Cytoprotective Properties of Thioketals Against Oxidative Stress Damage to Islets

Shammy Raj[1], Kosala D Waduthanthri[1], Gregory Korbutt[2], Andrew R Pepper[2], Larry D Unsworth[1]

[1]Dept. of Chemical and Materials Engineering, [2]Dept. of Surgery, University of Alberta

Introduction: Islet transplantation allows for glucose control in patients with Type 1 diabetes. During the transplantation process however, exogenous islets suffer oxidative stress caused by the presence of reactive oxygen species (ROS), which results in cell death. Furthermore, islets contain low levels of antioxidant enzymes which exacerbates the detrimental effect of ROS. Herein we have studied the use of an ROS sensitive thioketal (TK) molecule in protecting porcine islets against the detrimental effects of ROS. TK exhibited no cell toxicity at relatively high concentrations, and the cells retained normal function. In addition, TK neutralized exogenous oxidative stress and ultimately protected islets from ROS-induced insult.

Synthesis and Characterization

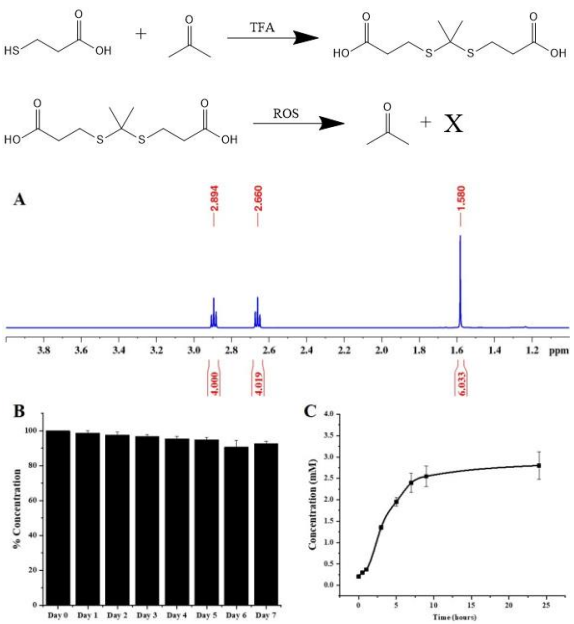

Figure: A) NMR spectrum of TK in $CDCl_3$. B) Stability of TK in PBS C) Time dependent evolution of acetone upon TK degradation in presence of ROS.

Toxicity Studies

Cells were incubated with 100 and 200 µM of TK for 48 h at 37 ^{O}C. Membrane integrity confirmed non-toxicity.

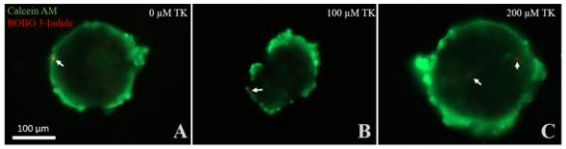

Figure: Live (green) and dead (red) fluorescence micrographs of islets incubated with A) PBS, B) 100 µM TK in PBS, and C) 200 µM TK in PBS.

Viable cells were quantified for islets incubated with 200 µM TK using fluorescent images. 79.8 ± 4.2% of the cells were viable in 200 µM TK, compared to 87.7 ± 3.2% in the control.

Functionality and Cytoprotective Studies

Porcine islets incubated with 200 µM of TK for 48 h at 37 ^{O}C were exposed to 800 µM H_2O_2 for 4 h.

1) Cell functionality was measured using the Oxygen Consumption Rate (OCR) normalized to total DNA.
2) Lipid peroxidation assay was used to analyze the oxidative damage caused to β-TC6 cells due to H_2O_2. Malondialdehyde (MDA) was quantified and normalized against total DNA.

Table: Protective effect of TK against oxidative stress represented by OCR and MDA analysis.

	OCR (nmol/min-mg DNA)	MDA (nmol/µg DNA)
C: No TK, no H_2O_2	86.3 ± 14.2	17 ± 1.1
G1: 0 µM TK + 800 µM H_2O_2	21.3 ± 13.7	27 ± 2.8
G2: 200 µM TK + 800 µM H_2O_2	85.5 ± 7.5	21 ± 1.2

Discussion and Conclusion: ROS degradation shows that TK is highly efficient in neutralising ROS. TK is stable as compared to other ROS scavenging molecules like bilirubin, and can be easily synthesized and scaled up for commercial purposes.

This results were translated to *in vitro* cell studies where it was shown that TK is not only non-toxic but also exerts cytoprotective properties against exogenous ROS as demonstrated by OCR and MDA analysis.

In conclusion, TK is an excellent molecule in scavenging ROS during islets cell culture. Its efficiency in animal models, and in other stages of islet transplantation are under study.

References

1) Shukla, A.K. *et al*, 2004. *Indian Journal of Chemistry, 43B.*

2) Miki, A. *et al*, 2018. *PloS one*, *13*(5).