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Effects of low-dose irradiation on mice with *Escherichia coli*-induced sepsis

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ABSTRACT

Although favorable immune responses to low-dose irradiation (LDI) have been observed in normal mice, i.e., a hormesis effect, little is known about the effects of LDI in infectious diseases. In this study, we examined the effects of LDI on mice with sepsis, a severe and often lethal hyperinflammatory response to bacteria. Female C57BL/6 mice were whole-body irradiated with 10 cGy 48 h before *Escherichia coli* infection, and survival, bacterial clearance, cytokines, and antioxidants were quantified. LDI pretreatment significantly increased survival from 46.7% in control mice to 75% in mice with sepsis. The bacterial burden was significantly lower in the blood, spleen, and kidney of LDI-treated mice than in those of control septic mice. The levels of pro-inflammatory cytokines, e.g., IL-1β and IL-6, as well as anti-inflammatory IL-10 were markedly reduced in pre-LDI septic mice. Nitric oxide production by peritoneal macrophages was also reduced in pre-LDI septic mice. Immune cells in the spleen increased and Nrf2 and HO-1 were induced in pre-LDI septic mice. LDI stimulates the immune response and minimizes lethality in septic mice via enhanced bacterial clearance and reduced initial proinflammatory responses.

1. Introduction

Whole-body exposure to low-dose irradiation (LDI) is increasing owing to its use in diagnostic imaging procedures and clinical applications. However, the acceptable range of LDI to ensure safety and minimize health risks is still debated. Epidemiological and experimental studies have long indicated that a high dose of ionizing radiation has deleterious effects, and particularly increases the risk of cancer. Despite applying the arbitrary definition of LDI regarding the dose and dose rate until recently, several studies have shown that less than or equal to 0.8 Gy LDI promotes tumor growth and metastasis (Sofia Vala et al., 2010) and long-term exposure to LDI increases the risk of leukemia (Leuraud et al., 2015; Robertson et al., 2013). The application of LDI to the brain for diagnostic purposes increases the risk of brain tumors in children and might cause cognitive alterations (Lumniczky et al., 2017). LDI induced a deficiency of hematopoietic stem cells and decreased blood lymphocytes in animal and human blood samples (Godekmerdan et al., 2004; Seed et al., 2002). In contrast to the above findings, LDI has various beneficial effects, e.g., it can promote the proliferation of normal cells (Kim et al., 2007), prolong life span (Ina and Sakai, 2004), decrease tumor incidence (Liu, 2007; Mitchel et al., 2003; Nambi and Soman, 1987; Wei et al., 1990), and enhance immune system function (Klug et al., 2013).

Therefore, it has recently been suggested that the dose–response curves for LDI against health risks cannot be linear, based on extrapolation from the effects detected at the maximum doses without a threshold, but are rather linear-quadratic or quadratic, indicating cellular hypersensitivity to LDI, hormesis, bystander effects, and adaptive responses (Azzam et al., 2001; Brooks, 2004; Ikushima et al., 1996; Kern et al., 1999; Rödel et al., 2007). Furthermore, LDI-mediated cellular mechanisms not only increase antioxidant activity, facilitate DNA damage repair, and stimulate immune systems, but also reduce malignant transformation and mutagenesis (Feinendegen et al., 1995; Koana et al., 2012; Song et al., 2015; Yamaoka et al., 1991). Based on these beneficial effects, many studies have investigated the application of LDI in various diseases. Although LDI has potential clinical benefits, e.g., for atherosclerosis, cancer, and inflammatory diseases (Cui et al., 2017; Mitchel et al., 2013), accurate data are continuously required to address concerns regarding the effects and to establish safety regulations.

Sepsis is a severe and systemic illness caused by a hyper-

Abbreviations: CD, cluster of differentiation; CFU, colony-forming unit; *E. coli*, *Escherichia coli*; IL, interleukin; iNOS, inducible nitric oxide synthase; LDI, low dose irradiation; LPS, lipopolysaccharide; PM, peritoneal macrophage

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inflammatory response to microbial infections (Ahn et al., 2006). Despite substantial progress in the development of countermeasures, sepsis remains a major public health issue, affecting millions of patients worldwide each year (Fleischmann et al., 2016). Some patients die from sepsis-induced hyper-inflammation, i.e., an uncontrolled over-activation of the innate immune system characterized by high amounts of circulating pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-10 (Paterson and Webster, 2000; Steinhauser et al., 1999; Zeng et al., 2013). Activated neutrophils and macrophages generate prodigious amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to an imbalance in the redox system (Hoetzenecker et al., 2012). Excessive production of ROS and RNS impairs the function of detoxifying and antioxidant enzymes, such as heme oxygenase-1 (HO-1), glutathione-*S*-transferase (GST), NAD(P)H quinine oxidoreductase 1 (NQO1), glutamate-cysteine ligase catalytic subunit (GCLC), and glutamate-cysteine ligase, modifier subunit (GCLM) (Noel et al., 2014; Xu et al., 2015). Nuclear factor erythroid 2-related factor 2 (Nrf-2), a basic leucine zipper oxidant-responsive transcription factor, regulates numerous important cytoprotective genes and maintains cellular redox homeostasis (Al-Sawaf et al., 2015). Animal studies have established the critical role of Nrf2 in improving survival during sepsis (Thimmulappa et al., 2006; Kong et al., 2011; MacGarvey et al., 2012). Restored Nrf2 is an important factor for therapeutic intervention in septic mice; it induces antioxidant enzymes to prevent the systemic accumulation of ROS and RNS (Bell et al., 2011; Kensler and Wakabayashi, 2010).

We previously demonstrated that total-body LDI modulates the immune response in mice, and the sensitivity and kinetics of the response vary according to the dosing method (Song et al., 2015). In addition, single- or fractionated-LDI contribute to different immune responses, i.e., a Th1-shift or Th2-shift. Therefore, we investigated whether the immunomodulatory effects of LDI influence the biological outcomes of bacterial infection.

2. Materials and methods

2.1. Experimental animals and whole-body irradiation

Female 6-week-old C57BL/6 mice were purchased from Orient Bio Inc. (Seongnam, Korea) and maintained in specific pathogen-free conditions. After acclimatization for 1 week, the mice were whole-body irradiated using a ^{137}Cs gamma LDI-KCCH 137 irradiator (dose rate 0.1 cGy/min) before bacterial inoculation. All animal experiments were approved by the Institutional Animal Care and Use Committees of the Korea Institute of Radiological & Medical Sciences (KIRAMS 2016-15).

2.2. Bacteria

Escherichia coli 018ac:K1:H7 Bort, purchased from ATCC (Manassas, VA, USA), were cultured at 37 °C in Trypticase soy broth (Difco, Detroit, MI, USA), and harvested at the mid-logarithmic growth phase. Bacteria were washed twice and re-suspended in phosphate-buffered saline (PBS). The concentration of bacteria was determined based on the optical density at 620 nm using a spectrophotometer.

2.3. Sepsis model

For the sepsis model, mice were *i.p.* injected with 5×10^5 or 5×10^6 CFUs of live *E. coli* cells after whole-body LDI. Survival was monitored for over 72 h. Blood, spleens, and kidneys were collected 24 h after *E. coli* infection. Serum was stored at − 80 °C until the measurement of cytokines.

2.4. Determination of colony-forming units (CFUs)

Blood, spleens, and kidneys were obtained from *E. coli*-infected or control mice under aseptic conditions 24 h after bacterial infection. The spleens and kidneys were mechanically homogenized and diluted in 10 mL of PBS. After 10-fold serial dilution with PBS, 0.1 mL of each suspended solution was smeared on LB agar plates. The plates were incubated at 37 °C for 24 h and CFUs were counted.

2.5. Cell culture

RAW264.7, a mouse macrophage cell line purchased from ATCC, was cultured in DMEM (HyClone, Logan, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin; Invitrogen, Carlsbad, CA, USA) at 37 °C in a humidified 5% CO_2 incubator.

2.6. Cell viability assay

RAW264.7 cells were seeded in 12-well plates (Corning Inc., Corning, NY, USA) at a density of 1×10^4 cells/well. After overnight growth, cells were exposed to the indicated doses of radiation at 48 h before stimulation with inactive-*E. coli* (1×10^5 CFU/well) after they were heat-killed at 65 °C for 30 min, and then washed once with PBS. After 24 h of incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) solution (5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) was added and absorbance at 540 nm was measured using a 96-well microplate reader (Thermo Scientific, Waltham, MA, USA).

2.7. Cytokine measurements

The concentrations of murine cytokines in sera were determined using enzyme-linked immunosorbent assay (ELISA) kits for IL-1β (R & D Systems, Minneapolis, MN, USA), IL-6 (BD Biosciences, San Jose, CA, USA), and IL-10 (R & D Systems) according to the manufacturers' protocols. The absorbance at 450 nm was obtained using a 96-well microplate reader (Thermo Scientific).

2.8. Determination of nitric oxide (NO) production

Peritoneal macrophages (PMs) were isolated from septic or control mice, and were seeded at a density of 1×10^5 cells/well into 96-well plates in the presence or absence of 1 μg/mL lipopolysaccharide (LPS) at 37 °C for 24 h. The culture supernatant of RAW264.7 cells was also collected after infection with or without pre-LDI. Each culture supernatant was mixed with the same amount of Griess reagent and incubated at room temperature for 10 min. The absorbance of the mixture was determined at 540 nm using a microplate reader. All measurements were performed in triplicate. The amount of nitrites was determined using a standard curve established with $NaNO_2$.

2.9. Flow cytometry

Isolated splenocytes (1×10^6 cells per experiment) were incubated with fluorescein isothiocyanate (FITC)- or allophycocyanin (APC)-conjugated monoclonal antibodies simultaneously for 30 min at 4 °C. Functional cell surface molecules, i.e., total T cells (CD3-FITC/TCRβ-APC), CD4-T cells (CD4-FITC/CD3-APC), CD8-T cells (CD3-FITC/CD8-APC), and dendritic cells (DCs) (I-A[*b*]-FITC/CD11c-APC), and activation molecules, i.e., T cells (CD25-APC, CD28-APC, CD44-FITC), B cells (CD19-FITC, CD69-FITC, CD86-FITC), natural killer (NK) cells (CD69-FITC), DCs, and macrophages (CD80-FITC, CD86-FITC) (BD Pharmingen, San Diego, CA, USA) were detected using a FACSCalibur flow cytometer (BD Biosciences) by collecting 20,000–30,000 events/tube. Results were analyzed using FlowJo software (v.10; FLOWJO, Ashland, OR, USA).