Transgenic Mouse Model for Reducing Oxidative Damage in Bone

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Introduction

Bone loss occurs due to age, radiation and microgravity and is characterized by alterations in the balance between bone formation and resorption. The production of Reactive Oxygen Species (ROS) and/or Reactive Nitrogen Species (RNS) plays a signalling role in responses to various intrinsic stimuli (such as hormones and mechanical loading) but when produced in excess after exposure to extrinsic stimuli (such as ionizing radiation) can lead to oxidative damage and tissue dysfunction.

Hypothesis

We hypothesized that excess reactive oxidative species (ROS) alter the tight balance between osteoclast and osteoblast activities, leading to accelerated skeletal remodeling and culminating in bone loss, resembling aging.

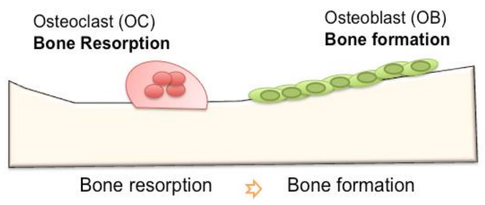
To test this, we first used an intrinsic approach with a transgenic mouse model. 1) We used the mCAT mouse model; these transgenic mice over-express the human catalase gene targeted to mitochondria (Schriner at al.), the major organelle contributing free radicals. Catalase is an anti-oxidant that converts a major reactive species, hydrogen peroxide (H_2O_2), into water and oxygen. This animal model was selected as it displays extended lifespan, reduced cardiovascular disease and reduced central nervous system radiosensitivity, consistent with elevated anti-oxidant activity conferred by the transgene.

Secondly we used an extrinsic approach by applying a treatment we know will incur bone loss and oxidative damage. 2) The treatment consists of hindlimb-unloading and total Body irradiation (TBI) combined. We reasoned that mice overexpressing catalase in mitochondria of osteoblast and osteoclast lineage cells would be protected from the bone loss caused by treatment causing bone loss, in this case simulated spaceflight.

Background

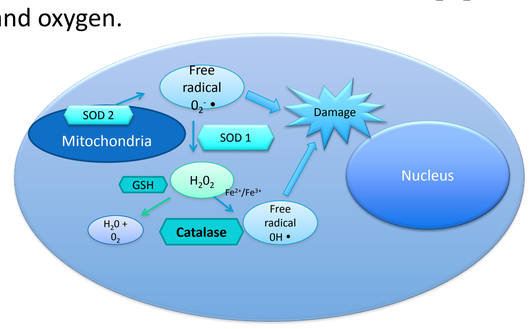
Bone remodeling

A balance between bone forming cells (osteoblasts) and bone resorbing cells (osteoclasts) in skeletal tissue is essential to maintain bone health.



Catalase and Reactive Oxygen Species

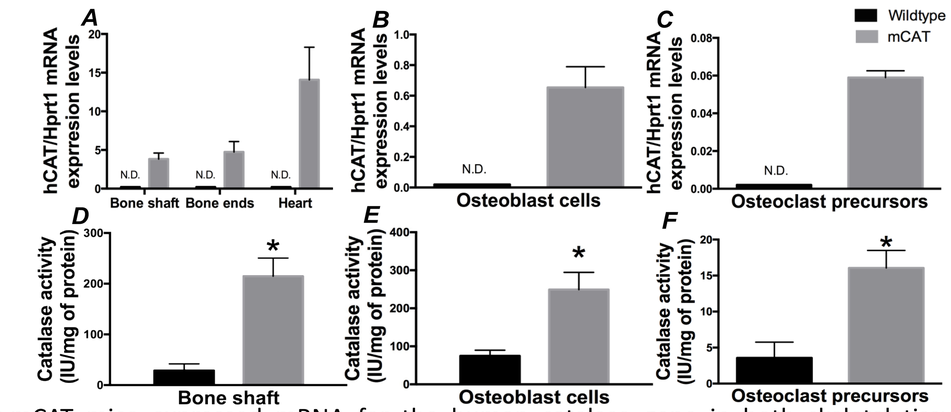
When detrimental reactive oxygen species (ROS) are formed in the cell, catalase converts H_2O_2 to water and oxygen.



Methods

Mice were bred to obtain groups of mCAT hemizygotes and wildtype (WT) littermates. Fourteen week old male mice of each genotype were separated into two groups, controls (untreated) or treated to simulate some aspects of spaceflight (n=7-9/group). Treatment consisted of hindlimb unloading (which simulates weightlessness) followed three days later by exposure to total body irradiation with ^{137}Cs 2Gy, 0.8Gy/min. After two weeks, tissues were harvested. Some bones were flushed to collect bone marrow for *ex vivo* cell culture to assess osteoblastogenesis and osteoclastogenesis. Other tibiae and femora were recovered to analyze gene expression, catalase activity and microarchitecture by 3D micro-computed tomography. Data were analyzed by one way ANOVA to compare all the groups with a Bonferroni correction to allow three comparisons (WT untreated vs mCAT untreated, WT untreated vs treated, and mCAT untreated vs treated) and p<0.017 was used as significance level using post-hoc Student t-test. Data are represented as mean +/- SEM.

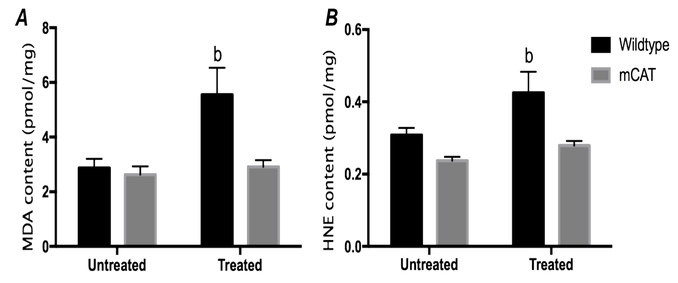
Figure 1: mCAT transgenic mice express the human catalase gene and display increased catalase activity.



• mCAT mice expressed mRNA for the human catalase gene in both skeletal tissue (A) and marrow-derived cultures of osteoblast (B) and osteoclast precursors (C). Hearts served as positive controls.

• mCAT mice displayed 3-4-fold greater catalase enzymatic activity compared to WT mice in bone extracts (D), osteoblastic cultures (E) and osteoclast precursors (F), thereby confirming that transgene was enzymatically active.

Figure 2: mCAT mice display reduced oxidative damage in skeletal tissue after treatment compared to WT

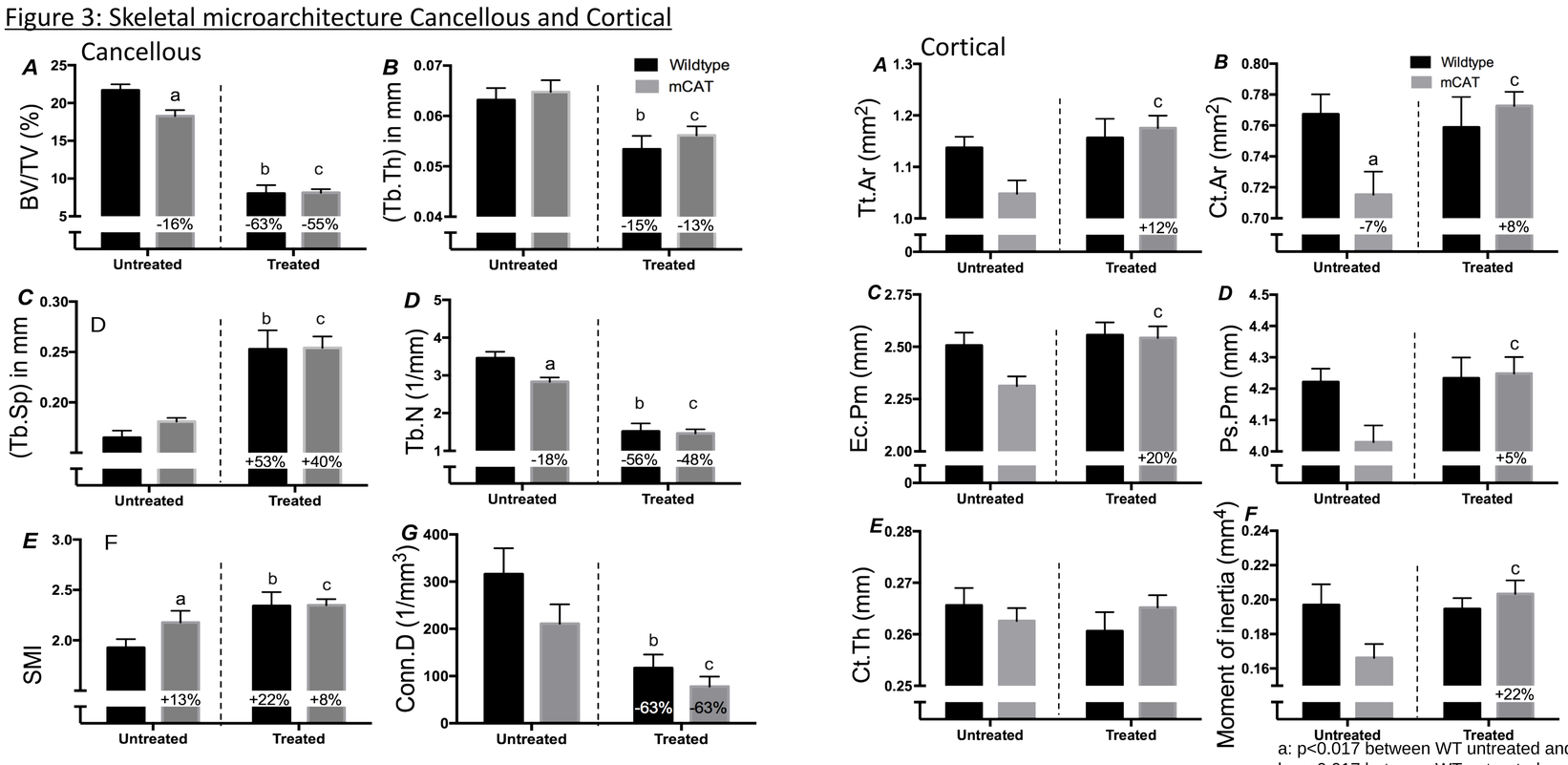


a: p<0.017 between WT untreated and mCAT untreated
b: p<0.017 between WT untreated and treated
c: p<0.017 between mCAT untreated and treated

• Oxidative damage was assessed in mineralized tissue using malondialdehyde (A, MDA) levels and 4-Hyroxynonenal (B, HNE) normalized to total protein content.

• Untreated Control WT and mCAT mice displayed no genotype-dependent differences in oxidative damage whereas treatment increased MDA and HNE content in skeletal tissue of WT but not mCAT mice.

Figure 3: Skeletal microarchitecture Cancellous and Cortical

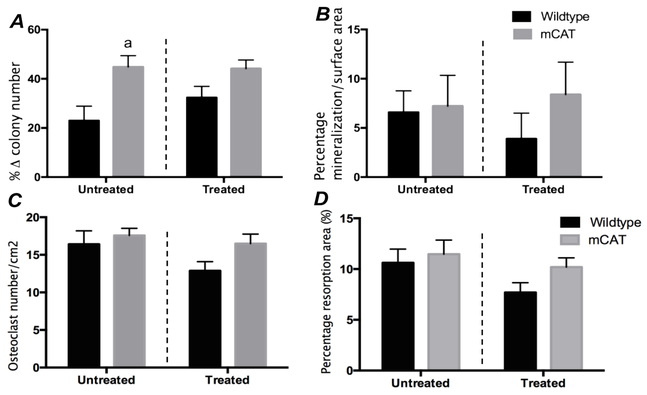


a: p<0.017 between WT untreated and mCAT untreated
b: p<0.017 between WT untreated and treated
c: p<0.017 between mCAT untreated and treated

1. Genotype effects: comparing Untreated mice (WT to mCAT)
- Cancellous: mCAT mice showed differences from WT mice in percent bone volume (BV/TV, A, -16%), trabecular numbers (Tb.N, D, -18%), and structural model index (SMI, E, +13%).
- Cortical bone: mCAT mice differed from WT in cortical area only (Ct.Ar, B, -7 %) but not other parameters.

2. Treatment effects
- Both the WT and mCAT mice showed significant bone loss after treatment in the cancellous bone.
- Interestingly, the mCAT mice, but not the WT mice, displayed radial growth in cortical bone after treatment, as seen in Tissue Area (Tt.Ar, A), Cortical Area (Ct.Ar, B), endocortical perimeter (Ec.Pm, C) and polar moment of inertia (MOI, F).

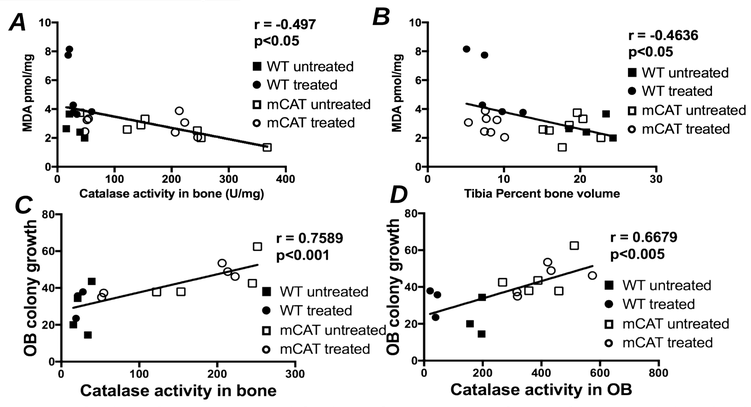
Figure 4: *Ex Vivo* Osteoblastogenesis and Osteoclastogenesis



a: p<0.017 between WT untreated and mCAT untreated
b: p<0.017 between WT untreated and treated
c: p<0.017 between mCAT untreated and treated

- Colony numbers during osteoblastogenesis: the number of colonies from marrow cells of mCAT mice increased more rapidly during growth than those from WT mice (A). Extent of mineralization at terminal differentiation was unaffected by genotype (B).
- No significant differences due to genotype or treatment were observed in *ex vivo* osteoclast formation (C), and activity (D).

Figure 5: Correlation analysis



- Negative correlation between oxidative damage and catalase activity in bone (A), which validates that this transgenic mice model is suitable for studying oxidative damage in bone-related studies.
- Negative correlation between oxidative damage and cancellous BV/TV (B), potentially confirming our hypothesis of the role of oxidative damage and bone loss.
- Positive correlation between catalase activity in bone tissue (B) and catalase activity in *ex vivo* osteoblasts (C) with osteoblasts colony growth, confirming the observation that mCAT mice have increased *ex vivo* osteoblast colony formation (Fig 4).

Summary & Conclusions

• mCAT mice over-expressed the transgene in bone tissue and bone cells *ex vivo* (marrow-derived marrow osteoblasts and osteoclast precursors); and mCAT mice also showed elevated catalase activity compared to WT in these same tissue and cells.
• Treatment (HU and IR) increased lipid peroxidation (MDA, HNE) in bone tissue of WT, but not mCAT mice.

★ ***Therefore, mCAT mice are useful models to analyse the influence of quenching mitochondrial ROS on bone phenotype.***

• mCAT mice (untreated) had lower cancellous percent bone volume, trabecular number, and structural integrity (SMI) compared to WT mice (untreated); and reduced cortical area.

★ ***Endogenous H2O2 generation is likely important for normal bone remodeling and skeletal integrity.***

•Treatment (HU and IR) of both WT and mCAT mice caused loss of cancellous tissue.
•In contrast, treatment of mCAT mice showed an unexpected increase in cortical bone area and other cortical parameters after treatment.

★ ***Overexpression of catalase in mitochondria disrupts typical skeletal responses to the challenges of simulated spaceflight (treatment with HU and IR).***

• Catalase overexpression in mCAT mice increased both *ex vivo* osteoblasts colony expansion, but not osteoclast differentiation, compared to WT mice.
• Statistical analysis revealed strong correlations between catalase expression in both bone tissue and *ex vivo* osteoblast and increased osteoblast colony formation. In addition, we observed a negative correlation between catalase activity, oxidative damage in bone and skeletal structure.

★ ***Oxidative defence pathways in both osteoblast and osteoclast-lineage cells are likely important for protection against skeletal challenges such as those imposed by irradiation and musculoskeletal disuse.***

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