Differential Effects of Radiation and Radioprotectant on Ewing's Sarcoma, Rhabdomyosarcoma, Bone Marrow Cells and Osteoblasts

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Introduction: Radiation therapy is a well-established adjunct to surgical excision for soft tissue sarcomas and an alternative to surgery for Ewing's sarcoma of bone in pediatric patients. Radiation negatively impacts the growth plate in a growing child, leading to long-term complications. Radioprotectant drugs that protect healthy cells and tissues from the negative effects of radiation may be of value in these pediatric patients. In previous work, we have established that one such radioprotectant, the free radical scavenger amifostine (WR-2721), provides effective radioprotection to growth plate chondrocytes in an in-vivo animal model1-3. In order to be clinically acceptable, however, radioprotectants must be selective in their protection of normal tissues at the expense of the irradiated tumor. This selectivity has not been demonstrated for musculoskeletal sarcomas. The specific aim of this study was to use a cell culture model to compare the individual effects of radiation and amifostine on two pediatric sarcoma cell lines versus normal osteoblasts and bone marrow. These experiments tested two hypotheses: (1) amifostine does not enhance the proliferation or survival of tumor cells and (2) tumor cells and bone marrow cells are relatively sensitive to radiation as compared with osteoblasts.

Methods: The TC-71 Ewing's Sarcoma cell-line, the RD rhabdomyosarcoma cell-line, and the MC3T3-E1 mouse osteoblastic cell-line were maintained in RPMI 1640, DMEM and α-MEM medium respectively. All media were supplemented with 10% fetal calf serum (FCS) and 1% penicillin-streptomycin-glutamine (PSG). Primary human bone marrow cells, obtained under an approved IRB protocol, were collected from adult subjects undergoing elective total hip replacement. Bone marrow monocytes, isolated by density gradient centrifugation, were cultured in α-MEM with 10% FCS and 1% PSG.

For the radioprotectant experiments, amifostine (0, 0.5, 1, 2, 5, & 10 mM) was added to 24-well plates containing 1x10⁴ cells/well. For the radiation experiments, 5x10³ cells/well were exposed to 0, 1, 2, 5, 10, and 20 Gy x-irradiation using a radiotherapy unit (MGC-30; Phillips, Shelton, CT) operating at 300 kV at 10 mA (effective dose rate = 2.94 Gy/minute at 15 cm source-object distance). Plates were harvested at 6, 12, 24, and 72 hours following exposure to radioprotectant and at 12, 24 and 72 hours following irradiation. The MTT assay was used to quantify the effects of treatment on cellular proliferation. A commercial assay for lactate dehydrogenase assay (CytoTox 96; Promega, Madison, WI) was used to quantify cytotoxicity. MTT data from treated wells were normalized to those in control wells. LDH data were expressed as the percentage of total LDH that was released into the medium. Statistical analysis was performed using ANOVA with post-hoc testing where appropriate. A significance level of p<0.05 was used in all cases.

Results: Clinically relevant doses of amifostine (1-2 mM) caused a profound (>80%) decrease in viable cell numbers in both tumor cell lines (Figure 1; p<0.001 vs. controls for all doses). This decrease in viable cell numbers was associated with statistically significant increases in LDH activity (indicative of overt cytotoxicity) in both the TC71 (Figure 2) and RD cell lines (p<0.001 for all doses). MC3T3 viability was decreased to a lesser extent (60%; p<0.005 for all doses) but this effect was not associated with cytotoxicity (Figure 2).

In contrast, amifostine caused a dose-dependent increase (up to 15-fold) in the proliferation of human bone marrow cells (p<0.001 for all doses) with concomitant modest increases in LDH activity (Figure 2) that probably reflect cell death due to exhaustion of the growth surface and/or available nutrients.

Data from the irradiation experiment confirmed the extreme radiosensitivity of bone marrow cells (75% inhibition at doses of 1 Gy or greater; p<0.001) and relative resistance of osteoblasts (35% inhibition at 20 Gy) (Figure 3). The two human sarcoma cell lines displayed intermediate sensitivity, with approximately 50-60% inhibition at all doses (Figure 3; p<0.001). Cytotoxicity appeared to be the predominant mechanism by which radiation modulated cell survival (data not shown).

Discussion: Three key findings have been identified. First, amifostine has differential effects on tumor cells versus normal cells. Amifostine has a clear and consistent toxic effect for both TC71 and RD cells. In contrast, there was no significant cytotoxicity in osteoblasts or bone marrow cells. Amifostine has an anti-proliferative effect on MC3T3 cells, but a proliferative effect on human bone marrow cells. The significance of (and mechanisms behind) these proliferative effects are currently unclear and merit further investigation.

The second important finding is confirmation of the radiosensitivity of two pediatric tumor cell lines that are generally considered to respond well to radiation therapy. This model therefore appears to be appropriate for future studies on combination therapy for pediatric musculoskeletal sarcomas.

The third and perhaps most intriguing finding is the relative sensitivity of bone marrow cells and resistance of osteoblasts to radiation therapy. These extreme differences in radiosensitivity offer a potential explanation for previous in-vivo observations that bone mineral density increases following irradiation in our Sprague-Dawley rat model4. These differences may help explain the clinical observation that successful radiation therapy is often associated with an osteoblastic “flare” response: this flare may reflect a brief imbalance in bone homeostasis caused by a loss of the relatively radiosensitive osteoclast precursors in bone marrow with concurrent sparing of the osteoblastic elements. These in-vitro data indicate that the amifostine does exert selective effects on tumor cells versus normal cells. Further in-vitro studies and, ultimately, animal studies are now needed to confirm that the use of amifostine as an adjunct to radiation results in protection of growth plate without concurrent protection of tumor cells.


Acknowledgements: Funded by the Children’s Miracle Network.