EFFECTS OF MITOCHONDRIAL-TARGETED HUMAN CATALASE IN SKELETAL TISSUE OF MICE EXPOSED TO SIMULATED SPACEFLIGHT

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During prolonged spaceflight, astronauts are exposed to both microgravity and space radiation and are at risk for increased skeletal fragility due to bone loss, Evidence from rodent experiments has established that both microgravity and ionizing radiation can cause bone loss due to increasd of bone-resorbing osteoclasts and decreased in bone-forming osteoblasts, although the underlying molecular mechanisms for these changes are not fully understood. We hypothesized that excess reactive oxidative species (ROS) produced by conditions that simulated spaceflight alters the tight balance between osteoclast and osteoblast activities, leading to accelerated skeletal remodeling and culminating in loss of mineralized tissue. To begin to explore this hypothesis, we used the mCAT mouse model [1]; these transgenic mice over-express the human catalase gene targeted to mitochondria, which are the major organelle responsible for cellular production of free radicals. Catalase is an anti-oxidant that catalyzes the conversion of the reactive species, hydrogen peroxide (H_20_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. We reasoned that mice overexpressing catalase the mitochondria of osteoblast and osteoclast lineage cells would be protected from the bone loss caused by simulated spaceflight.

Mice were bred to obtain groups of mCAT hemizyotes and wildtype (WT) littermates. At 14 weeks of age, mice of each genotype were separated into two groups, controls (untreated) or treated to simulate spaceflight (n=7-10/group). Treatment consisted of hindlimb unloading followed three days later by total body irradiation (2Gy of gamma-radiation). After a 2-week period, tissues were harvested. Bone marrow was processed for ex-*vivo* cell culture (ostoblastogenesis and osteoclastogensis) and the tibiae and femora were recovered to analyze gene expression, catalase activity and microarchitecture by micro-computated tomography. Data were analyzed by two-factor ANOVA, using genotpye (mCAT vs WT) and treatment (simulated spaceflight vs controls) as main effects, with $p<0.05$ accepted as significant.

There were no differences in body mass between mCAT and WT mice. As expected, mCAT mice expressed mRNA for the human catalase gene in skeletal tissue and marrow-derived cultures of osteoblasts and osteoclast precursors. Further, mCAT mice displayed 4-fold greater catalase enzymatic activity compared to WT mice in bone, 3-fold greater in osteoblastic cultures, and 2-fold greater in osteoclast precursors, thereby confirming that transgene is enzymatically active in bone. . Unexpectedly, when compared to control (untreated) WT mice, control mCAT mice displayed lower bone volume/tissue volume fraction (BV/TV) and lower trabecular numbers, and a higher structural model index (SMI), indicative of reduced mechanical properties.. Expansion of colonies in osteoblastogenic cultures from mCAT mice was greater than that of WT mice, although was not affected. No differences due to genotype were observed in *ex vivo* osteoclastogenesis or resorption activity.

Simulated spaceflight had a strong effect on the skeletal tissue microarchitecture in both mCAT and WT mice. Tretment caused a 63% decrement in BV/TV of WT mice and 55% decrement in mCAT mice, along with 56% reduction in trabecular numbers of WT animals whereas the loss was 48% for the mCAT mice. Finally, the WT mice had a 21% increase in SMI, but the mCAT only 11% increase. These results show a tendency for less bone loss and different geometry in the mCAT animals, which can be attributed to the increase in mitochondrial catalase activity. Ex-vivo osteoblastogenesis showed no treatment effect in terms of colony growth, although some differences emerged between WT mice and mCAT mice for mineralization, as the WT mice have 46% decreased mineralization due to the treatment, but the mCAT mice showed a 38% increase of mineralization after treatment. These data would correlate with a capacity of the osteoblast cells from mCAT mice to adapt to the treatment more efficiently than the WT mice.

In conclusion, over-expression of catalase targeted to mitochondria causes cancellous osteopenia and cortical bone structure differences; these unexpected findings suggest mitochondrial H2O2 is important for bone remodeling and skeetelal integrity. Further, overexpression failed to protect bone from the decrements in structure caused by simulated spaceflight. Note that this occurred despite an apparent effect of the transgene to stimulate osteoprogenitor growth. Further studies are needed to dissect the importance of ROS generation selectively in the osteoblast vs osteoclast lineages.

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