IN-VIVO MICRO-CT STUDY ON BONE MORPHOLOGY OF GROWING RATS: RADIATION EFFECTS

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Introduction

Micro-CT (µCT) is an efficient tool for the noninvasive examination of laboratory animals and for *in vivo* tracking of longitudinal changes in bone mass and microstructure due to disease or bone adaptation. Previous studies reported possible tissue damage due to low-dose radiation [1]. Several researchers [2, 3] investigated the effects of µCT radiation in adult animals and found minimal or no effects on bone microstructure. However, it is not known if weekly µCT scanning during the adolescent period would affect bone development and architecture. Therefore, our objective was to investigate whether a nine weeks *in-vivo* scanning of rat tibiae during the growing period would result in altered bone mass and structure.

Methods

An *in vivo* µCT scanner (Skyscan 1176) was used to perform weekly scans of the right tibia of five male Sprague-Dawley rats from their 4th to 12th weeks of age (9 weeks). An additional scan was performed two weeks later, after which rats were sacrificed. All scans were performed under anesthesia with a voxel resolution of 18 µm resulting in a dosage of 0.84 Gy. The left tibia was used as a control and scanned only on the first and last time points. Trypan blue tests were performed to assess cell radiation damage. Calcein labeling was used to measure bone growth rates (µm/day) (Figure 1). Bone structural parameters, including bone fractional volume (BV/TV), connectivity density (Conn.D), structure model index (SMI), trabecular number, thickness and spacing (Tb.Nr, Tb.Th, Tb.Sp), were determined for proximal tibial sections.

Results

For the structural parameters, no significant difference was seen between the ratio of right/left of the first (week 1) and the last (week 11) scans ($p>0.05$, Figure 2). Also, no significant difference was observed between the percentage living cells of the left and right tibia ($p=0.93$) (Table 1). Bone growth rate was also not significantly different ($p=0.14$) between the left and right tibia.

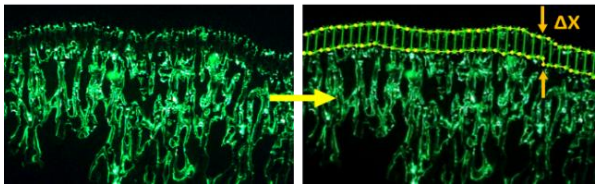

Figure 1: Bone growth rates (µm/day) measurement from calcein labeling (5X).

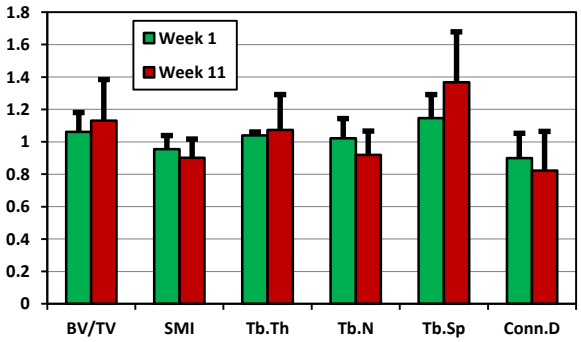

Figure 2: Right/left ratios for bone structural parameters (N=5) (mean + STD).

		Mean value & STD		p-value from t-Test
		Left tibia	*Right tibia*	
Trypan blue test	Cell viability (%)	93.2 ± 2.5	93.1 ± 1.4	0.93
Calcein test	Bone growth rate (µm/day) (week 11)	48.8 ± 9.2	44.2 ± 6.3	0.14

Table 1: Cell viability (trypan blue test) and growth rate (calcein labeling) at week 11.

Discussion

The induced radiation dosage for *in vivo* µCT measurements did not affect cell viability. Also, bone structural parameters showed no significant differences for the scanning time period. Overall, parameters BV/TV, Tb.Th and Tb.Sp increased with time, as a result of normal bone development in the growing rats. In conclusion, results indicate that the adapted protocol with its radiation dosage and scanning frequency can be used for studying the evolution of the rat tibiae during the adolescent period. The radiation damage also depends on other factors (scanning protocol, systemic effects, site-specificity, etc.), which are not µCT system specific. Careful consideration should be adapted for future studies.

References

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