Effect of Tartrate-Resistant Acid Phosphatase gene knockout on bone formation in mice

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Introduction

TRAP is a group of metalloenzymes which are closely associated with osteoclastic action, and is confirmed to participate in bone resorption. Bone resorption and formation are biologically interlinked in a dynamic equilibrium in order to maintain bone mass. Therefore, if there is a change in the rate of bone resorption, bone formation may also be affected. By TRAP gene knockout (-/-), bone resorption was reduced in mice models. We hypothesised that bone formation would be significantly reduced in the TRAP-/- mice compared to controls.

Aims

The aim of this research project is to determine the effect of TRAP-/- on bone formation in mice.

Materials & Methods

Control wild type mice and TRAP knockout mice (n=12, all female) were injected with calcein periodically to label newly formed bone. Transverse tibial bone sections of ~6 microns width were prepared using a Microtome with tungsten carbide tipped steel blades. All sections were processed and mounted onto slides for analysis. Slides with sections from each animal were randomised using a random number generator and labelled 1 to 12 to avoid measurement bias. Sections on the slides were assigned letters (A, B, C etc). The numbers and letters allowed direct identification of particular sections according to slide number and section letter. The effect of TRAP-/- on bone formation was analysed using histomorphometry. Calcein staining was not visible enough for accurate measurement of the labelled regions, and so could not be utilised. Other parameters, such as perimeter to area ratios, were used to determine the effects of TRAP on bone.

Results

TRAP-/- causes a significant decrease in cortical perimeter to area ratio compared to control.

Figure 2. Average perimeter/area ratio of cortical bone between wild type (control) mice and TRAP-/- (experimental) mice bone sections. Bars and error bars correspond to the mean ± SEM (Student’s t test, p<0.05 vs. control, n=6 per genotype).

TRAP-/- causes a significant increase in marrow perimeter to area ratio compared to control.

Figure 3. Average perimeter/area ratio of marrow between wild type (control) mice and TRAP-/- (experimental) mice bone sections. Bars and error bars correspond to the mean ± SEM (Student’s t test, p<0.05 vs. control, n=6 per genotype).

TRAP-/- does not affect total bone cross-sectional area compared to control.

Figure 4. Average total cross-sectional areas between wild type (control) mice and TRAP-/- (experimental) mice bone sections. Bars and error bars correspond to the mean ± SEM (Student’s t test, p<0.05 vs. control, n=6 per genotype).

TRAP-/- causes a significant increase in bone width on the left side only compared to control.

Figure 5. Average left width between wild type (control) mice and TRAP-/- (experimental) mice bone sections. Bars and error bars correspond to the mean ± SEM (Student’s t test, p<0.05 vs. control, n=6 per genotype).

Conclusions

Confirms that bone mass increases due to the decreased rate of bone turnover caused by TRAP-/-.

TRAP-/- does not significantly affect bone formation rate, but seems to have an effect on location of bone formation, causing primarily endosteal bone formation.

Further investigation of TRAP-/- models is necessary to elucidate whether TRAP antagonism truly contributes to bone strength in vivo, without causing significant side effects.

References