Extracellular Superoxide Dismutase (EC-SOD) R213G variant reduced mitochondrial ROS and preserved mitochondrial function in bleomycin-induced lung injury

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INTRODUCTION

Extracellular superoxide dismutase (EC-SOD), which catalyzes the dismutation of $\text{O}_2^-$ to $\text{H}_2\text{O}_2$ and $\text{O}_2$, is highly expressed in the lung and vasculature.

Human R213G polymorphism in EC-SOD alters tissue binding affinity, which results in the redistribution of R213G EC-SOD from tissue to extracellular fluids without changing enzyme activity.

Mice expressing knock in of R213G EC-SOD (R213G mice) are protected against bleomycin-induced lung inflammation and fibrosis. It is unknown how the redistribution of EC-SOD alters redox-regulated signaling relevant for protection. It is also unknown if the redistribution of EC-SOD impacts mitochondrial oxidative stress.

HYPOTHESIS

R213G SNP will influence the extracellular and intracellular redox environment, particularly thiol redox state and mitochondrial function.

METHODS

- Wild type (WT) and R213G mice were treated with a single intratracheal dose of bleomycin (0.1 U/mouse/bleo)
- Blood and lung were harvested and processed at 7 days post treatment
- ROS production was measured in lung, plasma, and BALF by Electron Paramagnetic Resonance (EPR) using CPH and CMH and mitochondrial ROS in lung was measured using mito-TEMPO-H
- GSH, GSSG concentrations were measured by high-performance liquid chromatography (HPLC); redox potential (Eh) was calculated using the Nernst equation
- Pathway analysis was performed by Ingenuity Pathway Analysis (IPA) software on our previously published RNAseq data
- Cardiolipin oxidation was evaluated by Electrospray Ionization LC Mass Spectrometry
- Respiration was evaluated by Oroboros O2x assay
- Data were analyzed by two-way ANOVA with Tukey's post hoc test analysis using Prism Graphpad

CONCLUSIONS

- Elevated EC-SOD in plasma and BALF in R213G mice lowers superoxide levels. In contrast, despite low EC-SOD content in R213G mice lung, ROS production was not elevated. We speculate that the protection against elevated lung ROS was due to reduced alveolar ROS production and decreased alveolar inflammation as previously described.
- The alterations in the extracellular redox environment in the R213G mice resulted in changes in critical intracellular redox-sensitive processes in the lung, including intracellular thiol redox status, and mitochondria ROS production.
- Bleomycin treatment suppressed mitochondrial respiration and increased mitochondrial cardiolipin oxidation in WT mice, but not R213G mice. Interestingly, mitochondrial function was mildly impaired at baseline in R213G mice despite these mice being resistant to the effects of bleomycin. This is consistent with our previous observation that R213G mice exhibit pulmonary hypertension (PH) at baseline through PH did not worsen post bleomycin. Further studies will determine if the altered mitochondrial function at baseline in the R213G mice is responsible for baseline PH.
- Despite increased mitochondrial oxidative stress measured by mitochondrial ROS and cardiolipin oxidation in WT mice, the total cardiolipin decreased and frequency of oxidized mtDNA lesions increased similarly in the two strains after bleomycin. The relationship between total CL, oxidized CL and oxidized mtDNA is complex and still incompletely understood.
- These data provide a novel link between the extracellular redox environment regulated by EC-SOD and mitochondrial dysfunction observed in bleomycin-induced lung injury and inflammation.

**Fig 1.** ROS in lung increased at 7 days post bleomycin similarly in both WT and R213G mice but lower ROS was observed in R213G mice in plasma and BALF cells

**Fig 2.** Lung GSH was preserved in the R213G mice and lower mitochondrial ROS observed 7 days post bleomycin compared to WT mice

**Fig 3.** Oxidation of mitochondrial phospholipid, cardiolipin is blocked in R213G

**Fig 4.** Mitochondrial respiration was suppressed significantly post bleomycin in WT mice but not R213G mice compared to each strain at baseline

**Fig 5.** Bleomycin reduced mtDNA damage similarly in both strains

**REFERENCES AND ACKNOWLEDGMENTS**


**EC-SOD variant (R213G) protects the intracellular redox status of the lung by blocking E/GSSG oxidation, mitochondrial ROS and cardiolipin oxidation**