Trityl Radical OX071, an EPR Oxygen Imaging Spin Probe, Is Non-Toxic to Cells

Zhengshan Zhao[1], Darwin Bodero[1], Boris Epel[1,2], and Mrignayani Kotecha[1*]
[1]Oxyegn Measurement Core, O2M Technologies, 2201 W Campbell Park Dr, STE 310, Chicago, IL 60612
[2]Department of Radiation and Cellular Oncology, University of Chicago, 5841 S Ellis Ave, Chicago IL 60637
*Contact: mkotecha@o2map.com

Statement of Purpose

The hydroxyethyl tetrathiatriarylmethyl radicals- OX063, or its deuterated analogue OX071 (or OX063-d24) (Figure 1), have been widely used as the oxygen reporting molecules for biomedical electron paramagnetic resonance oxygen imaging (EPROI) due to their physiological properties, including water solubility and stability with low toxicity, allowing a systemic delivery of the probe for *in vivo* application (1). Trityls have been known for low *in vivo* toxicity with the reported LD_{50} of 8 mM/kG (2). Recently, the new *in vitro* applications of EPROI especially in tissue engineering and regenerative medicine with emphasis on pancreatic islet transplantation therapies makes it necessary to test its toxicity with cells (3). The current study examined the OX071 toxicity in several different cell lines, including human fibroblasts, MIN6 cells, beta-TC6 cells, and human islets.

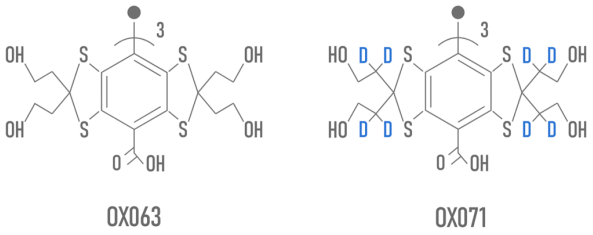

Figure 1: The chemical structure of OX063 and its deuterated analogue OX071 (also known as OX063-d24 in the literature).

Materials and Methods

The trityl OX071 (MW: 1384.96) in triacid powder form was purchased from N.N. Vorozthzov Novosibirsk Institute of Organic Chemistry, Siberian Branch of Russian Academy of Sciences Novosibirsk, Russia. The Deionized-Double Distilled Water Catalog # C993Y94) was purchased from Thomas Scientific, Swedesboro, NJ. Human fibroblasts (PCS-201-010), Beta-TC6 cells (Cat No: CRL11506) and Dulbecco's Modified Eagle's Medium (DMEM) (Cat No: 30-2002) were purchased from American Type Culture Collection (ATCC) (Manassas, Virginia). Human islets were purchased from Dr. Piotr Witkowski, The University of Chicago Medical Center, Chicago, IL 60637. ATPlite 1step Luminescence Assay System (Cat No: 6016736) was purchased from PerkinElmer (Downers Grove, Illinois). Spectrophotometry: Synergy LX Multi-Mode Reader (BioTek Instrument, Inc.).

Preparation of 72.2 mM OX071 stock solution: The 1.0 g of trityl powder was added in a 15 ml falcon tube and mixed with 8.0 ml of deionized-double distilled water. The mixture was vortexed thoroughly and pH was adjusted to 7.4 using 10 M NaOH, following which more deionized – double distilled water was added to a total volume 10 ml. The neutral trityl stock solution was sterilized by filtering the liquid using 0.22µm syringe filter. The stock was stored at 4 °C until further use. Before the experiments, the stock was diluted to the desired concentration in media.

Cell/Islet culture and treatment with OX071: Cells (Human fibroblasts (50,000 cells/well), beta-TC6 (80,000 cells/well), MIN6 (80,000 cells/well), or human islet (50 IEQ/well) were seeded onto 96-well plates in 100 µl cell culture medium. After overnight culture at 37 °C 5% CO_2 incubator, cells/islets were treated with OX071 at various concentrations (0, 0.05, 0.1, 0.25, 0.5, 1.0, 4.0, 8.0 mM) in 100 µl cell culture medium. The treatment of each concentration of OX071 was quadruplicated.

Cell toxicity evaluation: After 1hr, 24hr, or 48hr treatment, cell toxicities were evaluated according to the readings of luminescence intensities using ATPlite 1step Luminescence Assay System and Synergy LX Multi-Mode Reader.

Results

The luminescence showed that there was no significant change in intensities for all cells (Fig. 2) at 1hr, 24hr, and 48hr time. The luminescence intensities in beta-TC6 cells (Fig. 2B) in the 24h treatment had increased intensity at the trityl concentrations of 0.1, 0.25, 0.5 mM OX071 suggesting no toxicity. The 8.0 mM trityl treatment slightly decreased luminescence intensity, implying that it was likely caused by residual OX071 interfered luminescence intensity. In the 48h treatment for human islet cells, the concentrations of 0.05, 4.0 mM trityl increased luminescence intensity, suggesting no toxicity in the islets.

Conclusion: Our experimental results showed that trityl OX071 is non-toxic to cells, suggesting it can be used as an *in vitro* or *in vivo* spin probe for pO_2 measurements in islet transplantation therapies.

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References: 1. Epel et al., J. Mag Reson. 280:149-157, 2017. **2.** Golman et al., JMRI, 12(6): 929-938, 2000. **3.** Kotecha et al., Tissue Eng Part C Methods, 24(1):14-19, 2018.

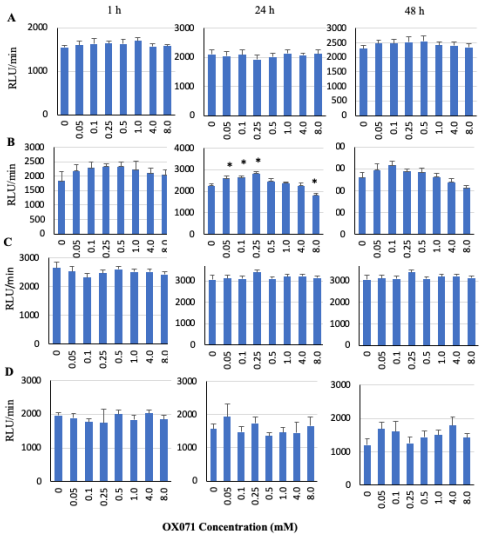

Figure 2: Luminescence intensities at 1hr, 24hr, and 48 hr for (A) Fibroblasts, (B) Beta-TC6, (C) MIN6 cells, (D) Human islets. Data are presented as mean ± SE. Statistical analysis was performed by student T-Test. *, p < 0.05 vs. Control.