The Endogenous Peptide Angiotensin-(1-7) Prevents Radiation-Induced Muscle Fibrosis: An In Vivo Murine Model

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Disclosures:

Introduction: Over 1,000,000 patients are diagnosed with cancer annually and nearly 67% of them receive radiotherapy (RT). Radiation-induced fibrosis (RIF) is a debilitating late effect of RT that causes muscle weakness, joint contracture, and functional limitations. Currently, no standardized therapy prevents RIF in muscle, and animal models for testing therapeutics are poorly established. Angiotensin-(1-7), an endogenous peptide hormone in the nonclassical renin-angiotensin system, has previously been shown to prevent collagen deposition associated with tumoral fibrosis. We hypothesize that Angiotensin-(1-7) prevents RIF in skeletal muscle after modeled sarcoma RT.

Methods: Seven-week-old Swiss Albino mice received either sham surgical procedure or subcutaneous osmotic minipump delivering angiotensin-(1-7) at 24 μg/kg/hr beginning three days before undergoing a two-week course of fractioned radiation (7.3 Gy/fraction; 2 fractions/week) using 300 kV x-rays targeting one hindlimb. This RT regimen provided the biological equivalent dose for sarcoma treatment (100.2 Gy) despite 29.2 Gy total dose. Controls received no irradiation or treatment. Animals were sacrificed at 1.5 and 4 months after RT. Fibrosis in the gastrocnemius was assessed with in vivo load-relaxation testing before sacrifice. Tension generated by displacing the immobilized muscle 5% and 10% of its resting length was recorded (IOX v2.8, EMKA Technologies) with a force transducer (National Instruments). Muscle relaxation properties were evaluated with Fung’s quasi-linear viscoelastic (QLV) model. Following testing, muscles were processed, sectioned and stained with picrosirius red for histological analysis. Interstitial and perivascular fibrosis were quantified by averaging the percentage of the field stained for collagen in five random sections taken from the mid portion of each gastrocnemius muscle.

Results: Radiotherapy (n=9) increased stiffness of the gastrocnemius significantly (107%, p<0.001) relative to control (n=12) at 1.5 months and 4 months, as determined from passive displacement muscle tension (figure 1). Angiotensin-(1-7) treatment (n=11) mitigated this response at 1.5 months (27% increased stiffness) and 4 months (37% increased stiffness), a significant reduction compared to sham treatment (p<0.001 and p<0.01, respectively). Relaxation data in the Fung framework showed no difference between groups (parameters C, τ1, τ2) suggesting that the increased peak tension found in irradiated animals was not compensated for by altered relaxation magnitude. Coincident with functional stiffening, histologic quantification (figure 2) at 1.5 months demonstrated that RT induced skeletal muscle fibrosis compared to control animals (0.6% fibrosis/field to 1.1% fibrosis/field, p<0.01) and perivascular fibrosis (7.4% fibrosis/field to 15.8% fibrosis/field, p<0.05). Ang-(1-7) treatment for 1.5 months prevented these fibrotic changes (skeletal muscle, 0.7% fibrosis/field; perivascular, 8.4% fibrosis/field) which was a significant reduction compared to sham treatment (p<0.05). At 4 months relative to controls (figure 2), irradiated mice showed more pronounced interstitial fibrosis (0.7% ± 0.1 to 1.8% ± 0.2, n=5, p < 0.001) as well as perivascular fibrosis (15.13 ± 1.8 to 28.5 ± 3.5, n=5, p < 0.01). Ang-(1-7) treatment for 4 months again mitigated both the interstitial fibrosis (0.9 ± 0.1, n=5, p < 0.001) and perivascular fibrosis (19.5 ± 2.0, n=5, p < 0.05) relative to the irradiated sham treatment group.

Discussion: Angiotensin-(1-7) mitigated RIF resulting from modeled sarcoma RT in a small animal model. The murine model was successfully used for study of an RIF therapeutic in the current investigation. Clinical translation of these findings will determine whether prophylactic angiotensin-(1-7) treatment can prevent RIF in patients who require adjuvant radiotherapy for treatment of extremity soft tissue sarcoma. Ang-(1-7) completed a phase I clinical trial in patients with solid tumors and was well tolerated with 4 of the 15 evaluable patients showing clinical benefit. A Phase II clinical trial is in progress to determine the efficacy of Ang-(1-7) in patients with metastatic sarcoma.

Significance: Prophylactic Angiotensin-(1-7) treatment prior to radiation therapy may prevent the development of fibrosis in muscles exposed to high dose radiation during sarcoma treatment.

Acknowledgments: The authors thank Dr. Dan Bourland for dosimetry, Dr. Beth Smith for study oversight and Mark Landrum for laboratory assistance.
