FREE RADICAL SCAVENGERS REDUCE THE BIOMECHANICAL AND BIOCHEMICAL IMPAIRMENT OF GAMMA IRRADIATED BONE ALLOGRAFTS

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INTRODUCTION

Prevention of viral and bacterial disease transmission by gamma radiation is widely used for sterilization of bone allografts (1). Despite its efficacy in sterility assurance, it has been shown that gamma radiation negatively affects the material properties of bone (2). The impairment in material properties is largely believed to stem from the cleavage of collagen molecules of bone via free radical attack(3). We hypothesize that the biochemical and biomechanical impairment of bone grafts during gamma radiation sterilization can be ameliorated via suppression of free radical generation using the free radical scavenger thiourea.

METHODS

Preparation of test specimens: Four fresh frozen bovine ulnae (3-4 years old) were machined into tensile test specimens. All specimens were obtained from the anterior mid-diaphyseal region. A low-speed metallurgical saw (South Bay Tech, San Clemente, CA USA) and a table-top milling machine(Sherline, CA USA) were used to machine specimens. The gage region measured 16 mm in length, 2 mm in width, and 1mm in thickness. Specimens were kept in calcium supplemented saline solution and stored at -40°C.

Treatment of Specimens: Thirty specimens were randomly placed into six treatment groups (n=5, each): control (C), irradiated (I), 0.5 [M] thiourea treatment (0.5 [M]), 1.5 [M] thiourea treatment (1.5 [M]), 0.5 [M] thiourea treatment and irradiation (0.5 [M] - I) and 1.5 [M] thiourea treatment and irradiation (1.5 [M] - I). Specimens were kept in aqueous thiourea solution supplemented with calcium at 4°C for two-weeks. The solution was supplemented with protease inhibitors to prevent microbial degradation and was changed every three days. Gamma radiation sterilization was performed using ^{60}Co source at an average dose of 30 kGy (Steris Corporation, Mentor, OH USA).

Biomechanical Tests: Specimens were monotonically loaded to failure under tension using an electromagnetic testing machine (ELF 3200, Enduratec, Minnetonka, MN USA). The loading was displacement controlled at a rate of 1%/s. Energy to fracture parameter was obtained from stress-strain curves.

Biochemical Analysis: The integrity of collagen molecules were assessed by SDS-PAGE analysis (n=2). The purification involved demineralization by formic acid, salt precipitation, dialysis to remove demineralization products, and solubilization of collagen molecules through pepsin digestion. In between purification steps of specimens, samples were lyophilized. Solubilized collagen molecules were loaded on 5% acrylimide-bis SDS-PAGE gels to determine the amount of intact alpha and beta chains.

RESULTS AND DISCUSSION

There were no statistically significant differences between the mechanical properties of any treatment groups for the bovine cortical bone. However, the trend in the data was as expected for most mechanical properties observed in the study such that (Fig 1.): The irradiated group demonstrated smaller energy to fracture than controls. Thiourea treated control groups were similar to controls suggesting that thiourea alone does not alter mechanical properties of bone. There was an apparent improvement in the mechanical properties of irradiated specimens treated with thiourea such that mechanical properties of irradiated specimens treated with 1.5 [M] thiourea was greater than irradiated specimens treated with 0.5 [M] thiourea and irradiated alone. Also the mechanical properties of irradiated specimens treated with 0.5 [M] thiourea were greater than those specimens irradiated alone. It appeared that the mechanical properties of irradiated specimens treated with 1.5 [M] thiourea were similar to the mechanical properties of unirradiated controls. This observation suggests that thiourea may have a radioprotective effect on the mechanical properties of bone.

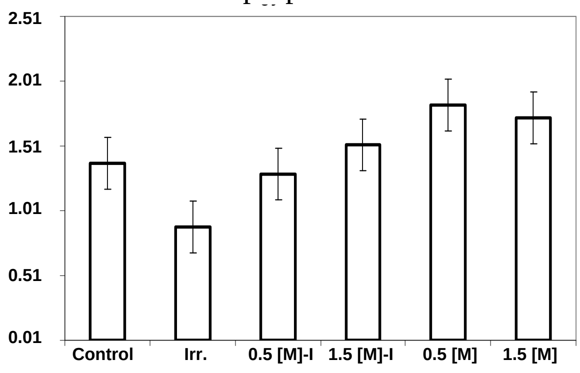

Fig. 1 Energy to fracture (Joules) of treatment groups. Error bars illustrate the standard error of the mean.

Biochemical results showed that there was a decrease in the intensity of α-chains following sterilization. It is shown in lane profiling that the irradiated samples treated with thiourea demonstrated intact α-chains (Fig. 2).

It is known that gamma radiation embrittles bone tissue by impairing the post-yield properties rather than the pre-yield properties (2,4). As bovine bone already suffers in terms of post-yield deformability in its natural state, further embrittlement due to gamma radiation sterilization is highly limited. Therefore, we do not suggest bovine ulna as a model for investigation of gamma radiation induced embrittlement.

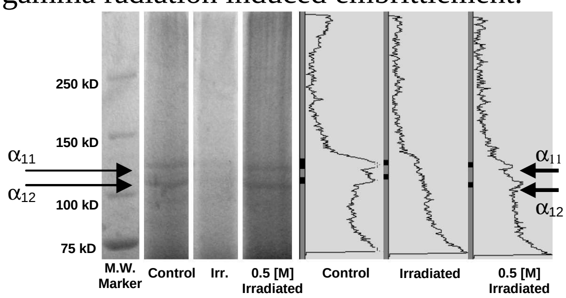

Fig 2:SDS-PAGE and lane profiles of the control, irradiated and 0.5 [M] irradiated samples.

SUMMARY

The trend observed in these results supports the hypothesis that the suppression of free radical generation has a radioprotective effect on the mechanical and biochemical properties of gamma radiation sterilized cortical bone tissue. The results of this preliminary study suggest that suppression of free radical damage via thiourea treatment may yield stronger and more durable grafts.

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