Reducing Oxidative Stress in Retinitis Pigmentosa Mouse Model

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Background

As a byproduct of oxygen consumption, the body creates highly reactive free radicals called reactive oxygen species (ROS). These reactive molecules include superoxides (O_2^-), peroxides(H_2O_2), hydroxyl radicals (•OH), and singlet oxygens.

- When our body produces ROSs, there is a system of antioxidant enzymes and vitamins which can maintain a homeostatic level of ROSs that do not damage and interfere with normal processes.
- Oxidative stress, an increase in ROSs beyond homeostatic levels, can be caused by systemic disease or environmental factors (diet, smoking, etc). The increase in ROSs overwhelm the normal levels of enzymes in our body and can no longer mitigate their damaging effects.
- The retina is more prone to production of ROSs and oxidative stress because it is a highly metabolic tissue, contains light activated molecules, and photoreceptor membranes contain a large amount polyunsaturated fatty acids (PUFA), which are highly susceptible to attack by ROSs.
- The higher intake of oxygen creates an environment that is rich in oxygen to be used for cellular respiration, but, as a consequence, there is an increase in production of ROSs which is a byproduct of oxygen metabolism.
- The hydrogens associated with the allylic positions of PUFAs are attacked by ROSs. Resonance stabilization of the affiliated double bond and other surround functional groups, cause an electron withdrawing effect making the PUFA susceptible to radicalization.

The extracellular matrix (ECM) contains key structural proteins such as collagen to hold the cells in place; It allows for cellular communication, provides crucial extracellular environmental cues, and serves as a track for nutrient delivery.

- Fragmentation of extracellular proteins by ROSs can be debilitating to cellular morphology and function.
- Oxidative stress in the ECM can damage membranes, and completely inhibit ECM activity and cell-cell interconnectivity.
- SOD3 is an extracellular enzyme responsible for the breakdown of superoxides into hydrogen peroxide and water. Overexpression of the protein has shown to improve healing after wounding or ischemic injury.

Our objective here is to overexpress the SOD3 protein in mouse retina to understand if it can improve photoreceptor degeneration cause by a genetic mutation in the opsin protein, known as P23H. We chose to work with the heterozygous model of P23H because it best resembles the patient phenotype.

Methods

Electroretinography

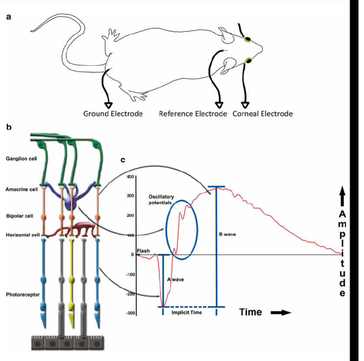
Electrodes were placed on dark-adapted, anesthetized mice, then the mice were flashed with light to record the scotopic data. Mice were light-adapted and then flashed to record the photopic data.

Histology

Mice eyes were fixed in Davidson, dehydrated, and embedded in paraffin for sectioning. Sections were stained with H&E and examined for their photoreceptor health.

Western Blotting

Retinal proteins were extracted from mice eyes. Membrane was probed with anti-Bcl-x monoclonal primary antibody and anti-RDS monoclonal primary antibody.



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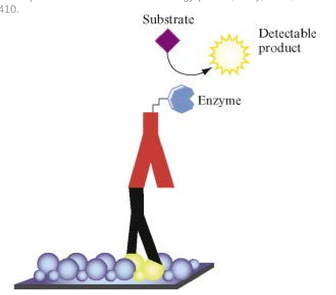

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Results

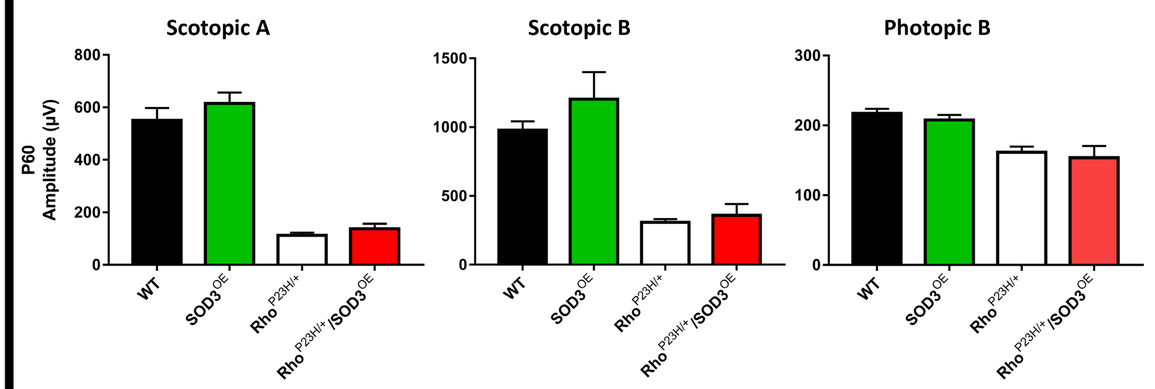


Figure 1. Electroretinography is a method to measure the photoreceptors' retinal response. We performed scotopic and photopic electroretinography (ERG) on P60 in animals with the genotypes $SOD3^{OE}$, $Rho^{P23H/+}$, and the compound $SOD3^{OE}$/ $Rho^{P23H/+}$. Rod photoreceptor function was analyzed using Scotopic A and B ERG at P60, while cone photoreceptor function was analyzed using Photopic ERG. All three waveforms shows lower ERG in the Rho^{P23H} and $Rho^{P23H/+}/SOD3^{OE}$ compared to the WT. However, there are negligible difference between Rho^{P23H} and $Rho^{P23H/+}/SOD3^{OE}$. N=3-4 mice per genotype.

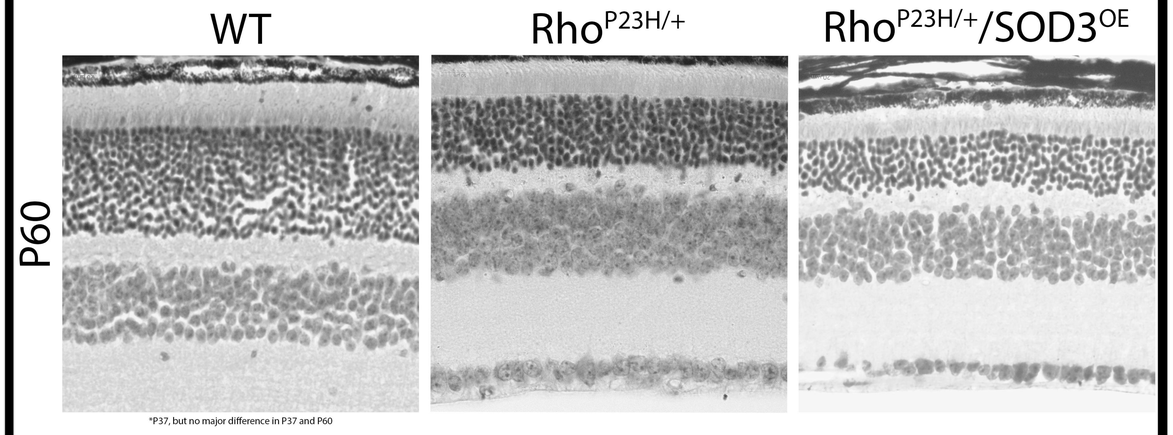


Figure 2. Hematoxylin and Eosin staining were done on P60 retinas to see the structures of the retina. In Rho^{P23H} and $Rho^{P23H/+}/SOD3^{OE}$, the outer nuclear layer width is reduced compared to the WT.

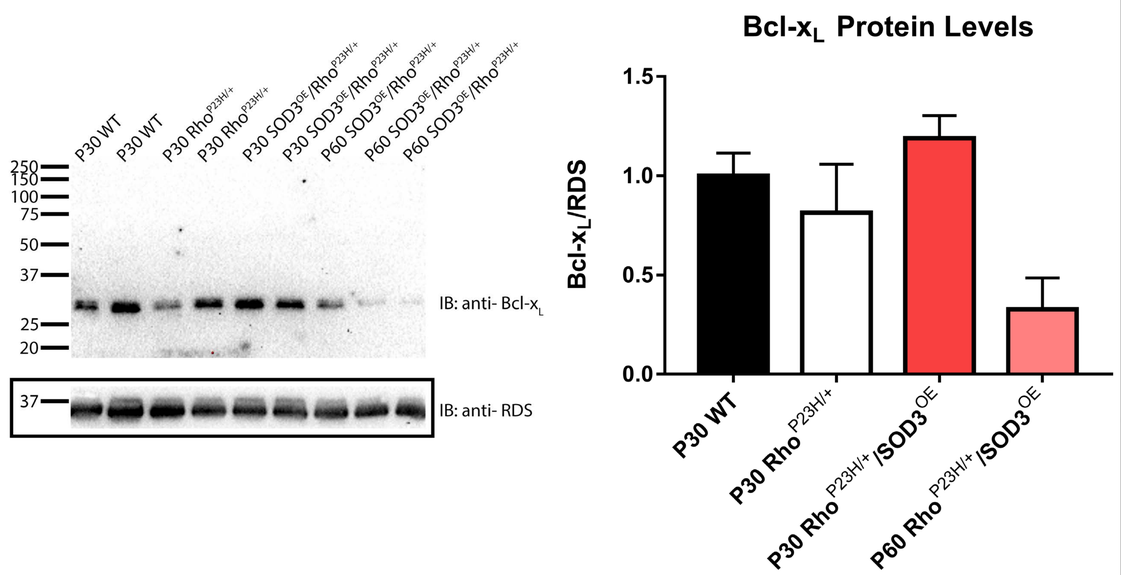


Figure 3. Bcl-x_L is an anti-apoptotic protein and we are investigating the expression levels of Bcl-x_L in the compound mice versus the control to see how the mutation affects the mechanism of cell death. The total retinal extract were collected from P30 and P60 mice and ran on SDS-PAGE and transferred to the membrane. The blot was probed with anti- Bcl-x_L antibody, and their band densities were normalized against the RDS protein. RDS was used as a loading control and to determine relative quantitation of the protein. There is a decrease in Bcl-x_L expression from P30 to P60 in $Rho^{P23H/+}/SOD3^{OE}$. N=2-3 retina per genotype.

Discussion

Electroretinography

The photoreceptor response signals were recorded through ERG. The Scotopic A wave reflects the function of the photoreceptors responsible for signal transduction and the Scotopic B wave reflects the function of the inner layer cells responsible for signal transmission.

- There is reduced scotopic ERG in $SOD3^{OE}$/ $Rho^{P23H/+}$ at P60.
- The lower rod function, indicated by the ERG, could be caused by photoreceptor degeneration.

Histology

H&E staining can show structural differences with each mutation. Retinas which more photoreceptors are able to transduce light signal better.

- The number of photoreceptor cells in the outernuclear layer is reduced significantly in retinas of Rho^{P23H} and $Rho^{P23H/+}/SOD3^{OE}$
- In $Rho^{P23H/+}/SOD3^{OE}$, where we were trying to rescue some of the degenerative photoreceptors, we see an even greater decline in outer nuclear layer cell count than P23H alone.

Western Blot

Bcl-x_L is a protein that binds and inactivates proapoptotic factors such as Caspase-3 and Cytochrome C. In cells that are fighting to survive, it would upregulate Bcl-x_L to help prevent apoptosis.

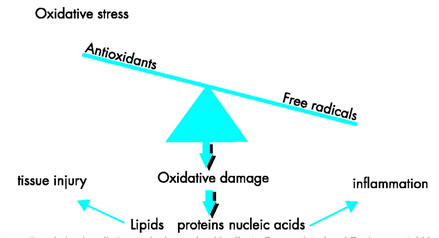
- There is a slight decrease in Bcl-x_L in the $Rho^{P23H/+}$ mouse model but higher in the compound at P30.
- The expression of Bcl-x_L decreases in the compound at P60 compared to P30.

Conclusion

Overexpression of SOD3 in the retina in conjunction with the P23H rhodopsin mutation was able to slightly delay the degenerative effects of Retinitis Pigmentosa at post-natal day 30. However, this is not a viable long-term strategy as seen P60 ERG and histology.

- As seen in $SOD3^{OE}$ alone, the upregulation of SOD3 was able to reduce the apoptosis of cells in the early stages of growth.
- It was only effective in reducing apoptosis in P23H models at early time points. The mutation overwhelms the effectivity of SOD3 overexpression at a later time point.

Although the overexpression of the anti-oxidant has not entirely counteracted $Rho^{P23H/+}$, this study advances the understanding of the importance of antioxidant enzymes in the extracellular space and how this strategy could be implemented to counteract negative effects of retinal diseases.



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