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Thioredoxin system and HIF1a pathway activation by oxidative stress in human iPSC-RPE cells

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Purpose

The accumulation of reactive oxygen species (ROS) is a critical factor contributing to the pathogenesis of both dry and wet forms of age-related macular degeneration (AMD). The objective of this study was to investigate the concentration-dependent activation and response of the thioredoxin system to ROS induced by sodium iodate ($NaIO_3$).

Methods

Human iPSC-derived retinal pigment epithelium (RPE) cells were cultured at 100,000 cells/cm². Oxidative stress was induced with $NaIO_3$ at 5% CO_2, 37°C for 24 h. VEGF levels in media samples were measured using ELISA. ROS production was assessed using CM-H_2DCFDA (5 µM). Cell viability was measured using resazurin reduction (0.01 mg/ml) and lactate dehydrogenase (LDH) release.

Cells were washed with DPBS and lysed in TRIzol. RNA was purified (PureLink RNA Mini Kit), converted to cDNA (High-Capacity RNA-to-cDNA Kit) and analyzed via qRT-PCR (QuantStudio 3). Targets for qPCR included VEGFA, HIF1A, TXN and TXNIP. Standard curve analysis and mRNA quantification were performed using Design & Analysis Software.

Results

ROS levels significantly increased with $NaIO_3$, peaking at 1 mM and decreased at 2–4 mM, with a significant reduction at 4 mM compared to 1 mM (One-way ANOVA, post-hoc Tukey's test, $P < 0.001$). VEGF release showed a significant increase at 1 mM ($P = 0.02$) and 4 mM ($P < 0.001$) compared to 0 mM (One-way ANOVA, post-hoc Dunnett's test). LDH release was significantly elevated at 3 mM and 4 mM compared to 0 mM ($P < 0.001$). Resazurin cell viability had no statistically significanct changes ($P < 0.11$).

Gene expression analysis revealed:

- **VEGFA**: Significant **upregulation** at 2, 3, and 4 mM ($P < 0.001$)
- **HIF1A**: Significant **upregulation** at 2 mM ($P = 0.003$), 3 mM, and 4 mM ($P < 0.001$)
- **TXN**: Significant **upregulation** at 2, 3, and 4 mM ($P < 0.001$)
- **TXNIP**: Significant **downregulation** at 2 mM ($P = 0.03$), 3 mM ($P = 0.001$), and 4 mM ($P < 0.001$) (One-way ANOVA, post-hoc Dunnett's test)

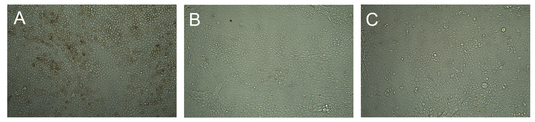

Figure 1. Morphology human iPSC-derived RPE cells effected by $NaIO_3$ at (**A**) 0 mM, (**B**) 2 mM (**C**) 4 mM concentration.

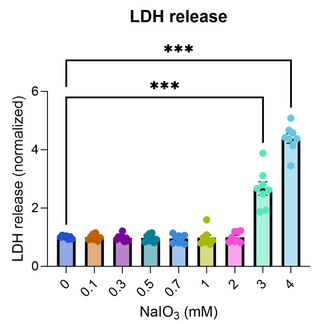

Figure 2. LDH release of human iPSC-derived RPE cells effected by $NaIO_3$.

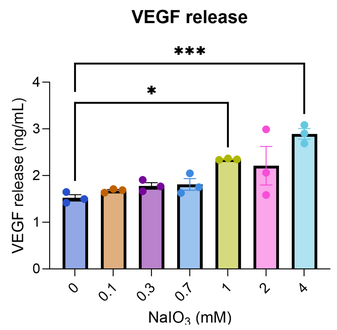

Figure 3. LDH release of human iPSC-derived RPE cells effected by $NaIO_3$.

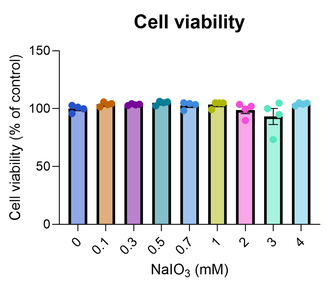

Figure 4. Cell viability of human iPSC-derived RPE cells effected by $NaIO_3$.

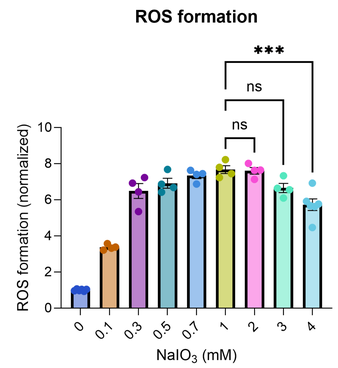

Figure 5. Reactive oxygen species formation in human iPSC-derived RPE cells effected by $NaIO_3$.

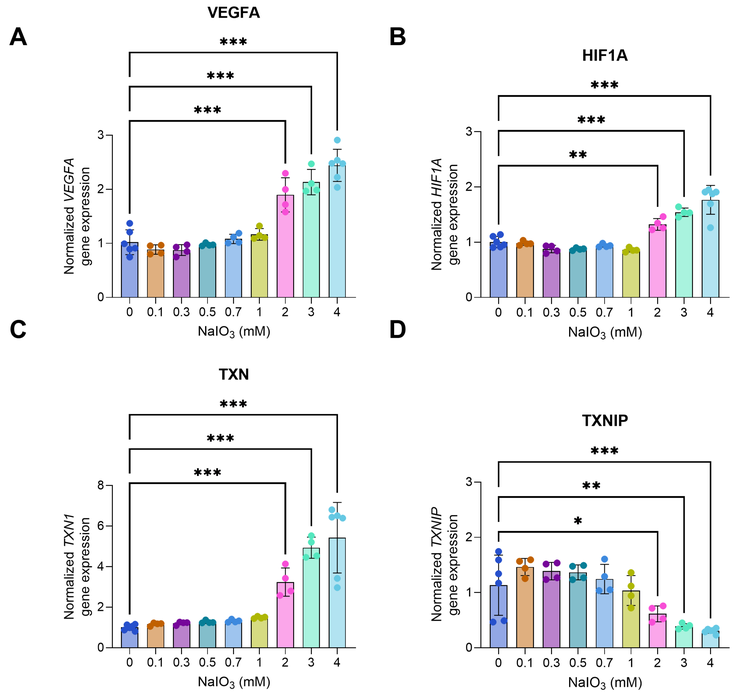

Figure 6. Gene expression of (**A**) VEGFA, (**B**) HIF1A (**C**) TXN and (**D**) TXNIP in human iPSC-derived RPE cells effected by $NaIO_3$.

Conclusion

$NaIO_3$ induction altered HIF1A, TXN, and TXNIP expression, highlighting pathways linked to cellular stress and angiogenesis.

These biomarkers provide actionable targets for anti-angiogenic or gene-editing therapies.

Disclosures
RC, OV, AK, DL: none
GK: Experimentica Ltd. (I,S)
JJH: Experimentica Ltd. (I,S)

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