The Effects of External Beam Irradiation on Ear Cartilage: An Animal Model

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Citation

Abstract

Adjuvant therapy of the head and neck has become the mainstay for treatment of head and neck malignancies. Although important for local control, complications do arise with the use of this adjuvant therapy. We have recently noted changes in the ear architecture in patients that have undergone postoperative irradiation therapy. In an attempt to identify changes in the cartilage thickness, we conducted an animal model to determine the etiology of our clinical observation.

Eighteen New Zealand white rabbits were utilized. Three experimental irradiated groups were designed for single dose external cobalt60 beam therapy at 20, 25 and 30 Gy. The right ear served as matched nonirradiated control. After two months the cartilage underwent quantitative histomorphometric analysis using an inverted microscope, digitized camera and computer software.

Irradiation did not significantly affect the thickness of cartilage upon comparing control and irradiated groups. There was no change seen in vascularity or tissue composition that could be identified.

In conclusion, it appears that alterations in thickness of the cartilage did not result in the clinical examples demonstrated. The etiology to the changes noted clinically are unknown, but may be related to fibrosis and scarring of the dermis/epidermis. Further evaluation is warranted.

INTRODUCTION

With the discovery of X-rays by Wilhelm Konrad Roentgen (1895), ionizing radiation became both a diagnostic tool and therapeutic modality. The goals of ionizing radiation are local regional tumor control combined with functional preservation alone or in conjunction with surgical extirpation. High doses of irradiation can be delivered currently with specialized techniques in an attempt to minimize the late complications of normal tissue. Radiation portals precisely outline tissues at risk and exclude uninvolved regions by applying individualized treatment blocks.[1],[2] With the advent of this adjuvant therapy, the approach to the patients with surgical extirpation has changed. Local control has been improved, however, complications related to wound healing have increased.

The daily dose of radiation is considered to be one of the most important factors in the development of sequelae. We have recently noted that patients undergoing pre and/or postoperative radiation therapy of the head and neck have presented alterations in their ear architecture (Figure 1). This presentation is irrespective of surgical alterations within the area. Figure 1 demonstrates the clinical appearance seen in many of these patients. Unfortunately we can not correlate this effect with dose or other factors clinically. Protrusion of the ear anteriorly with a constriction similar to congenital ear anomalies has lead us to explore this clinical observation in an animal study. Consequently, it was the purpose of this study to determine alterations in cartilage thickness in a rabbit model with animals undergoing single dose irradiation therapy. It was hypothesized that cartilage thickness would be effected in a radiation dose dependent manner.

MATERIAL AND METHODS

Eighteen New Zealand white rabbits (3.0-3.5 kg) were used in this study. Three experimental irradiated groups were designed. Under general anesthesia, radiation was applied to the left ear using a cobalt60 external beam source. The dose

was not fractionated and three distinct dosing levels were employed. It is a common practice to utilize single dose irradiation as a determinate of an effect. Further studies with fractionated dosing can then be employed.[2-6] These included six animals each that received 20, 25 and 30 Gy. The right ear served as matched, nonirradiated control. The right ear was protected during the irradiation dosing to abrogate scatter irradiation. Preoperative dosimetry was determined allowing the maximum dosing to the area in question, and to prevent associated morbidity and damage to the surrounding tissues. After two months, rabbits were euthanized. Commonly 6 weeks are utilized for the effects of chronic radiation to begin.[2-6] The ears were harvested and placed in 10% neutral formalin for histological processing. All animal care and use adhered to institutional, NIH, and AAALAC guidelines.

HISTOLOGY

A section was grossed from each portion of the ear provided. The sections were processed for paraffin and sectioned at 4 m. They were then stained using hematoxylin and eosin.

ANALYSIS

Quantitative histomorphometic analyses were carried out using an inverted microscope (Olympus), digital camera (Olympus), computer-controlled stage (Ludl Electronic Products), brightfield optics, G3 CPU (Apple), and IPLab imaging software (scanalytics). Thickness metrics was utilized. For these cartilage samples, the thickness of the cartilage was measured using a 10x (1.2 m/pixel) objective.

STATISTICS

Data are presented as mean standard error of the mean with n=6. A p 0.05, as described by Student's t-test assuming equal variance and null hypothesis, denotes statistical significance.

RESULTS

As demonstrated in Figure 2, irradiation did not significantly affect the thickness of cartilage upon comparing control and irradiated groups. No significant alterations in quantification of vascularity or cellular infiltration was noted. In addition, no significant changes in the epidermis or dermis were identified.

Figure 1

Figure 1: 54 year old man who underwent resection of a SCC to the tongue. He was reconstructed with a vertical rectus abdominis free tissue transfer for total glossectomy and partial phayrngectomy. The patient was treated postoperatively with 60 Gy of radiation therapy. Note the difference in ear protrusion with the right (radiated) being more prominent than the left (nonirradiated). No surgery on the ear was performed at the time of the original resection

Figure 2

Figure 2: Grafts demonstrating irradiated and control cartilage thickness. There was no statistical difference between the 3 irradiated groups and control.
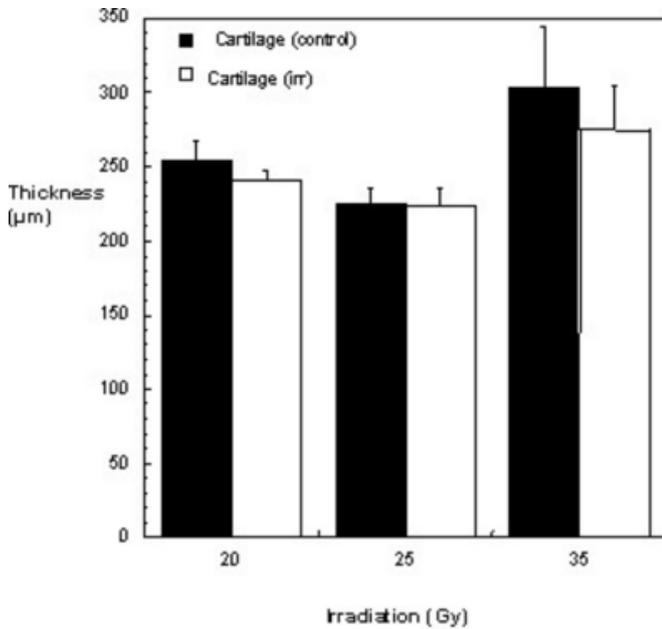
DISCUSSION

The results of this study indicate that elastic cartilage is not affected within a two-month period by a single dose of irradiation between 20 and 35 Gy. We have selected to use nonfractionated dosing in this study for several reasons. 1) Fractionating dosing within this animal population would be difficult subjecting the animal to numerous anesthetic procedures. 2) The study was an attempt to examine if radiation therapy would effect cartilage. Further studies with fractionation would be planned if demonstrated changes were noted. 3) Previous studies with a variety of tissue types have indicated that single dosing for examination of changes appears appropriate in order to determine potential effects of irradiation.[2,3,4,5,6]

External beam irradiation remains the most widely available treatment approach to cancer therapy. The concept of fractionating irradiation allows treatment of the cancer while not exceeding the tolerance of the surrounding normal tissue providing adequate time for repair. In general the larger the daily dose of irradiation, the lower the total dose that can be administered due to normal tissue tolerance.[1,2] Unfortunately, the dose necessary for tumor irradiation is indexed to the tumor volume. Consequently the larger the irradiation dose, the more tumor cells are killed. Thus a balance between tumor cells killed and total dose administered which is limited by radiation effects on normal tissue, must be struck.

Standard treatment regimens attempt to exploit the differences in cell kinetics between tumor cell populations and the normal tissue. Delivery fractions of conventional radiation normally involve 180 cGy-200 cGy over 24 hour intervals. A total dose of 50-70 Gy is usually achieved over a 6-7 week period. It is unclear what the components to fractionating the radiation dose would be in this study. It can be hypothesized however, that if a single dose did not produce the hypothesized effects, then fractionation would probably not demonstrate alterations in cartilage architecture and thickness.[2-6]

The nature of tissue injury from ionizing radiation is thought to be caused by two discrete mechanisms. 1) DNA disruption, which can cause a lethal injury if genes critical to routine cellular processes are affected. More commonly however cell death occurs from the disruption of normal cell division. In general, cells are most sensitive to radiation in the G2-M phase and most resistant in the last S phase.[6] 2) The generation of oxygen free-radicals which has a direct toxic effect on the cell, culminating in cell death. Consequently radiosensitivity is partially based upon the oxygen tension in the tumor.[2]

Acute radiation effects occur during or immediately after the course of radiation therapy. They are characterized by an inflammatory response in rapidly proliferating tissues like the skin and mucosal surfaces.[7] Late radiation effects can be exhibited by any tissue. There is no association between the intensity of the acute reaction and the late radiation effects. However, factors that appear important in the development of chronic changes are total dose and fractionation. Tissue fibrosis appears to be the most common histologic effect of late radiation changes however alopecia and xerostomia may also occur. Late radiation changes produce an irregular epidermis with areas of atrophy alternating with variable hyperplasia. Pigmentary changes may been seen along with thickening and fibrosis of the skin and subcutaneous tissues.[1,2,7]

The National Cancer Institute task force has determined the tolerance doses (TD) of tissues and organs as a function of the likelihood of a treatment related complication in 5 years using conventional fractionation. Thus the minimal tolerance dose is defined as that dose that will create a complication in 5% of the patients at 5 years for that specific tissue (TD 5/5). As the dose increases the TD ratio also increases. For example, the dose causing 50% complications at 5 years is expressed as (TD 50/5).[1,2,8]

The mechanism of wound healing is a complex process that involves cellular migration, proliferation, differentiation, and a variety of growth factors used to accelerate the dermal and epidermal process. Radiation induces adverse effects on soft tissue by modifying the normal environment. [9,10,11]

Becker has recently demonstrated in 19 of 1950 patients, radiologic features of inflammatory swelling adjacent to the area of involvement, noting sclerotic fragmentation and sloughing of necrotic bone or cartilage.[12] Large dose irradiation applied to rats resulted in a highly significant reduction of cartilage.[13] This was demonstrated in the rat with 5 x 1 Gy doses of irradiation. Further, this reduction appeared to be dose dependent. Becker also noted that low doses of irradiation interfered with nitric oxide synthase and hem oxygenase [1]. This effect does not appear to be through a macrophage mechanism.[14,15] It is interesting to note that in our study we utilized a higher dose of irradiation and a larger animal model. Despite this increased dose, we did not identify changes in the cartilage. This could be due to the age of the rabbit (adult) or other factors such as cartilage turn-over which is slower in the ear than other locations such as the long bones or epiphyseal plates.

Hiranuma isolated chondrocytes from the growth plate of the ribs of 4 week old rabbits. The study demonstrated that 10 Gy or less of irradiation to growth plate condrocytes impaired terminal differentiation.[16] In contrast to this study, we utilized adult rabbits. The use of fetal or young rabbits may have proved alternative results. Alternatively, actively dividing cartilage may be more susceptible. Both of the above studies however indicate that radiation dose alters cartilage structure in an animal model.

The etiology to the clinical changes seen in ear architecture in not known. It appears from this study that alterations in cartilage thickness does not occur. However scarring and fibrosis does occur within the soft tissue and thus may be the hypothesis for change of formation of the normal ear shape.[2] Herskind demonstrated more terminal differentiation in radiation induced fibrosis and imply that the progenitor population surviving radiotherapy might be more prone to terminal differentiation than before radiotherapy.[17,18,19] Further, the risk of fibrosis increases with the progression of the differentiation of untreated progenitor fibroblasts, indicating that the progression of fibroblast differentiation may be a co-factor in the development of radiation induced fibrosis.[17-19] Radiation induced inactivation of fibroblasts was paralleled by an increase in terminally differentiated fibrocytes, demonstrating that premature terminal differentiation is an important response to irradiation of fibroblasts from radiotherapy patients. Further more, increased collagen production was observed after irradiation.[17-19] Other results indicate increased local collagen synthesis and accumulation of connective tissue in irradiated skin.[20]

SUMMARY

In conclusion, it appears that in this animal model, alterations in cartilage thickness did not result in an explanation of the clinical examples of deformation. The etiology of the changes noted clinically are unknown but may relate to secondary fibrosis, thickening and scarring of the dermis/epidermis.[21] The lack of stable structural components to the cartilage then allows movement of the ear secondary to this scarring.

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