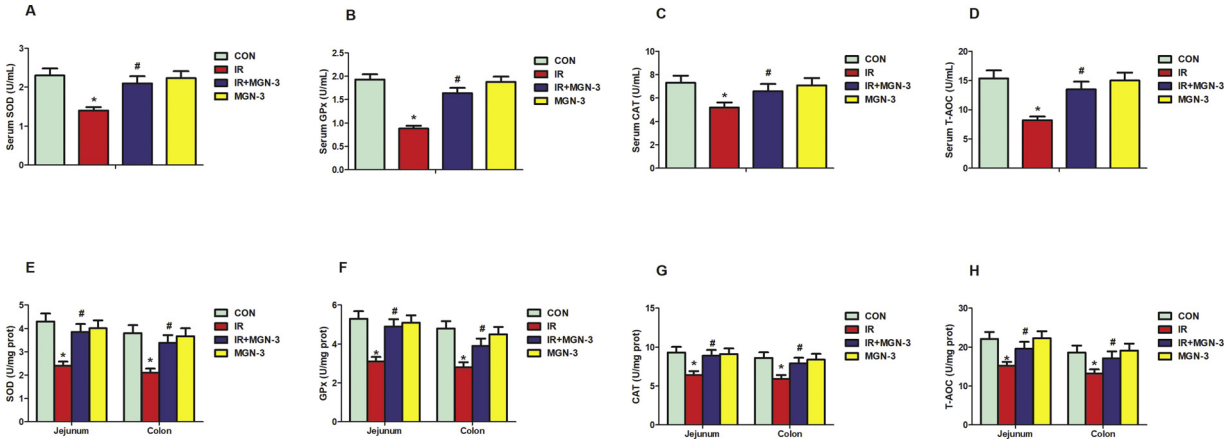

Fig. 4. Effect of MGN-3 on antioxidative status after irradiation. Serum SOD (A), GPx (B), CAT (C) and T-AOC (D) levels. Jejunal and colonic SOD (E), GPx (F), CAT (G) and T-AOC (H) levels. Values are the mean ± standard error (n = 10). $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. IR group.

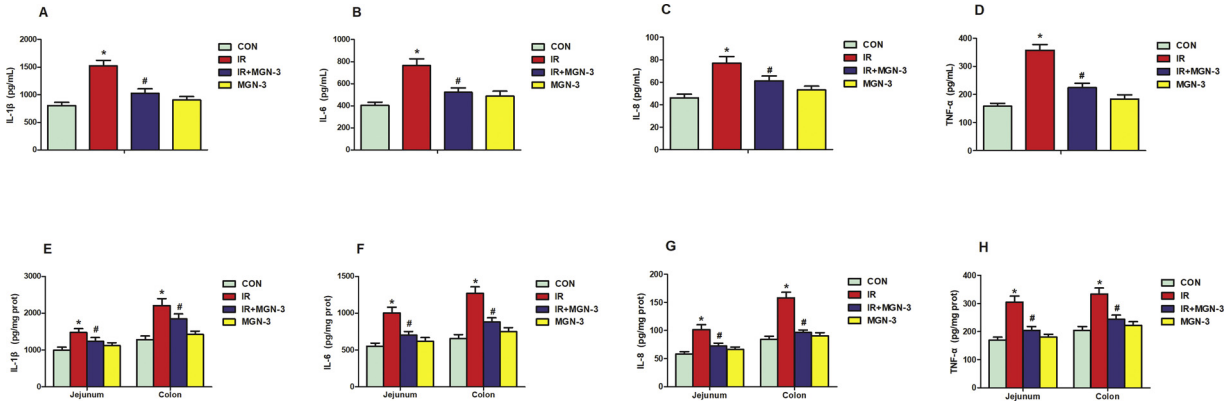

Fig. 5. Effect of MGN-3 on proinflammatory cytokines after irradiation. Serum IL-1β (A), IL-6 (B), IL-8 (C) and TNF-α (D) levels. Jejunal and colonic IL-1β (E), IL-6 (F), IL-8 (G) and TNF-α (H) levels. Values are the mean ± standard error (n = 10). $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. IR group.

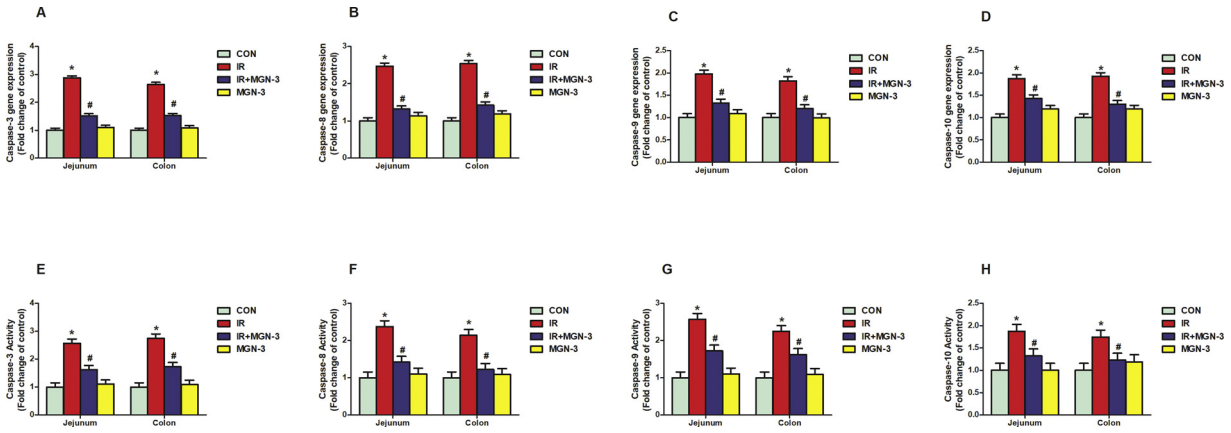

Fig. 6. Effect of MGN-3 on apoptotic status after irradiation. Jejunal and colonic gene expression (A–D) and enzyme activity (E–H) of caspase. Values are the mean ± standard error (n = 10). $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. IR group.

phosphorylation and ATP synthesis. Oxygen is converted to ATP when it passes through the mitochondrial respiratory chain complex, and the mitochondria supply 95 % of the energy required for cellular activities [24,30,31]. The mitochondrial respiratory chain is the most important source of ROS. Excessively produced peroxides, if not eliminated by endogenous antioxidants, can cause oxidative stress damage and mitochondrial dysfunction [32]. Previous studies have shown that the mitochondria are among the organelles most sensitive to IR, and IR induces mitochondrial dysfunction [33,34]. In the present study, IR significantly reduced the activity of the intestinal mitochondrial respiratory chain complexes I, III, and IV, while MGN-3 restored the normal activity of these complexes. The mitochondrial respiratory chain is divided into the main and secondary respiratory chains, wherein the main respiratory chain is composed of complexes I, III and IV, and the secondary respiratory chain is composed of complexes II, III and IV [35]. The mitochondrial respiratory chain complex V is an ATP synthase [36]. In this study, MGN-3 had an excellent mitigating effect on IR-induced reduction of mitochondrial respiratory chain complex V

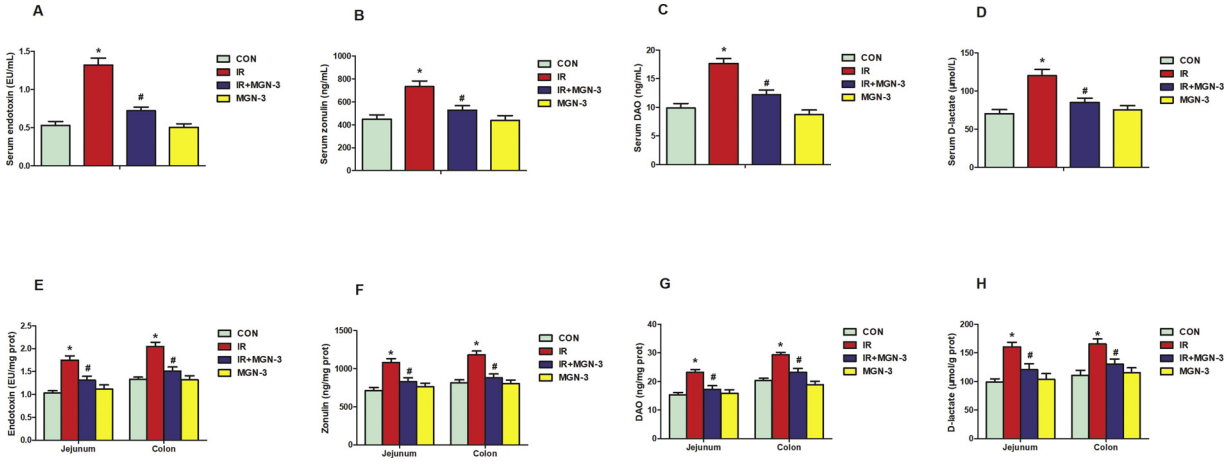

Fig. 7. Effect of MGN-3 on intestinal permeability after irradiation. Serum endotoxin (A), zonulin (B), DAO (C) and D-lactate (D) levels. Jejunal and colonic endotoxin (E), zonulin (F), DAO (G) and D-lactate (H) levels. Values are the mean ± standard error (n = 10). $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. IR group.

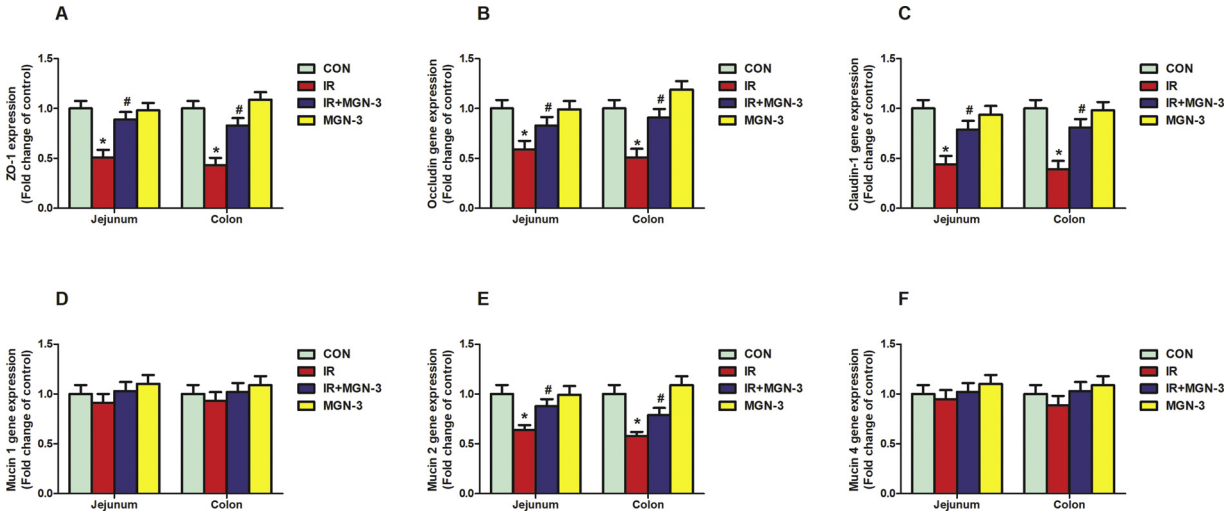

Fig. 8. Effect of MGN-3 on intestinal barrier function after irradiation. Jejunal and colonic gene expressions of ZO-1 (A), occludin (B), claudin-1 (C), mucin 1 (D), mucin 2 (E) and mucin 4 (F). Values are the mean ± standard error (n = 10). $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. IR group.

and intracellular ATP levels. MGN-3 also alleviated the adverse effects of IR on mitochondrial coding genes and mitochondrial copy numbers. These findings indicated that MGN-3 has a superior therapeutic effect on IR-induced mitochondrial dysfunction and ATP synthesis disorders.

IR-induced organ damage is mainly caused by free radicals, which play important roles in the radiation effects on biological tissues and organisms. ROS produced under physiological conditions are important signaling molecules that regulate the biochemical processes of cells, and excess free radicals produced by IR have harmful effects [37]. Consistently, our data showed that IR caused changes in the oxidative status, which was characterized by elevated ROS, RNA, MDA and H_2O_2 levels in the serum, jejunums and colons of mice. Organisms have antioxidant defense systems that scavenge free radicals, including enzymes and nonenzymatic antioxidant defense mechanisms. For example, SOD converts O_2 into H_2O_2 through disproportionation, and GPx and CAT convert H_2O_2 into H_2O and O_2 [38,39]. In the current study, the antioxidant capacities of mice were inhibited in the IR group, mainly due to significantly decreased SOD, GPx, CAT and T-AOC enzyme activities in the serum, jejunum and colon. The exact mechanism underlying the effect of MGN-3 is uncertain but may be associated with the effects of the antioxidant properties of MGN-3 [22]. Regarding antioxidant activity, our data showed that MGN-3 significantly reduced the ROS, RNA, MDA and H_2O_2 levels in the serum, jejunum and colon, while the SOD, GPx, CAT and T-AOC activities were simultaneously significantly increased after MGN-3 pretreatment. These findings showed that MGN-3 exerted antioxidant activity to regulate the oxidant-antioxidant balance in the radiated mice.

Apoptosis is programmed cell death, including controlled breakdown of intracellular components. The apoptotic mechanisms are highly complex, and the mitochondria-induced apoptotic pathway is one of the classic mechanisms [30]. The main feature of the mitochondrial pathway is the loss of mitochondrial integrity and transmembrane potential, leading to caspase-3 protein activation, DNA fragmentation, and cell death [40]. Previous studies have shown that IR-induced tissue damage, such as intestinal damage, increases with apoptotic cells [26,41]. Our results showed that IR significantly upregulated the gene expression and enzyme activity of the mitochondrial apoptotic pathway-related proapoptotic caspases (caspase-3, 8, 9 and 10) in the jejunal and colonic tissues of mice, while MGN-3 countered these IR-induced effects. These findings indicate that MGN-3 attenuated IR-induced apoptosis of intestinal epithelial cells by inhibiting the mitochondrial apoptotic pathway.

Because intestinal epithelial cells proliferate rapidly, the intestine is one of the most sensitive organs to radiation. The biological functions of the intestine include absorbing water and nutrients, regulating ion homeostasis, and preventing the invasion of harmful pathogens. Therefore, the intestinal mucosa must have a complex barrier function to selectively permeate the substrates in the intestinal lumen. Radioprotectors can protect against IR-induced damage by regulating intestinal permeability [42,43]. The chemical markers, endotoxin, DAO, D-lactate and zonulin, are usually circulated in low levels in healthy individuals but will significantly increase in the circulation when the intestinal barrier is destroyed [44]. Our data showed that the levels of endotoxin, DAO, D-lactate and zonulin were significantly increased in the serum and jejunal and colonic tissues of radiated mice. In addition, the endotoxin, DAO, D-lactate and zonulin contents in the serum and jejunal and colonic tissues of MGN-3-treated mice were significantly lower than those in radiated mice. Moreover, IR significantly inhibited the expression of tight junction proteins and mucins, whereas MGN-3 effectively reversed these biological processes. These results indicate that MGN-3 can rescue IR-induced intestinal barrier dysfunction.

In summary, our results indicate that MGN-3 is a safe drug with free radical-scavenging abilities. Owing to its excellent antioxidant capacity, MGN-3 effectively protected mice against radiation-induced intestinal barrier dysfunction. These results suggest that MGN-3 can alleviate radiation-induced intestinal injury.

Authors' contributions

Z.Z. performed the experiments and drafted the manuscript; W.C. performed the experiments and analyzed the data; W.Q. contributed to the experimental design and revised the manuscript; and K.W. conceived the ideas, designed the experiments, and finalized the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare no financial, personal, or professional interests that would have influenced the content of the manuscript or interfered with objectively assessing the results.

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