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Sulindac Confers Protection Against Oxidative Stress Induced Damage In Retinal Pigment Epithelial Cells

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Introduction

Purpose –The retinal pigment epithelial (RPE) layer is one of the major areas affected by oxidative stress in ocular diseases. In our study we tested the non-steroidal anti-inflammatory compound (NSAID) sulindac for protection against oxidative stress induced damage in RPE cells. Besides its known anti-inflammatory activity, recent studies have shown that sulindac can protect cardiac cells against oxidative damage by a preconditioning mechanism.

Methods - The ability of sulindac to protect RPE cells against oxidative stress was determined by treating cultured RPE cells with sulindac, before exposing them to oxidative stress. Following 48h exposure of RPE cells to sulindac, cells were exposed to either a range of *tert*-Butyl Hydrogen peroxide (t-BHP) concentrations or Hydrogen peroxide (H2O2) to induce oxidative stress. For inducing Hypoxia, RPE cells were exposed to less than 0.5% oxygen environment in a hypoxia chamber. After the treatments cellular viability was determined using the MTT assay.

Results- The results show that exposure of cultured RPE cells to oxidative stress using t-BHP or H2O2 or Hypoxia causes significant decrease in cell viability. Pretreatment of RPE cells with sulindac for 48hrs, protects them against these insults and enhances survival.

Conclusion- In future experiments we plan on showing that the mechanism of protection, and also test whether, sulindac is functioning as a preconditioning agent. To understand this protective mechanism we will evaluate changes in preconditioning markers, role of ROS scavengers and mitochondrial function. In our future studies we plan on investigating the efficacy of combining sulindac in a dual therapy along with the reducing agent methionine sulfoxide reductase A (MsrA). We will also furhter extend our investigation to animal model of photooxidation where mice will be treated with UV light and the ability of sulindac to prevent UV induced damage will be evaluated. In conclusion we believe that sulindac may represent a novel therapeutic agent for oxidative stress induced ocular diseases.

Methods

For protecting RPE cells from oxidative stress induced damage we used the NSAID, sulindac. For our experiment we used ARPE-19 cells cultured in 96 well plates. The cells were preincubated with sulindac for 48 hours prior to exposing them to oxidative stress. Our oxidizing agents of choice were TBHP and Hydrogen peroxide (H_2O_2). We also stressed the ARPE-19 cells with 24 hours of Hypoxia treatment. After the stress the cellular viability was evaluated using the MTS assay. This assay uses a tetrazolium compound that is bioreduced into a formazan product in the cells. The signal generated (color intensity) is directly proportional to the number of viable (metabolically active) cells in the wells.

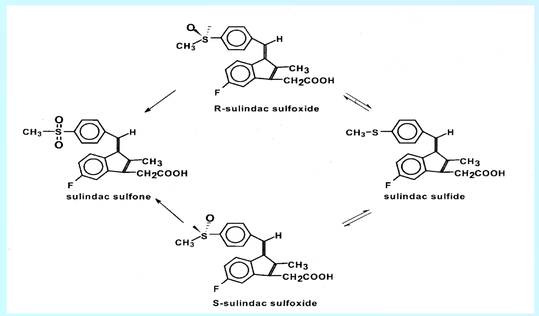

Figure 1: Structure of sulindac and its analogues. Sulindac can be reduced to an active NSAID sulfide form or irreversibly oxidized to a sulfone analogue. The sulfoxide form of sulindac retains the functions of NSAID through COX inhibitory properties

Results

When ARPE-19 cells grown in 96 well plates were exposed to TBHP without prior incubation to sulindac they exhibited loss of cell viability.

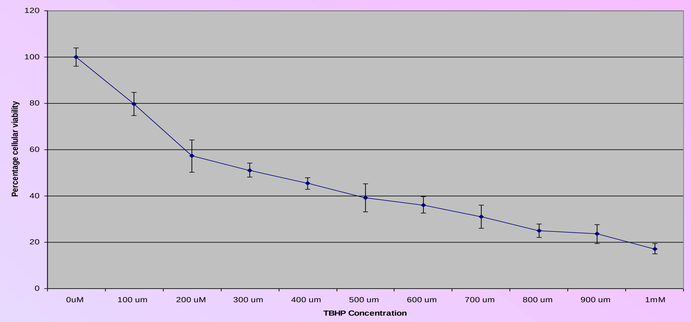

Figure 2 – "Kill curve ". Loss of cell viability in retinal pigment epithelial cells exposed to oxidative stress by TBHP treatment

ARPE-19 cells were preincubated with different concentrations of sulindac ranging from 50uM to 400µM. Our results showed that preincubation with sulindac protected the ARPE-19 cells against loss of cell viability due to oxidative stress. However, sulindac concentrations of 200µM or higher was found to be toxic to ARPE-19 cells as shown by acute reduction of cell viability even without any TBHP (Figure-3)

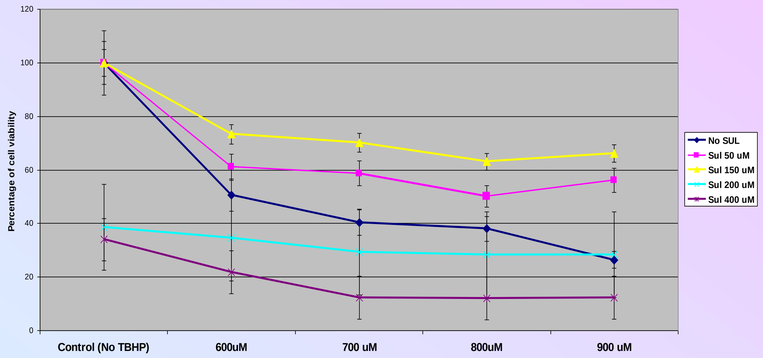

Figure 3 Treating ARPE 19 cells with Sulindac prior to TBHP exposure prevents cell death caused by oxidative stress.

Our next experiment was designed to determine if preincubation with sulindac is also effective in protecting ARPE-19 cells against oxidative stress induced by treatment of retina cells with H2O2. We incubated cells with Sulindac for 24hrs and then exposed them to 50µM H2O2. The experiment showed that sulindac is capable of protecting retina cells against H2O2 induced oxidative stress. We also tested the protective effect of sulindac sulfone under similar conditions and it offered only partial protection (Fig 4).

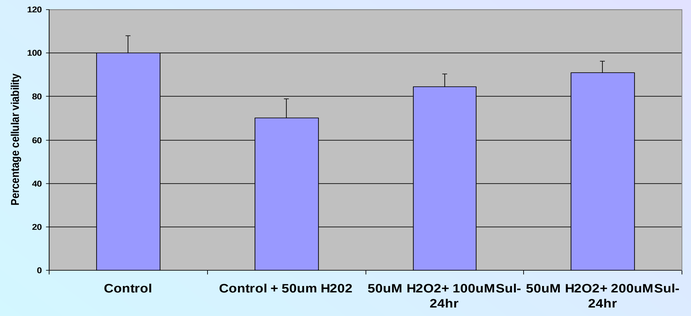

Figure 4 - Loss of cell viability by H_2O_2 induced oxidative stress can be reduced by preincubating retina cells with sulindac

Results

To test the ability of sulindac in protecting against a variety of stresses we exposed ARPE-19 cells to 24 hours of hypoxic stress following sulindac preincubation. We kept ARPE-19 cells grown in 96 well plates inside a hypoxia chamber having only 5% oxygen for 24 hours to induce hypoxic stress. Prior to exposing the cells to hypoxia one set of cells were incubated 24 hours with sulindac. When compared with control cells the results show that sulindac pretreatment confers significant protection against hypoxia induced stress.

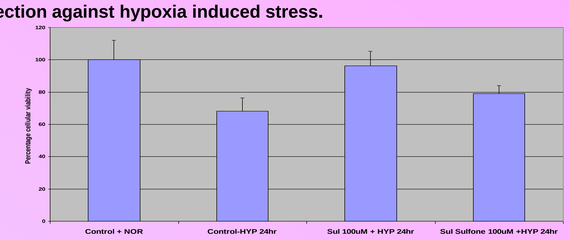

Figure 5 shows comparative cellular viability of ARPE-19 cells treated with sulindac, sulindac sulfone and untreated (control) cells. The cells preincubated with sulindac showed higher viability when exposed to 24hr hypoxic stress. The difference in survival between control and sulindac treated cells was statistically significant (P<0.05)

Clinical significance

Oxidative stress has been implicated as the underlying cause in the damage to various ocular diseases such as cataract, age related macular degeneration (AMD), glaucoma and diabetic retinopathy. AMD is the leading cause of legal blindness in 50 years of age or older. It damages the macula leading to loss of central vision.

Normal vision

Vision affected by AMD

Figure 6: The two images shown here highlight the difference in vision caused by damage to the macula in the eyes of patients suffering from AMD.

Summary

- **Sulindac increases cell viability in RPE cells exposed to the oxidizing agents TBHP and H_2O_2.**
- **Sulindac protects RPE cells against hypoxia-induced cell death.**
- **Sulindac sulfone, an analogue of Sulindac, shows partial protection in hypoxic conditions.**
- **Protection provided to RPE cells by Sulindac possibly involves a preconditioning mechanism that influences the mitochondria.**

References

Moench, I., Prentice, H., Rickaway, Z., Weissbach, H. Sulindac confers high level ischemic protection to the heart through late preconditioning mechanisms. *Proc. Natl. Acad. Sci. USA.* (2009) 106 (46), 19611-19616.

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