2001 Protective Effect of Ramipril, Inhibitor of Angiotensin Converting Enzyme on Radiation-Induced Normal Tissue Damage Without Tumor Protection

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Purpose/Objective: The effectiveness of radiation therapy (RT) is limited by inadvertent acute and late tissue injury. One strategy being investigated in animal models is the use of angiotensin converting enzyme (ACE) inhibitors to reduce normal tissue injury. The present study was designed to investigate the possible reduction in radiation induced damage of acute and late responding tissues by the ACE inhibitor, ramipril. Ramiprils effectiveness on damage was evaluated in a mouse model along with an assessment whether the reduction in radiation injury was just limited to normal tissue or would also reduce effectiveness of radiation on tumor response.

Materials/Methods: Skin damage was accessed using a semiquantitative scale, scored 1–5, (adapted from Dion et al., Int J Rad Onc Biol Phy, 1988) which includes moderate erythema with dry skin, dry desquamation, moist desquamation and full thickness skin loss. RT induced, 30 Gy x 2 and 6 Gy x 10 skin damage in the right hind limb of the BALB/c mouse was evaluated in a RT alone and RT plus ramipril 2.5mg/kg/day (administrated after RT) group for greater than three months.

In the same groups of animals as above, muscle damage was evaluated weekly after day 60 by measuring leg contraction in the irradiated leg as compared to the contralateral leg.

The effect ramipril has on radiation induced (8 Gy x 2) tumor growth delay (GD) was tested in a murine tumor model. A549 lung adenocarcinoma xenographs were grown intramuscularly in hind right leg of Athymic mice. Tumor size was recorded in groups including no treatment, RT alone, and RT plus ramipril over time.

Results: RT induced, 30 Gy x 2, skin damage was consistently decreased ($p < .05$) in the RT plus ramipril ($n = 10$) vs. RT alone ($n = 8$) starting approximately 30 days after radiation and continuing through 90 days post RT. These skin effects do not appear to be specific to the 30 Gy x 2 fractionation schedule because this trend continued ($p < .06$ at multiple time points, $n = 4$) with the fractionated schedule of 6 Gy x 10.

The leg retraction data also showed a consistently significant ($p < .001$) decrease in radiation induced retraction in the RT plus ramipril group (50%) vs. the RT alone group (30%) at all time points. This trend ($p < .05$ at most time points) also continued in the 6 Gy x 10 fractionation schedule.

There was tumor GD in the RT alone group of approximately 8 days vs. no treatment (additional time to reach 3x initial tumor volume relative to untreated controls). Tumor GD with RT and ramipril was extended from the RT alone by another 8 days as compared to RT alone, showing an approximate GD of 16 days from the no treatment control. This result was independent of whether ramipril was administered during or after the radiation treatment. In both experiments, the RT plus ramipril groups showed significantly ($p < .05$) smaller tumor size at the last two time points when compared to the RT alone group.

Conclusions: In this animal model the ACE inhibitor ramipril protected both acute (skin) and late responding (muscle) tissues in radiation induced tissue damage. This effect is also appears to be consistent in different fractionation schedules. Interestingly, the data not only supported the hypothesis that ramipril does not confer any tumor protection with RT but indicates further experiments are necessary to confirm if ACE inhibitors can contribute to greater tumor control when combined with radiation. The potential to translate this animal data into a clinical trial is exciting. Ramipril is a relatively non toxic medication presently prescribed safely in many hypertensive patients.

2002 Preferential Damage to an AT Island and Matrix Attachment Region (MAR) in Fragile Site FRA16D Induced by Ionizing Radiation in Cancer Cells

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Purpose/Objective: Long-range distribution of DNA lesions induced by ionizing radiation (IR) is believed to be influenced by the spatial organization of DNA in the nucleus. No specific genomic regions, however, have yet been found to be preferentially affected by IR. We propose that matrix attachment regions (MARs) could be such preferential targets. MARs are responsible for periodic association of DNA loops to the nuclear matrix. Strong MARs, which are critical for replication, transcription, and mitosis, comprise stretches of 200–1000 base pairs (bp) of highly AT-rich repetitive DNA, referred to as AT islands. We have suggested that AT-island-containing MARs can overlap with known sites of genomic instability. Fragile site region 16D (FRA16D)is an example of cancer relevant site of genomic instability. FRA16D contains a substantial AT island and coincides with a putative tumor suppressor gene WWOX (a frequent target for loss of heterozygosity in various cancers). The goal of this study was to establish the MAR properties of the AT island in FRA16D and characterize IR-induced damage to this region in comparison to lesions in a non-MAR DNA.

Materials/Methods: MAR properties of FRA16D AT island were analyzed in silico and verified using binding of radiolabeled probe to isolated nuclear matrices. Lesions in specific regions of DNA from irradiated leukemic CEM cells were monitored using quantitative PCR stop assay, which detects DNA damage that prematurely terminates PCR primer extension. DNA double-strand breaks (DSB) in total cellular MARs and total loop DNA were examined using field inversion gel electrophoresis (FIGE) analysis of matrix-associated DNA fraction and loop DNA fraction prepared from irradiated cells.

Results: Consistent with a high computed MAR potential, FRA16D AT island was directly confirmed to have MAR properties. A strong signal of [^{32}P]FRA16D probe bound specifically to isolated nuclear matrices was observed in the presence of ~500-fold excess of non-specific DNA competitor but was nearly eliminated, as expected, by an 8-fold excess of unlabeled probe. A model non-MAR loop DNA domain, a segment from the β-globin gene, showed no significant binding to nuclear matrices. FRA16D AT island and the β-globin segment were also used as model regions to examine IR-induced damage in cellular DNA. Analysis of CEM cells irradiated with 0–30 Gy and post-incubated for 24 h showed that IR-induced persistent