6-Shogaol ameliorates injury to the intestinal mucosa and increases survival after high-dose abdominal irradiation

Jinhan Wang a,b,1, Ming Yao a,1, Yan Wang a, Chi-Tang Ho c, Shiming Li c, Yang Shi d, Qiang Liu a,b, Hui Zhao b,a

a Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300192, China
b Tianjin Key Laboratory of Food and Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China
c Department of Food Science, Rutgers University, New Brunswick, NJ 08901, USA
d Tsingdao Lihe Extrat Science & Technology Co. Ltd, Tsingdao 26611, China

ARTICLE INFO

Article history:
Received 30 March 2017
Received in revised form 23 June 2017
Accepted 25 June 2017
Available online 30 June 2017

Keywords:
6-Shogaol
Radiation-induced intestinal injury
Anti-inflammatory
Antioxidant
Bacterial translocation

ABSTRACT

Gastrointestinal mucosal damage is a catastrophic effect of abdominal or pelvic radiation used in cancer therapy or pretreatment for hematopoietic stem cell transplantation. 6-Shogaol is the constituent of ginger biophenolic, possesses both anti-inflammatory and antioxidant effects. We therefore investigated 6-shogaol’s candidacy as a protector against radiation-induced intestinal injury. Herein, we found that pretreatment with 6-shogaol improved animal survival and intestinal function following irradiation injury. Furthermore, the potential radioprotective role of 6-shogaol may be partially attributed to its antioxidant and anti-inflammatory properties, which alleviated radiation toxicity to the gastrointestinal tract. Additionally, we observed that 6-shogaol might reduce bacterial translocation and endotoxin levels following abdominal irradiation, and thereby protect against radiation-induced intestinal injury. Our results show a potential role for 6-shogaol as a protective agent to obviate the treatment-limiting intestinal side-effects and thereby may be useful in radiotherapy of patients.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Intestinal radiation injury most often develops following abdominal or pelvic radiation therapy provided as part of the treatment for cancer or pretreatment in hematopoietic stem cells transplantation (Guo et al., 2014; Hauer-Jensen, Denham, & Andreyev, 2014; Stacey & Green, 2014). This type of injury not only affects quality of life but can also be life threatening in some cases, like when patients receive high doses of acute radiation or accumulated dosage of radiation. Although radiation can cause a variety of gastrointestinal (GI) injury, because the tissue of the small intestine is enriched with highly proliferating cells that are typically more radiosensitive, the doses required to cause small intestinal injury are very close to therapeutic doses. The nature of radiation therapy including its mobility and the difficulty in defining the treated area, increases the risk of injury to the small bowel. Patients suffering from radiation-induced small bowel disease usually present with abdominal discomfort or cramping pain, nausea, vomiting, diarrhea, and fecal urgency during or shortly after a course of radiotherapy (Nair, Parida, & Nomura, 2001).

Ionizing radiation induces biological effects via a series of molecular events, set off by reactive oxygen species (ROS). These free radicals, including \( \cdot \text{OH}, \cdot \text{HO}_2, \cdot \text{O}_2, \) and \( \cdot \text{H}_2\text{O} \), can cause oxidative damage to biological molecules, including DNA and many proteins in membranes, resulting in the dysfunction/mal-functioning of biological processes and sometimes even cell death (Nair et al., 2001). An increased rate of cell death in the intestinal epithelium results in villous denudation, the damage of mucosal barrier, and physiological inflammation. When the mucosal barrier becomes disrupted, bacterial-derived products are able to invade tissues. More seriously, translocation of bacteria may result in fatal problems such as cytokine-mediated systemic inflammatory response syndrome (SIRS), multiple organ failure (MOF) and eventually death (Kainthola, Gupta, & Agrawala, 2016).
Synthetic radioprotectors like sulfhydryl-containing free-radical-scavenging compounds were discovered in the beginning of the nuclear era, yet due to various reasons, an ideal radioprotector remains elusive (Chen et al., 2016; Hosseinimehr, 2007). In the last two decades, some natural plant-derived compounds have been demonstrated to possess properties that provide promising radiation countermeasures based on their proven therapeutic potential and the combination of efficacy (Song, Yan, & Cai, 2006). Ginger is not only a spice for food processing, but also a traditional Chinese medicine (TCM). As a spice, ginger has been widely used as a food condiment in many food industry, including cocoa-cola, gingerbread, candy and wine (Nandagopal & Nair, 2013; Nath, Deka, Jha, Paul, & Misra, 2013; Ravindran & Babu, 2016). Moreover, ginger is one of the useful drugs in TCM, and it has been shown to be effective in the prevention of radiotherapy-induced nausea and vomiting (Haniadka et al., 2013). When the daily intake reaches up to 2.0 g in both animals and humans, Ginger and its constituents have showed very low levels of toxicity and high levels of tolerability (Chrubasik, Pittler, & Roufogalis, 2005). Shogaols are the constituent ginger biophenolics and possess both anti-inflammatory and antioxidant effects (Brahmbhatt, Gundala, Asif, Shamsi, & Aneja, 2013). Recently, 6-shogaol, a pungent constituent of ginger, has been shown to possess a gastro-protective effect against HCl/ethanol-induced gastric lesions (Haniadka et al., 2013). Therefore, it is worth investigating whether or not 6-shogaol may be a candidate protector against radiation-induced intestinal injury.

In the present study, we isolated 6-shogaol from ginger oleoresin and designed to evaluate its radioprotective effects in a model for abdomen irradiation (ABI). We found that 6-shogaol protected against radiation toxicity in the intestinal mucosal barrier. More importantly, 6-shogaol reduced the release of cytokines and inflammatory mediators and translocation of bacteria in small intestinal.

2. Material and methods

2.1. Preparation and identification of 6-shogaol

Ginger oleoresin was isolated from ginger as previously described (Tao et al., 2013). 6-Shogaol from ginger oleoresin was subjected to preparative HPLC using a Pursuite ERS C18 (250 × 4.6 mm) stationary phase column with a particle size of 5 μm. The flow rate was 1.0 mL/min, fractions (10%) in acetonitrile were injected in 500–1000 mL, the isocratic mode was acetonitrile: water (40:60 v/v), and the UV detector was at 280 nm. The fractions eluted were collected, separately, pooled, and evaporated under N2 to obtain purified components. An HPLC fingerprint chromatogram was established for quality control and the purity of the 6-shogaol obtained was confirmed to be 98.4% (Fig. 1) by using the area normalization methods.

2.2. Cells

Rat intestinal epithelial IEC-6 cells (CRL-1592, passage 28) were obtained from the National Platform of Experimental Cell Resource for Sci-Tech (http://cellresource.cn/) (Beijing, China). IEC-6 cells used in all experiments were at or before the 32th passage.

2.3. Radiation-induced intestinal injury model

Male C57BL/6j mice, 6–8 weeks old, weighing 20–22 g, were purchased from Vital River Laboratory Animal Technology Co. Ltd. (China). All mice were housed in a temperature controlled, with a 12 h light/dark cycle, and fed standard chow and water.

Mice were maintained in the certified animal facility of the Institute of Radiation Medicine of the Chinese Academy of Medical Sciences (CAMS). All animal experiments have been approved by the ethics committee of Tianjin University of Commerce and Certification Materials will be provided upon request.

6-Shogaol was dissolved in 3% dimethyl sulfoxide (DMSO) and 6% Tween-20 in 1× phosphate-buffered saline (PBS). PBS (including both 3% DMSO and 6% Tween-20) was used as the vehicle. ABI was performed as described previously (Gong et al., 2016).

2.4. Health status monitoring

Post irradiation survival analysis was carried out by exposing mice to ABI 15 Gy. The mice were monitored up to 30 days after ABI and the body weight of mice was recorded twice daily. Diarrhea scores were recorded as 0, 1, 2, or 3, where 0 is normal stool consistency, 1 is loose stools, 2 is overt diarrhea with perianal soilage, and 3 is severe liquid feces and possibly bloody diarrhea with substantial tail soilage. These observations typically present on days four to five post-irradiation. Stool consistency was measured daily, twice daily from day 1–7 when diarrhea is prevalent.

2.5. Histological evaluation

Formalin-fixed tissue samples were embedded in paraffin and cut into 5 μm sections then stained with hematoxylin and eosin (H&E). Villus length, defined as the distance between the mouth of the crypts and the tip of the villi, was measured in 4 different fields per tissue section, and the average was calculated to avoid the potential impact of patchy necrosis.

2.6. Bacteriological study

Radiation-induced translocation of gut bacteria from the intestine to other organs (mesenteric lymph nodes (MLNS) or liver tissue) was measured 3.5 days after ABI at 15.0 Gy. DNA was extracted from samples using a genomic DNA purification kit from Tiangen (Beijing, China) and quantified spectrophotometrically. Briefly, real-time PCR was performed using Power SYBR green PCR master mix (Bio-rad, CFX-96) and 16S rRNA gene-targeted primers, forward (5’-AACGCGAAGAACCCTAC-3’) and reverse (5’-CGGTGTGTAACAGACC-3’). Serially diluted bacterial genomic DNA was used to generate a standard curve. Results were expressed as nanogram bacterial DNA per gram mouse tissue. The endotoxin level of serum was tested with a Chromogenic
were as follows:

2.7. FITC dextran permeability experiments

After 3.5 days following the 15 Gy or sham abdominal irradiation, mice were gavaged with a FITC (fluorescein isothiocyanate)-dextran solution (4 kDa Sigma #46944) at 0.6 mg/g body weight, 4 h prior to sacrifice.

Serum was obtained by cardiac puncture, and the levels of FITC-dextran in the sera were obtained. A standard curve was constructed using serum from age-matched C57BL/6J male mice spiked with increasing amounts of FITC dextran. Samples were measured in a 96-well plate with a microplate reader (Synergy HT; Bio-Tek Instruments).

2.8. Transepithelial electrical resistance (TEER)

IEC-6 cells were seeded to the upper layer of 0.33-cm² Transwell (Corning Costar, NY, USA) at a density of 8 × 10^4 cells/well. TEER assays were carried out as described previously (Prisciandaro et al., 2012). The resistance across confluent monolayers was measured by a Millicell-ERS volt-ohm meter (Millipore, MA, USA) with electrodes. Values were expressed as ohms per square centimeter (Ω/cm²), taking into account the surface area of the filter.

2.9. Measurement of intracellular reactive oxygen species (ROS) generation

IEC-6 cells at a concentration of 1 × 10^4 cells per well of a 6-well plate were incubated with 20 μM 6-shogaol at 37 °C for 4 h. Thereafter, cells were exposed to 4 Gy γ-rays/H₂O₂, followed by 24 h of culture, then the addition of DCF-DA (10 μM) for 30 min, and finally, the fluorescence intensity was analyzed by flow cytometry (Mindray, Br cyste E6, China) using a laser excitation and emission wavelengths of 492–495 nm and 517–527 nm, respectively.

2.10. Apoptosis analysis

Mice were sacrificed 3.5 days after 15 Gy ABI, and the duodenum was harvested for histology and fixed.

Formalin-fixed tissue samples were cutted into 5 μm then stained with TUNEL according to the manufacturer’s protocol (Beyotime, Shanghai, China). Then, sections were counterstained with DAPI. Slides were then washed and observed under a fluorescence stereomicroscope.

2.11. Immunohistochemistry

Immunohistochemistry staining was performed according to kit instructions (PV-6000, ZSGB-NIO, China). Sections were dewaxed and treated with citrate buffer. Antibodies including used included those against TNF-α (ProteinTech, 17590-1-AP), iNOS (ProteinTech, 18985-1-AP), and IFN-γ (ProteinTech, 15365-1-AP).

2.12. Quantitative reverse transcription (RT)-PCR analysis

Total RNA was extracted from the small intestines using TRIzol Reagent (Invitrogen). A 10 μg aliquot of each RNA sample was reverse transcribed into cDNA using oligo-dT random primers and reverse transcriptase (Takara, Dalian, China). RT-PCR was performed using SYBR Premix Ex Taq(TM) II (Takara). Primers used were as follows:

TNF-α: 5'-CTACCTGTGGCCTCCTTTT-3' (forward) and 5'-GAG CAGAGGGTAGTAGTAG-3' (reverse); iNOS: 5'-TCTACACCA CACAAAC-3' (forward) and 5'-CTCAATCTCTGCTATCC-3' (reverse); IFN-γ: 5'-GCTCTGAGAACATGACCCTAC-3' (forward) and 5'-TTAGGCTTCAATGACTTG-3' (reverse).

2.13. Statistical analysis

Data are displayed as the mean ± SEM from 3 to 5 independent experiments. Differences between groups were carried out by one-way ANOVA. p < 0.05 was considered statistically significant.

3. Results

3.1. Pretreatment with 6-shogaol improves animal survival and intestinal function following IR injury

The chemical structure of 6-shogaol is illustrated in Fig. 1. An HPLC fingerprint chromatogram was established for the quality control of 6-shogaol production, which resulted in 98.4% purity (Fig. 1). A significant improvement in survival was seen with daily administration of 100 mg/kg/d 6-shogaol prior to lethal irradiation (Fig. 2A). At an ABI dose of 15 Gy, 90% of 6-shogaol-treated animals survived to 30 days compared with 40% of irradiated animals. Particularly, the animals in the 15 Gy ABI group began to die at 4 days post-irradiation. At 8 days post-irradiation, the declining number of surviving animals reached a plateau. In 6-shogaol pretreated mice, mortality was 10% at 15 Gy of ABI. We observed only one death in the group treated with 6-shogaol (n = 10) after receiving the irradiation (Fig. 2B).

It is known that exposure to ABI induces signs of radiation sickness, including diarrhea, black stools, and weight loss. We also recorded changes in body weight for 30 days (Fig. 2C). Sixteen days after irradiation, the mean body weights were significantly higher in the 6-shogaol + 15 Gy ABI group than in the 15 Gy ABI group, and 6-shogaol treatment continued to improved body weight up until the end of the study period. These results suggested that, in mice following radiation-induced intestinal injury, 6-shogaol pretreatment affected the survival rate and the change in body weight.

Animal survival was highly correlated with diarrhea severity following ABI (Booth, Tudor, Tudor, Katz, & MacVittie, 2012). To determine the method by which 6-shogaol improved gut physiology prior to radiation, we treated mice with vehicle or 6-shogaol, according to the radioprotection protocol depicted previously in Fig. 2A. At 15 Gy ABI, diarrhea was first manifest on day 3. After suffering from this symptom for roughly one week, the animals had either recovered or reached a moribund state and then was euthanized humanely. Diarrhea typically was observed as soft or very loose stools, even with some evidence of hemorrhage visible on day 4, 5, and 6 after exposure to ABI (Fig. 2D, Fig. S1). An effective radioprotectant of the GI tract should thus reduce diarrhea and normalize the amount of formed stool. Irradiated animals had almost no formed droppings 4 days after radiation, whereas animals pretreated with 6-shogaol had more formed stool (Fig. 2D, Fig. S1). Likewise, there was no evidence of hemorrhaging and diarrhea improved in approximately 50% of mice in the 6-shogaol pre-treatment group. These results indicated that 6-shogaol might be of value in the treatment of radiation-induced diarrhea.

3.2. Oral gavage with 6-shogaol protects against the histological intestinal damage provoked by IR

The impact of IR on the integrity of the intestinal wall was demonstrated by histological evaluation of duodenum tissue. To examine the putative role 6-shogaol has in protecting against...
radiation-induced intestinal injury, we compared the histological manifestations within the duodenum of 6-shogaol-treated or vehicle-treated mice prior to 15 Gy ABI, as depicted in Fig. 3A. At day 3.5, evidence of mucosal architecture destruction, such as villous denudation, was observed in the small intestines of 15 Gy ABI-treated mice, whereas the crypt-villi structures of the duodenum were preserved in 6-shogaol-treated mice (Fig. 3B and C).

Upon harvesting intestinal samples, we found that, among the different parts of the intestine, the duodena were the most sensitive. As shown in Fig. S2, the red circle depicts the intestinal fistula and impaired duodenal mucosal integrity in a duodenum from the 15 Gy ABI-treated mice, whereas the crypt-villi structures of the duodenum were preserved in 6-shogaol-treated mice (Fig. 3B and C).

Death from GI radiotoxicity may also stem from compromised epithelial integrity and barrier function, which facilitates both electrolyte disturbances and possible parenteral access of enteric pathogens. We also investigated the epithelial integrity of the GI tract with a FITC–dextran assay, wherein mice are gavaged with dextran covalently coupled to FITC, which cannot cross the GI epithelium unless the epithelial barrier is compromised (Dawson et al., 2009). Four hours after gavage, FITC-dextran levels were measured in the blood. The results showed that pretreatment with 6-shogaol decreased FITC-dextran uptake in the bloodstream of ABI-treated mice (Fig. 3E and F).

Transepithelial electrical resistance (TEER) is a widely accepted method that quantitatively measures the dynamics of epithelial integrity in vitro models (Fig. 3G). TEER assays based on measuring ohmic resistance to obtain real-time values without cell damage. We used IEC-6 monolayers to mimic the normal intestinal epithelial barrier in vitro. γ-radiation (4 Gy) induced a rapid decrease in TEER at 24 h, which was not restored in 72 h (Fig. 3H). However, pretreatment of IEC-6 cells with 6-shogaol for 4 h prior to radiation exposure, protected against the radiation-induced decrease in TEER at 24 h, and TEER was maintained as in the vehicle control.

These results suggested that 6-shogaol pretreatment may have the potential to improve radiation-induced damage to epithelial integrity, both in vivo and in vitro.

3.3. Pretreatment with 6-shogaol reduces apoptosis of intestinal epithelial cells following radiation

Radiation-induced DNA damage can trigger cell phenotypic modifications and/or apoptosis. Intestinal epithelial cell apoptosis results in disruption of intestinal epithelial barrier integrity. Meanwhile, the radiation-induced inflammatory response is cascaded by the induction of apoptosis, presumably by recruited immune cells. Therefore, we evaluated the effect of 6-shogaol in radiation-induced apoptosis on the duodenum. At 3.5 days post-irradiation, a significant increase in TUNEL positive cells among the intestinal epithelial barrier was intact. These phenomena were also seen in the H&E staining, which revealed the breakdown of the intestinal mucosa (Fig. 3D).

Death from GI radiotoxicity may also stem from compromised epithelial integrity and barrier function, which facilitates both electrolyte disturbances and possible parenteral access of enteric pathogens. We also investigated the epithelial integrity of the GI tract with a FITC–dextran assay, wherein mice are gavaged with dextran covalently coupled to FITC, which cannot cross the GI epithelium unless the epithelial barrier is compromised (Dawson et al., 2009). Four hours after gavage, FITC-dextran levels were measured in the blood. The results showed that pretreatment with 6-shogaol decreased FITC-dextran uptake in the bloodstream of ABI-treated mice (Fig. 3E and F).

Transepithelial electrical resistance (TEER) is a widely accepted method that quantitatively measures the dynamics of epithelial integrity in vitro models (Fig. 3G). TEER assays based on measuring ohmic resistance to obtain real-time values without cell damage. We used IEC-6 monolayers to mimic the normal intestinal epithelial barrier in vitro. γ-radiation (4 Gy) induced a rapid decrease in TEER at 24 h, which was not restored in 72 h (Fig. 3H). However, pretreatment of IEC-6 cells with 6-shogaol for 4 h prior to radiation exposure, protected against the radiation-induced decrease in TEER at 24 h, and TEER was maintained as in the vehicle control.

These results suggested that 6-shogaol pretreatment may have the potential to improve radiation-induced damage to epithelial integrity, both in vivo and in vitro.

3.4. Antioxidant activity of 6-shogaol

ROS are generated by IR and can directly damage DNA. Failure to repair damaged DNA may involve the initiation of apoptosis. Previous research has shown that ginger extracts possess the ability to scavenge free radicals, as well as superoxides and hydroxyls (Haniadka et al., 2013). Our experiments indicated that 6-shogaol exhibited strong antioxidant capacity as shown by the scavenging of hydroxyl radicals and DPPH radicals (Fig. S3). This finding
indicated that the hydroxyl-radical-scavenging ability of 6-shogaol is greater than that of the natural antioxidant (vitamin C) (Fig. S3).

Furthermore, we used DCF fluorescence Assay to test the antioxidant of 6-shogaol in IEC-6 cells. Firstly, normal intestinal cell line (IEC-6) was used to investigate the cytotoxic effect of 6-shogaol by the MTT assay. We found that when IEC-6 cells were treated with 25 μM 6-shogaol for 72 h, there is no significant difference between control and 25 μM 6-shogaol treatment cells (Fig. S4). Thus, in the following experiments, we used 6-shogaol at a lower concentration of 20 μM.

DCF fluorescence was used to test a variety of ROS (Wang & Joseph, 1999). Then, we established an H2O2-induced oxidative stress model to determine the antioxidant activity of 6-shogaol by the MTT assay. We found that when IEC-6 cells were treated with 25 μM 6-shogaol for 72 h, there is no significant difference between control and 25 μM 6-shogaol treatment cells (Fig. S4). Thus, in the following experiments, we used 6-shogaol at a lower concentration of 20 μM.

DCF fluorescence was used to test a variety of ROS (Wang & Joseph, 1999). Then, we established an H2O2-induced oxidative stress model to determine the antioxidant activity of 6-shogaol in IEC-6 cells. In these experiments, cells were treated with H2O2 (100 μM) or 6-shogaol (20 μM) 4 h prior to H2O2 treatment. IEC-6 cells showed a significant increase in DCF fluorescence 24 h after H2O2 treatment. In contrast, pretreatment with 6-shogaol significantly reduced the ROS levels in H2O2-treated IEC-6 cells (Fig. 4C and D). The DNA damage induced by ionizing radiation is similar to oxidative stress. Furthermore, results from DCF fluorescence assays also highlighted the antioxidant abilities of 6–shogaol in reducing ROS levels in the radiation-induced oxidative stress model (Fig. 4E and F).

3.5. Pretreatment with 6-shogaol impedes the pro-inflammatory response provoked by IR

The release of pro-inflammatory cytokines exerts an important impact on the pathogenesis of IR-induced intestinal injury, which is why we then examined the effects of pretreatment with 6-shogaol on the expression of major inflammatory cytokines in the duodenum of irradiated mice. On day 3.5 after 15-Gy radiation, there was a 2-fold, 10-fold and 6-fold induction of IFN-γ, TNF-α and TNF-α mRNA, respectively (Fig. 5C). These inductions were significantly curtailed by 6-shogaol pretreatment, and given that pretreatment with 6-shogaol prior to radiation resulted in a
significant suppression of pro-inflammatory cytokines in the duodenum (Fig. 5A and B), these results demonstrated that 6-shogaol inhibited expression of IR-induced inflammatory cytokines in duodenal tissues.

3.6. Pretreatment with 6-shogaol preserves intestinal permeability and prevents endotoxin translocation following IR

Villi injury due to radiation is followed by the release of pro-inflammatory factors, mucosal disruption, and increased intestinal permeability. The integrity of gut mucosa does not remain intact when there is a disturbance in the microcirculation. Damage of the mucosal barrier as an outcome of irradiation and compromised gut associated lymphoid tissue allows bacterial toxins and bacteria to access to penetrate deeper into tissues. The mesenteric lymph nodes (MLNs) were the first tissues to show a positive culture, followed by the liver. Then, bacterial translocation, as determined by real-time PCR of bacterial DNA in the MLNs and liver, was measured on day 3.5 after exposure to 15 Gy ABI, as depicted in Fig. 3A. Further displayed in Fig. 6A, no bacterial translocation was observed in the control group. Whereas significant bacterial loads in the MLNs from the 15 Gy ABI group were detected on day 3.5 after ABI. Animals pretreated with 6-shogaol exhibited a marked reduction in bacterial translocation, when compared to irradiated mice. In addition, no significant bacterial translocation was observed in liver tissues. The reason for this may be that, in the model of high-dose ABI-induced acute gastrointestinal syndrome, the bacteria from MLNs were not translocated to the liver at this point in time, and mice had died gradually on day 4.

Following irradiation, the permeability of the intestinal mucosal barrier increases (Fig. 4E, Fig. 52), and the bacteria enter the blood from the intestine and release large amounts of endotoxin into the blood, causing endotoxemia and bacteremia. The presence of endotoxin has been observed in the liver and blood of mice 1–3 days after irradiation (Wilson, Barry, & Bealmear, 1970). To determine whether 6-shogaol attenuated the endotoxin level at this phase of acute gastrointestinal syndrome, designated groups of mice were sacrificed on 3.5 days after irradiation and the plasma levels of endotoxin were quantified. There was a significant increase in the endotoxin level in the irradiated mice when compared to unirradiated animals (Fig. 6B). In contrast to the result seen in the 15 Gy ABI group, pretreatment with 6-shogaol significantly reduced plasma levels of endotoxin (Fig. 6B).

Our results suggest that 6-shogaol might reduce bacterial translocation and endotoxin levels following ABI to protect against radiation-induced intestinal injury.

4. Discussion

The two most radiosensitive human tissues, GI epithelium and the hematopoietic progenitor cells in the bone marrow, are also essential for sustaining life (Jagetia, 2007; Jagetia & Baliga, 2003). Intestinal radiation injury may most often develop following abdominal or pelvic radiation therapy for cancer, or pretreatment of hematopoietic stem cells transplantation. Patients undergoing radiotherapy are at a greater risk of having radiation injuries in the form of ARS and radiation enteritis as a result of disruption of the intestinal microflora and damage to the mucosal barrier. Clinically, total body irradiation was still a classic pretreatment for hematopoietic stem cell transplantation. Cytokines are highly relevant to radiation injury. Upon exposure to ionizing radiation, pro-inflammatory cytokines such as TNF-α and IL-1 become...
the main components of immediate early gene programs, converging with the generation of ROS in irradiated tissues (Schaue, Kachikwu, & McBride, 2012). The imbalance between pro-inflammatory and anti-inflammatory factors exacerbates tissue injury and systemic inflammatory response. In addition, the inflammatory processes of acute injury can be considered a distortion of the intestinal cellular response to bacterial infections (Ferrara, 2000). Moreover, GI damage was associated with greater amounts of endotoxin entering the systemic circulation because the GI tract is colonized by gram-negative bacteria and is a potent source of endotoxin. Exposure to ionizing radiation induced enhanced intestinal permeability, leading to bacterial and endotoxin translocation. The occurrence of GI-ARS results from the destruction of the mucosal lining of the gut, resulting in diarrhea, dehydration, and transmigration of enteric bacteria into the bloodstream. Recent research has shown that mice harboring a normal microbiota exhibit enhanced intestinal radiosensitivity as compared with germ-free animals, and bone marrow transplantation (BMT) cannot rescue lethality due to radiation enteritis in the mouse model (Crawford & Gordon, 2005). Therefore, development
of effective countermeasures against radiation-induced intestinal injury and bacterial translocation is imperative for the treatment of IR injury and remains a significant unmet need and shortcoming in current treatment options (Hill et al., 1997).

Traditionally, ginger has been the most popular plant material in China, and has been used not only as a condiment for food processing, but also as a medicinal food for gastrointestinal symptoms, such as nausea and vomiting related to pregnancy (Rashmi & Tiwari, 2016). In addition, Shen Nong’s Herbal Classic, originating more than 2000 years ago, documented the use of ginger for treatment of gastric ulcers. Nowadays, ginger and its extracts have been used widely as a folk remedy for gastrointestinal complaints, especially in response to the emergence of radiotherapy for the treatment of cancer (Baliga et al., 2012; Marx et al., 2017). For example, research (Jeena, Liju, Ramanath, & Kuttan, 2016) indicated that ginger essential oil (GEO) protected mice exposed to whole body γ-irradiation from oxidative stress and clastogenic damage. 6-Shogaol is one of the constituent ginger biophenolics and possesses anti-inflammatory and antioxidant effects. It plays an important protective role in different gastrointestinal damage models. In the HCl/ethanol-induced gastric ulceration model, 6-shogaol protects the gastric mucosa against ethanol-induced injury (Haniadka et al., 2013). In the TNF-α-induced barrier impairment model, 6-shogaol prevents TNF-α-induced barrier loss via inhibition of PI3K/Akt and NF-κB signaling (Luettig, Rosenthal, Lee, Krug, & Schulzke, 2016). In this study, we observed that 6-shogaol pretreatment reduced intestinal bacterial/endotoxin translocation in lethally irradiated mice and improved their survival. Particularly, mice that were pretreated with 6-shogaol had a significant mitigation of intestinal fistula and impaired mucosal integrity after exposure to radiation. Moreover, 6-shogaol pretreatment inhibited the release of IR-induced inflammatory cytokines and alleviated apoptosis in duodenal tissues.

To summarize, 6-shogaol has the pharmacological effects that could contribute to maintaining the intestinal integrity from the damage induced by irradiation, partially considering its antioxidant and anti-inflammatory properties, which evidently alleviated radiation toxicity to the gastrointestinal tract. Our results demonstrated that 6-shogaol potentially played a role that as a protective agent in obviating the treatment-limiting intestinal side-effects, and therefore 6-shogaol is feasible to be adopted in the radiotherapy of patients.

Conflict of interest

The authors have declared no conflicts of interest.

Acknowledgments

We thank Kaihua Ji, Liqing Du, Chang Xu and Ningning He for excellent technical support. We also thank Qin Wang and Wei Gong for their support in the study. This work was supported by the National Natural Science Foundation of China (31571832 and 31670859); Natural Science Foundation of Tianjin (15KPXM01SF056); Fundamental Research Funds for CAMS & PUMC (2016ZX310068 and 2016RC310017); Research Funds for the Innovation Team of IRM-CAMS (no. 1650); and Tianjin Innovative Research Team Grant (TD-12-5049).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2017.06.054.


