Original Paper

CpG-Oligodeoxynucleotides Improved Irradiation-Induced Injuries by G-CSF and IL-6 Up-Regulation

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Key Words

Irradiation • Radioprotection • CpG-ODN • G-CSF • IL-6

Abstract

Background/Aims: This study investigated the radioprotective properties of three classes of CpG-oligodeoxynucleotides (CpG-ODNs) and the underlying mechanisms. **Methods:** Mice irradiated at different doses (7Gy or 9Gy) were treated with or without ODNs (50µg via intraperitoneal injection). Assays were performed to determine survival rate and the number of white blood cell in peripheral blood. The levels of granulocyte-colony stimulating factor (G-CSF), interleukin 6 (IL-6) and interferon-α (IFN-α) were measured using enzyme-linked immunosorbent assay (ELISA). **Results:** Survival rate of mice irradiated in 7Gy was increased from 50% to about 100% with ODNs pretreatment. ODNs administration increased the number of WBCs of irradiated mice. G-CSF, IL-6 and IFN-α levels were up-regulated with ODNs treatment. **Conclusion:** All three classes of ODNs protected mice from irradiation-induced injuries. B-class ODNs exhibited the most potent radioprotective property via the up-regulation of G-CSF and IL-6.

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Introduction

Exposure to ionizing radiation (IR) often causes severe damages to radiosensitive tissues. However, no effective safe drugs are currently available to protect IR-induced injuries. This inadequacy must be addressed.

Burdelya et al. reported that the ligand of toll-like receptor 5 (TLR5) exerted potent radioprotective effects on mice by activation of TLR5 and its downstream signaling pathway [1]. Our experiment subsequently demonstrated that TLR2, TLR4 and TLR9 exhibited

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potent radioprotective properties [2-4]. We also demonstrated that the TLR9 ligand CpG-oligodeoxynucleotides (CpG-ODN) alleviated IR-induced injuries in mice.

The pattern recognition receptor toll-Like Receptor 9 (TLR9) recognizes bacterial DNA and synthetic ODN containing unmethylated CpG-ODN [5]. CpG-ODN activates B-lymphocyte and innate immune cells to secrete various cytokines and promote acquired immunity [6, 7]. Three different kinds of CpG-ODN classes have been described [8, 9]. Phosphorothioate (PS)-modified B-Class ODNs can effectively stimulate B and NK cells and exhibit antitumor activity [10, 11]. A-class ODNs could induce considerably high levels of IFN- α from plasmacytoid dendritic cells [12]. The recently discovered C-class ODNs combine the properties of the A- and the B-classes in different species and induces potent B-cell activation comparable to B-Class ODN with IFN- α secretion comparable to A-Class ODN [9, 13].

Previous studies revealed that ODNs can protect mice from IR-induced injuries [14]. However, the functional difference between the three classes of ODNs is not clear. The present study compared the radioprotective properties of the three classes of ODNs and examined the underlying mechanisms. We found that the B-class exhibited the most potent alleviation of IR-induced injury via the up-regulation of G-CSF and IL-6.

Materials and Methods

Oligodeoxynucleotides

All ODNs (Fig. 1) were purchased from Sangon Biotech (Shanghai, China). ODNs were suspended in DEPC treated water, stored at -20°C and handled under aseptic conditions. All dilutions were performed using pyrogen-free normal saline.

Irradiation

⁶⁰Co- γ rays at the Irradiation Center (Faculty of Naval Medicine, Second Military Medical University, China) were used for irradiation. Mice were exposed to varying doses of irradiation depending on the requirements of the study.

Mice and treatments

The Second Military Medical University in China approved all experiments in accordance with the Guide for Care and Use of Laboratory Animals published by the NIH (Publication No. 96-01, United States). Male wild-type BALB/c mice (6-8 weeks old) were purchased from the China Academy of Science (Shanghai, China). Mice were housed in individual cages in a temperature-controlled room with a 12h light/dark cycle. Food and water were provided ad libitum.

White blood cell (WBC) count

Peripheral blood was collected from the retro-orbital sinus/plexus using EDTA-coated blood collection tubes at different times after irradiation. White blood cells (WBCs) were detected using blood cell analyzer.

ELISA

Blood was collected and kept at room temperature for 30 minutes followed by centrifugation at 3000 rpm/min for 10 min. Serum was removed and stored at -20°C. The levels of G-CSF, IFN- α , and IL-6 were detected in all samples by ELISA. All steps were performed in accordance with the manufacturer's instructions.

Statistical analysis

Data are expressed as means $\pm$ the standard error of mean (SEM) for each experiment. The number of samples is indicated in the description of each experiment. We used an analysis of variance (ANOVA) followed by a Student-Newman-Keuls post hoc test for statistical analysis. Experiments for quantification were performed in a blinded fashion and all experiments were repeated at least 3 independent times.

Fig. 1. Sequences of three classes of ODNs used in the study. A-class ODNs: ODN 1585 and ODN 2216. B-class ODNs: ODN 1826, ODN 2005, ODN BW006 and ODN D-SL01. C-class ODNs: ODN M362 and ODN D-SL03.

Results

Sequences of oligodeoxynucleotides used in the study

Three classes of ODNs with different properties were used in our study. ODN 1585 and ODN 2216 were A-class ODNs. ODN 1826, ODN 2005, ODN BW006 and ODN D-SL01 were B-class ODNs. ODN M362 and ODN D-SL03 were C-class ODNs. Fig. 1 shows the structure of all ODNs used.

Survival rates of mice

Mice were treated with different ODNs before or after different irradiation doses to evaluate the protective effects of ODNs on radiation injury. Mice were treated with 50 µg ODNs via intraperitoneal injection (i.p.) 24 h and 2 h prior to whole body irradiation with a dose of 7 Gy. The data indicated that 50% of irradiated mice without ODNs treatment died in 30 days, and more than 50% of mice pretreated with different ODNs survived (Fig. 2). Therefore, ODN pretreatment protected from radiation injury. B-class ODNs (ODN 1826 and ODN 7909) were the most potent property of the ODN classes.

We further investigated the protective effects of ODNs in mice treated with lethal irradiation (9 Gy). The results demonstrated that all irradiated mice without ODN treatment died in 14 days. Mice pretreated with ODNs 24 h and 2 h before irradiation exhibited increased survival rate or prolonged survival time (Fig. 3). Administration of B-class ODNs (ODN 1826 and ODN 7909) increased survival rate most potently.

We also examined whether ODN treatment after irradiation mitigated radiation injury. Mice were treated with ODNs 30 min after 9 Gy radiation. Our data demonstrated that A- and C-class ODNs exerted no protective effects, and B-class ODNs markedly increased the survival rate (Fig. 4).

Fig. 2. Survival rate of irradiated mice with 7Gy was increased by the pretreatment with ODNs.

ODNs improved irradiation-induced decrease in peripheral WBC count

Treatment with 7Gy irradiation obviously decreased peripheral WBCs relative to the non-irradiated mice, and ODNs administration considerably improved WBC counts. B-class ODNs more potently improved the decrease in WBC compared to the other ODNs (Fig. 5A). Simple regression was used to detect the relationship between the number of WBCs and survival rate. Our data demonstrated that survival rate positively correlated to the number of WBCs (Fig. 5B).

ODNs increased the G-CSF, IL-6 and IFN- α levels

G-CSF, IL-6 and IFN- α are key factors in radiation injuries. We examined the levels of G-CSF, IL-6 and IFN- α in the serum of mice treated with different ODNs in a radiation injury mice model to investigate the underlying mechanisms involved in the radioprotective properties of ODNs. We found that different ODNs induced different levels of G-CSF, IL-6 and IFN- α in mice (Fig. 6A). We analyzed correlations between mouse survival rates and the three cytokines levels and found that G-CSF and IL-6 positively correlated with mouse survival separately (Fig. 6B). However, IFN- α levels exhibited an insignificant negative correlation with mouse survival (Fig. 6C). Notably, removal of the outliers (blank and PBS-treated group) revealed that the IFN- α levels negatively correlated with mouse survival (Fig. 6D).

Fig. 3. Survival rate of irradiated mice with 9Gy was increased by the pretreatment with ODNs.

IFN- α and IL-6 deletion reduced the radioprotective property of B-class ODNs

The aforementioned results demonstrated that ODNs alleviated radiation injuries via cytokine regulations. *In vivo* experiments were performed to further confirm the result. Irradiated mice treated with B-class ODNs were treated with or without IFN- α . The results demonstrated that the administration of IFN- α decreased survival rate (Fig. 7A). IL-6 knockout mice were treated with PBS or B-class ODNs, and the protective effect of B-class ODNs was not observed (Fig. 7B).

Discussion

Previous studies demonstrated that activation of the TLR signaling pathways possesses radioprotective properties. The TLR9 ligand CpG-ODN, also exerts radioprotective effects [4]. The present study demonstrated that all three classes of ODNs alleviated IR-induced

Fig. 4. Survival rate of irradiated mice with 9Gy was increased by the posttreatment with ODNs.

injuries in mice. ODNs increased the survival rate of mice treated with different doses of radiation. IR also resulted in severe injuries to radiosensitive tissues such as bone marrow tissue, and ODN treatment of ODNs markedly protected the number of WBCs in peripheral blood.

This study investigated the difference between the three classes of ODNs and found that B-class ODNs showed the most potent radioprotective properties. Irradiated mice treated with B-class ODNs (ODN 1826 and ODN 7909) exhibited the highest survival rate and number of WBC.

Fig. 5. ODNs improved irradiation-induced injuries of the peripheral white blood cell count. (A) Shown are the bar graphs of numbers white blood cell. (B) Simple regression of WBC number and survival rate.

Fig. 6. ODNs increased the level of G-CSF, IL-6 and IFN-α. (A) Shown are the bar graphs of concentration of different cytokines. (B) Simple regression of survival rate and different cytokines (G-CSF and IL-6). (C) Simple regression of survival rate and IFN-α in groups treated with or without ODNs (P > 0.05). (D) Simple regression of survival rate and IFN-α in groups treated with different ODNs (except blank and PBS-treated group) (P > 0.05).

We examined the mechanisms of the radioprotective effects of ODNs. IR induced severe injuries in hematopoietic tissue which may lead to death [15, 16]. G-CSF and IL-6

Fig. 7. Survival rate of mice. (A) Administration of IFN- α decreased the survival rate of irradiated mice with the treatment of B-class ODN. (B) Treatment of B-class ODNs showed no protective effect on the survival rate of irradiated IL-6 knock-out mice.

Fig. 8. Underlying mechanism of different classes of ODNs in radioprotection.

are critical in hematopoiesis [17-20]. We demonstrated that G-CSF and IL-6 levels positively correlated to the survival rate of irradiated mice. Therefore, we determined the effects of ODNs on G-CSF and IL-6 levels. The results demonstrated that all three classes of ODNs up-regulated G-CSF and IL-6 levels, which explained the radioprotective effects. B-class ODNs exhibited the most potent properties. ODNs induce moderate level of interferon (IFN), which is a cytokine implicated in virus defense and the resolution of infectious diseases [21-23]. Previous studies revealed that IFN- α potentiated radiation injury [24, 25]. The level of IFN- α was measured and we found that IFN- α was negatively correlated to the survival rate of irradiated mice. A- and C-class ODNs more effectively up-regulated IFN- α than B-class ODNs. These data demonstrated that B-class ODNs presented significant radioprotective properties resulting from the stronger ability to up-regulate G-CSF and IL-6 and poorer ability to up-regulate IFN- α than other classes of ODNs (Fig. 8).

Conclusion

This study demonstrated that ODNs effectively protected mice from IR-induced injuries. ODNs obviously increased the survival rate and the number of WBCs. B-class ODNs exhibited the most potent radioprotective properties and the most potent ability to up-regulate G-CSF and IL-6 levels.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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