The mitochondrial superoxide scavenger MitoTEMPO appears to not prevent ethanol-mediated inhibition of osteoblast differentiation and function but may prevent upregulation of RANKL in bone

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Abstract

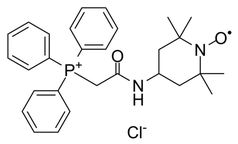
The molecular basis of ethanol (EtOH) toxicity on bone turnover has yet to be fully understood. It is known that chronic consumption increases bone resorption by RANKL mediated stimulation of osteoclast differentiation while at the same time decreasing bone formation and function. Our previous work suggested that EtOH-associated oxidative stress and reactive oxygen species (ROS) generation play a role, as dietary antioxidants such as N-acetyl cysteine blocked EtOH-induced bone loss in female rats and mice. Mitochondria are one source of ROS such as superoxide and hydrogen peroxide. MitoTEMPO is a superoxide dismutase mimic that accumulates in mitochondria and scavenges superoxide radicals. Our aim was to test if the mitochondrial superoxide scavenger MitoTEMPO could ameliorate ethanol's toxicity on bone. MitoTEMPO or saline were provided by osmotic minipumps implanted subcutaneously six days ahead of an EtOH challenge of male C57BL/6 mice consisting of gavage for four consecutive days with 3, 3, 4 and 4.5 g/kg EtOH. Osteoblast markers and osteoclast differentiation marker RANKL mRNA in the femoral shaft were determined by qRT-PCR. The amino-terminal propeptide of type 1 procollagen (P1NP) a marker of bone formation was also determined in serum by ELISA. A marker of oxidative stress, 8-hydroxy 2-deoxyguanosine, was determined in bone marrow DNA. MitoTEMPO failed to rescue most effects of ethanol, except for potential repression of RANKL mRNA induction in the femoral shaft. Further studies are needed to evaluate the effect of MitoTEMPO and mitochondrial superoxide in skeletal ethanol toxicity.

Introduction

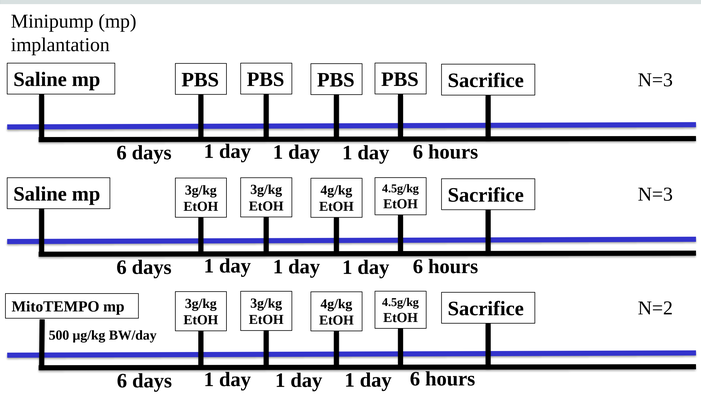
- EtOH consumption increases risk of osteopenia and osteoporosis.
- Binge EtOH in mice represses expression of genes for osteoblast differentiation and function and induces expression of gene such as RANKL involved in osteoclast differentiation
- The dietary antioxidant N-acetylcysteine partially blocks EtOH-induced bone loss in female mice.
- Mitochondria are a major source of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide.

Hypothesis

MitoTEMPO, a superoxide dismutase mimetic accumulating in mitochondria can prevent bone toxicity mediated by binge ethanol as a result of suppressed ROS formation.

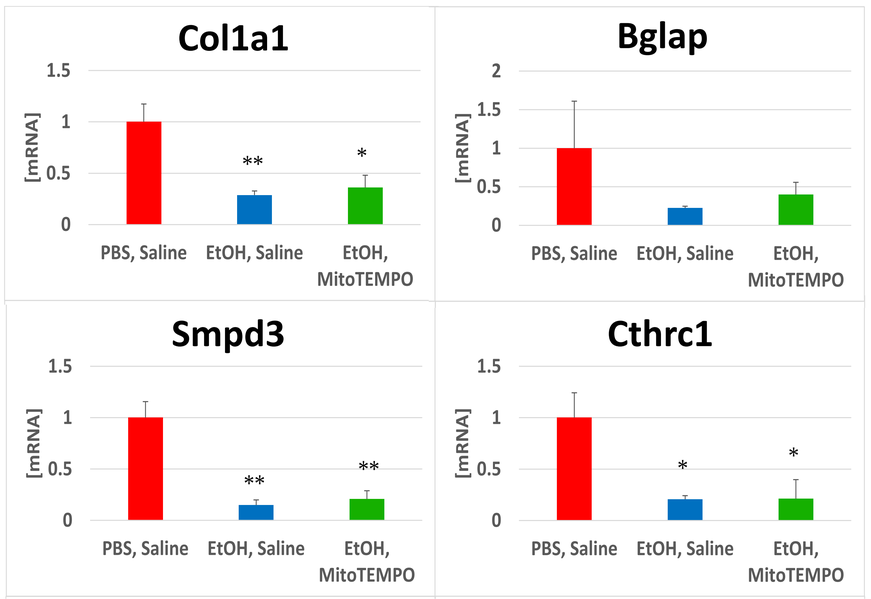


Mouse model

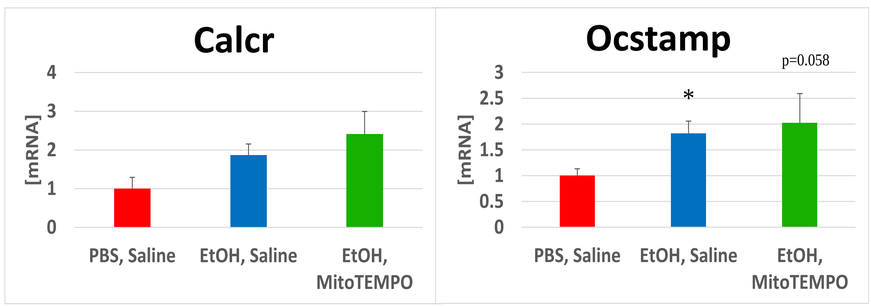


Femoral shaft gene expression

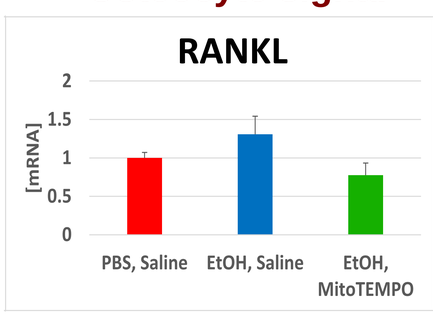
Osteoblast markers



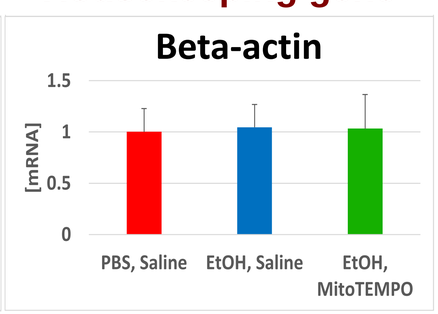
Osteoclast differentiation markers



Osteocyte signal

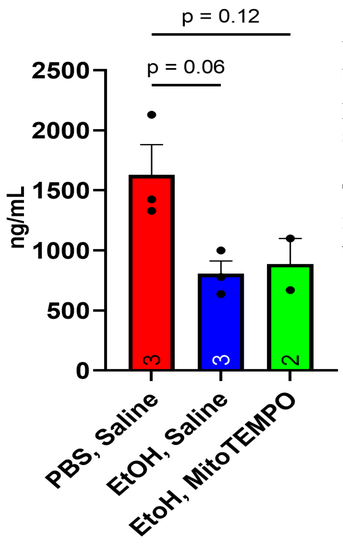


Housekeeping gene



RNA was isolated from the femoral shaft and subjected to qRT-PCR. We determined expression of mRNA for osteoblast markers collagen 1a1 (Col1a1), osteocalcin (Bglap), collagen triple helix repeat containing 1 (Cthrc) and sphingomyelin phosphodiesterase 3 (Smpd3); RANKL that is an osteocyte-secreted factor promoting osteoclastogenesis and osteoclast differentiation markers calcitonin receptor (Calcr) and Ocstamp. mRNA concentration is set to 1 for the average of the PBS, Saline group *, **: P < 0.05, 0.01 vs. PBS, Saline. As previously reported (Denys et al. Toxicol Sci. 2022. 185:232-245, EtOH downregulates osteoblast markers and upregulates RANKL and osteoclast differentiation markers Calcr and Ocstamp. MitoTEMPO did not prevent ethanol effects on osteoblast and osteoclast differentiation markers. MitoTEMPO and EtOH-treated mice did not show upregulation of RANKL, although difference with saline and ethanol-treated mice was not significant as a result of small numbers.

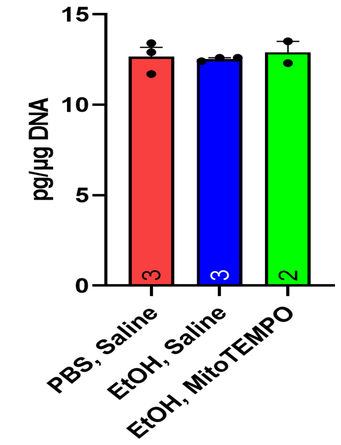
Serum procollagen 1a1



Whole-body bone formation was estimated by measurement of the amino-terminal propeptide of type 1 procollagen (P1NP) in serum by ELISA.

There was a trend (P < 0.1) of ethanol-mediated reduction in bone formation that was not prevented by MitoTEMPO.

8-hydroxy 2-deoxyguanosine



Oxidative stress was assessed by 8-hydroxy 2-deoxyguanosine (8-oxo-dG). DNA was isolated from bone marrow 8-oxo-dG determined by ELISA.

There were no significant differences between the three groups.

Conclusion and Discussion

- *In vivo* MitoTEMPO treatment in the current experiment did not prevent binge EtOH effects on serum procollagen 1a1, osteoblast or osteoclast markers in the femoral shaft.
- Ethanol and MitoTEMPO did not affect content of ROS-mediated 8-oxo-dG in DNA from bone marrow. It is possible that EtOH does not produce oxidative DNA damage in bone marrow. It has previously been shown that EtOH DNA damage in liver is CYP2E1 dependent and there is very little CYP2E1 expression in bone.
- MitoTEMPO appears to block EtOH induction of RANKL mRNA in the femoral shaft.
- A major limitation is the low number of animals for this study. Conclusions need to be validated with a larger mouse sample size. Determination of other markers of ROS is also required to validate the effectiveness of this dose of MitoTEMPO as a ROS suppressor in bone and appropriately address the hypothesis.

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