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Biology Contribution

Radioprotection and Cell Cycle Arrest of Intestinal Epithelial Cells by Darinaparsin, a Tumor Radiosensitizer

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Summary

Darinaparsin, an organic arsenic compound, has been reported to radiosensitize cancer cells, but interestingly protects intestinal epithelial cells from radiation. Here we report in vivo data that demonstrate this tissue-selective effect of radiomodification in a clinically relevant orthotopic xenograft tumor model, a model of acute radiation gastrointestinal syndrome, and the potential mechanism through DNA damage repair. These data may provide a novel paradigm for radiomodification that significantly increases the therapeutic index of radiation therapy.

Purpose: It was recently reported that the organic arsenic compound darinaparsin (DPS) is a cytotoxin and radiosensitizer of tumor cells in vitro and in subcutaneous xenograft tumors. Surprisingly, it was also found that DPS protects normal intestinal crypt epithelial cells (CECs) from clonogenic death after ionizing radiation (IR). Here we tested the DPS radiosensitizing effect in a clinically relevant model of prostate cancer and explored the radioprotective effect and mechanism of DPS on CECs.

Methods and Materials: The radiation modification effect of DPS was tested in a mouse model of orthotopic xenograft prostate cancer and of IR-induced acute gastrointestinal syndrome. The effect of DPS on CEC DNA damage and DNA damage responses was determined by immunohistochemistry.

Results: In the mouse model of IR-induced gastrointestinal syndrome, DPS treatment before IR accelerated recovery from body weight loss and increased animal survival. DPS decreased post-IR DNA damage and cell death, suggesting that the radioprotective effect was mediated by enhanced DNA damage repair. Shortly after DPS injection, significant cell cycle arrest was observed in CECs at both G1/S and G2/M checkpoints, which was accompanied by the activation of cell cycle inhibitors p21 and growth arrest and DNA-damage-inducible protein 45 alpha (GADD45A). Further investigation revealed that DPS activated ataxia telangiectasia mutated (ATM), an important inducer of DNA damage repair and cell cycle arrest.

Conclusions: DPS selectively radioprotected normal intestinal CECs and sensitized prostate cancer cells in a clinically relevant model. This effect may be, at least in part, mediated by DNA damage response activation and has the potential to significantly increase the therapeutic index of radiation therapy.

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Introduction

The therapeutic index of radiation therapy is limited by the resistance of tumors and sensitivity of normal tissues to irradiation. Optimization of the therapeutic index would theoretically be achieved by simultaneously and selectively radiosensitizing tumor cells and protecting normal tissues from the effects of radiation. Traditional radioprotective strategies rely on countermeasures that prevent ionizing radiation (IR) damage (such as free radical scavengers) or stimulate post-IR proliferation and recovery of normal tissue, for example, with growth factors (1). These approaches typically do not discriminate between normal and cancer cells. For instance, the use of antioxidants such as alpha-tocopherol and betacarotene during the course of radiation therapy was associated with evidence of poorer tumor control in randomized trials (2, 3).

A detailed understanding of the radiobiology of normal and cancer cells, particularly in terms of the mechanisms of post-IR DNA damage response, enables development of radiosensitizers/protectors with selectivity for cancer and normal tissues. Activated by DNA strand breaks following IR, DNA damage response comprises a complex signaling cascade orchestrated by PI3K-related kinases [ATM, ATR (ATR ataxia telangiectasia and Rad3 related), and DNA-PK (DNA protein kinase)] and downstream factors, which eventually leads to multifaceted events (DNA repair, cell cycle arrest, and apoptosis) and maintains genomic stability. It is known that some DNA repair pathways (such as homologous recombination) are deficient in some tumors, which provides the rationale for targeting complementary repair pathways [eg, with PARP (Poly-(ADP-ribose) polymerase) inhibitors] in combination with IR (4, 5). In another instance, cancer cells are often defective in G1/S cell cycle arrest due to mutations in retinoblastoma protein and CDK (cyclin dependent kinase) inhibitors (p27 and p16), and/or high levels of cyclin D1 (6). As a result, drugs that enhance G1/S cell cycle arrest do not affect these cancer cells but enhance the survival of normal hematopoietic cells after IR (7).

It was previously reported that darinaparsin (DPS, an organic arsenic compound) sensitizes solid tumor cells to IR and increases the post-IR survival of radiosensitive intestinal crypt epithelial cells (CECs) in the crypt microcolony survival assay (8). The results presented here build on these findings and help elucidate the underlying mechanisms of action of DPS-mediated radioprotection. Moreover, the results demonstrate that the previous findings of radioprotection in the crypt assay are biologically meaningful in terms of decreasing the death rate in mice from IR-induced gastrointestinal syndrome.

Materials and Methods

Animal tumor models and reagents

Prostate cancer cells that express firefly luciferase were mixed with MatriGel (BD Bioscience, Franklin Lakes, NJ) and injected (20×10^5/mouse) into the left dorsal prostate of nude mice (male, 8 weeks old). The implanted tumors were imaged (1×/wk) by D-luciferin (3 mg/mouse in 15 mg/mL solution, intraperotineal [IP]) using an IVIS 200 system (Caliper, Hopkinton, MA). The tumor was quantified by the average radiance. The experiments were started 4 weeks after implantation when the tumors reached an average radiance of $1.53*10^8$ (photos/second/cm^2/sr) and showed exponential growth. For irradiation, the mice were anesthetized with ketamine (80 μg/g, IP) and xylazine (16 μg/g, IP) and placed in a jig with head and upper limbs shielded from IR. A Phillips RT250 200kVp x-ray unit with a 1.0-mm Cu filter was used to deliver the IR dose to the mice at the dose rate of approximately 126 cGy/min.

To assess the effect of DPS on acute gastrointestinal radiation syndrome and DNA damage response, female BALB/C mice (8 weeks old) were used for studying effects of DPS on CECs. DPS was provided by ZIOPHARM Oncology (Boston, MA) and stored at −80°C before dissolving in phosphate-buffered saline at a concentration of 20 mg/mL for each injection. All other reagents were obtained from Sigma-Aldrich (St Louis, MO) unless otherwise specified. The IR doses were delivered by subtotal body irradiation as described above. The change in mouse body weight and survival were monitored twice daily. In some experiments, bromodeoxyuridine (BrdU; 100 mg/kg, IP) was injected 1.5 hours before mouse euthanization. To assess the effects of DPS and IR on CEC survival, we used an established microcolony assay that measured the number of viable crypts/cross-section in the duodenum 3.5 days after radiation as previously described (9).

Immunohistochemistry

Formalin-fixed and paraffin-embedded duodenal tissue sections were autoclaved for 10 minutes in 40 mM citrate buffer (pH 4), and then placed in 3% H_2O_2 (in 70% ethanol) for 30 minutes. The slides were incubated with primary antibodies (Supplemental Table 1) overnight at 4°C. After washing, tissues were incubated with secondary antibodies (Supplemental Table 1) for 1 hour at room temperature and stained with a horseradish peroxidase (HRP) substrate (Diaminobenzidine [DAB]) kit (DAKO, Carpinteria, CA) and then counterstained with hematoxylin.

Histologic and immunohistochemical scoring

The slides were scored in a blinded manner independently by 2 experimenters. For the scoring of histological (micronuclei, aberrant mitosis) and immunohistochemical (cleaved caspase 3, BrdU, pH3, and pATM) features, the scoring was done by counting the number of positive cells and calculating the number of positive cells/the total number of cells counted. The CECs of at least 15 crypts were counted as either positively or negatively stained, and the ratio of positive/total cells was used for each sample as the index of activation. For cytoplasmic staining such as that of p21 and growth arrest and DNA-damage-inducible protein 45 alpha (GADD45A), the scoring was expressed numerically on a scale from 0 to 3 for each sample, and an average value was derived from all of the samples scored for each group.

Statistics

All data are expressed as the mean ± SE. The statistic analyses were carried out using the software Prism (La Jolla, CA). Exponential growth modeling was used to model tumor growth and to compare the growth of tumors in mice treated with the combined DPS/IR treatment compared with all other treatment