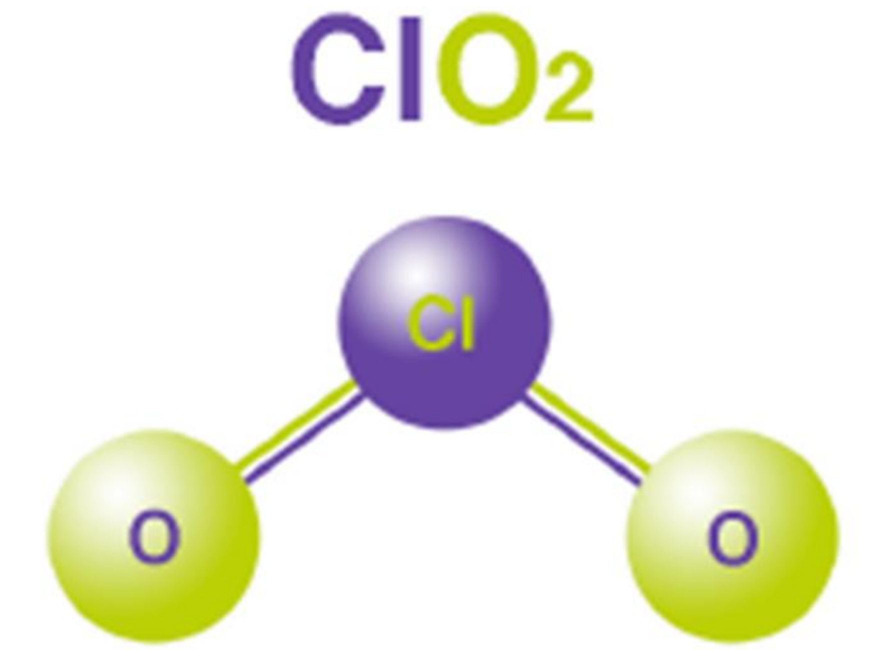
UNIVERSITY OF NEW HAVEN

"Evaluating cell survival and DNA damage of cells that are deficient in a DNA repair gene exposed to disinfectant chlorine dioxide"



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INTRODUCTION

Chlorine dioxide (ClO2) is an effective biocidal product used to destroy or control the growth of many living organisms. Several scientific investigations, including those performed by the Environmental Protection Agency have suggested that ClO2 is an oxidizing agent that eradicates pathogens without creating a harmful byproduct unlike other chemicals, such as chlorine. As a result, this chemical is used as a disinfectant and for the treatment of drinking water in many countries, including the United States. The use of chlorine dioxide has become prolific in the treatment for drinking water, as well as for sterilization of medical utensils, industrial use and the decontamination of swimming pools. However, the ability of this chemical to cause DNA damage or decrease cell survival is still uncertain because the mechanism of action is not known. The purpose of this research is to expose wild and mutant MEF_1 cells with ClO_2 and document their response based on their survival and potential DNA damage from the treatment. This is important because cancer cells usually lack important DNA repair genes, therefore they are known to be more vulnerable to oxidation.

MATERIALS AND METHODS

Cell Culture:
Mouse Embryonic Fibroblast (MEF) wild type (WT) and mutant (Pol β) cells were used in this research. The cells were cultured in DMEM high glucose medium containing 10% Fetal Bovine Serum and 1% PSG.

Preparing ClO_2 solution:
Chlorine dioxide was produced by reacting sodium chlorite ($NaClO_2$) with citric acid. To treat the cells with ClO_2 50 µl of each reactant are mixed with 9.9ml of DMEM to create a 250mM concentration. From this concentration a serial dilution was performed to create 50mM, 25mM, 5mM, and 2.5mM. ClO_2 was prepared on ice then immediately added to the cells. The cells were treated for 30 minutes on ice and in the dark because the reagents are light sensitive.

MTT:
MTT is a colorimetric assay for assessing cell survival and metabolic activity. After the cells were treated with ClO_2, they recovered for 24 hours then the MTT solution was added for one hour. The MTT solution contains tetrazolium salts that are reduced in the mitochondria of viable cells. Formazan is the product of this reaction, displaying a purple color when solubilized with buffer. A darker color means more viable cells present and it is confirmed with an absorbance reading.

Flow Cytometry:
Flow cytometry allows cells that are suspended in a fluid to be analyzed under a beam of light or laser. Data from this research used an anti-phosphor H2AX antibody which is a DNA double strand break marker. Cells were also stained with propidium iodide (PI/RNASE) which binds to DNA and shows the stage of the cell cycle.

RESULTS

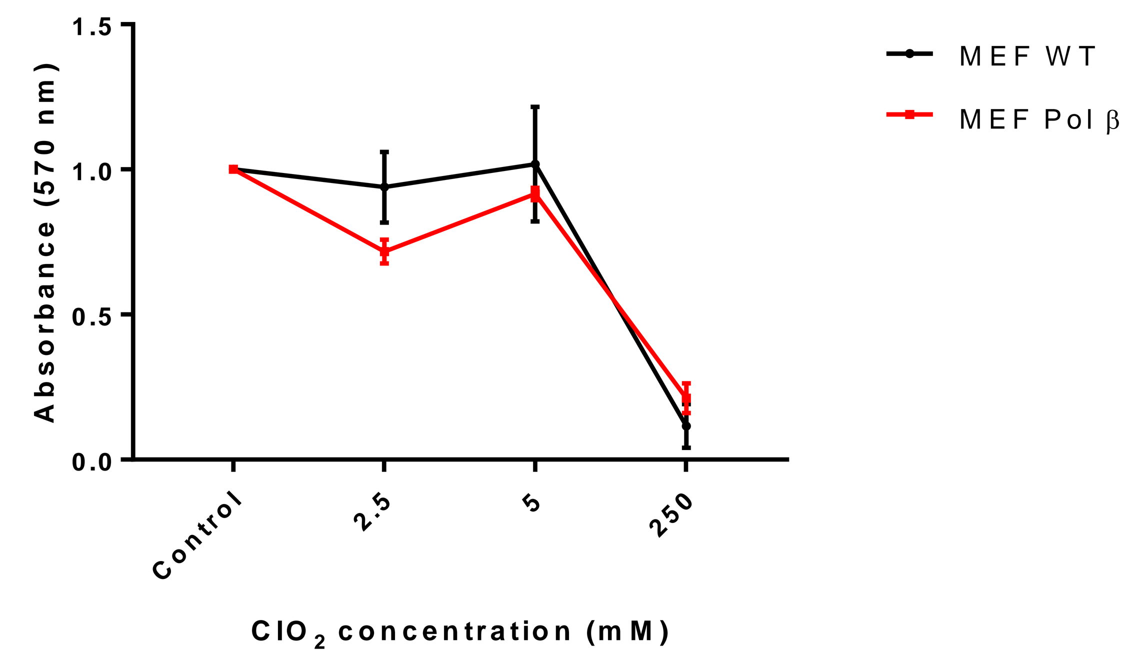

Figure 1: Mouse embryonic fibroblast cells that are deficient in DNA polymerase beta are more vulnerable to ClO_2 treatment than Mouse embryonic fibroblast wild type cells.

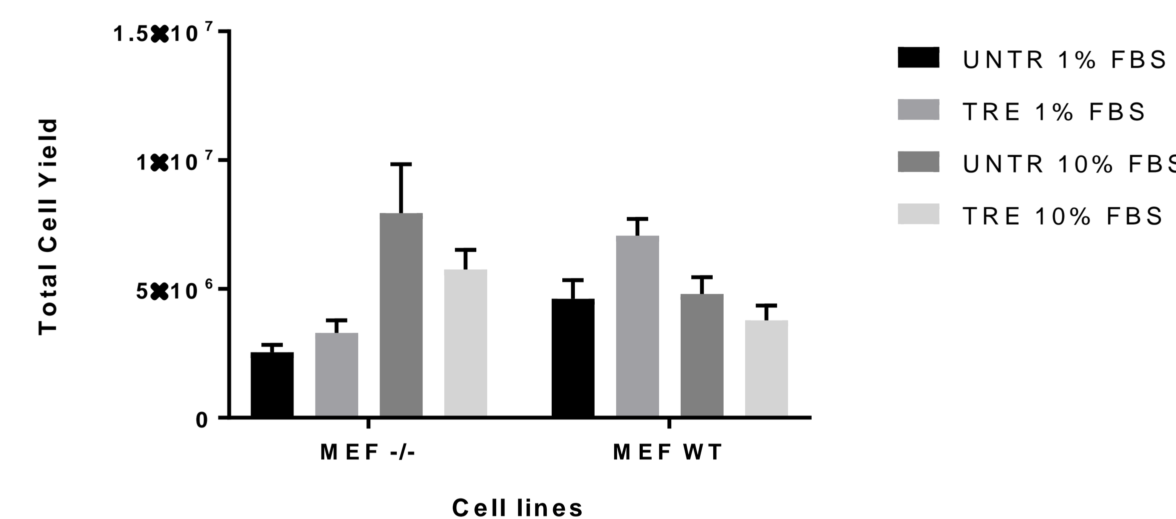

Figure 2: MEF Pol β and MEF WT cells were treated with ClO_2 (2.5mM), counted after 24 hours of recovery and compared to untreated cells. The cell lines were cultured in DMEM high glucose with 1% FBS (serum starved) and 10% FBS.
*This experiment was done once, data from cells cultured in DMEM high glucose with 1% FBS are inconsistent and needs to be repeated

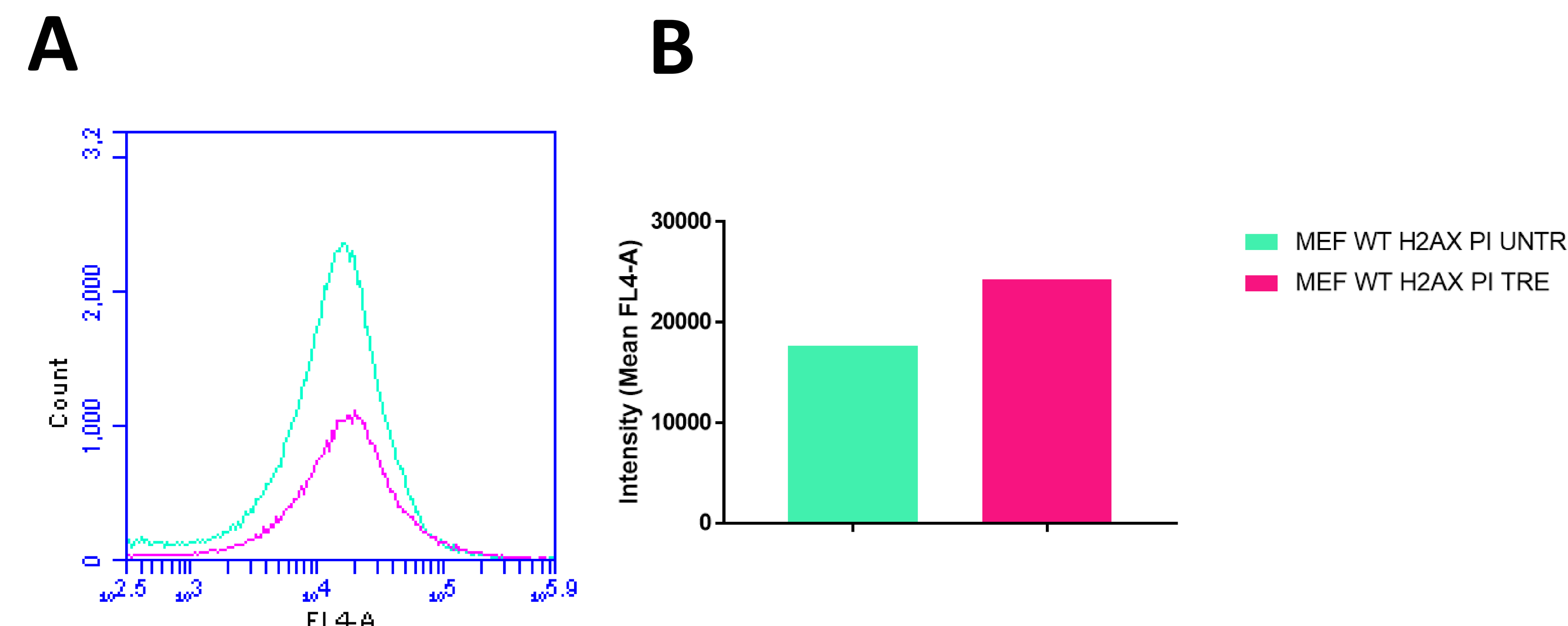

Figure 3: MEF WT cells analyzed with H2AX and PI/RNASE. (A) Data from FL4-A displays DNA double stranded breaks. *This experiment was done once and needs to be repeated. (B) The intensity of the fluorescence for MEF WT UNTR is 37% lower than the intensity of the MEF WT TRE because there are less DNA double stranded breaks.

CONCLUSION AND FUTURE RESEARCH

- MTT data (Figure 1) shows that MEF cells lacking essential DNA repair polymerase beta (Pol β) protein are more vulnerable to chlorine dioxide treatment than MEF WT cells.
- Data from the starvation assay (Figure 2) shows that the cell lines left untreated in 10% FBS had a higher total cell yield than treated cells.
- Flow cytometry data (Figure 3) confirms that MEF WT cells treated with ClO_2 experienced more double stranded DNA breaks than the untreated cells.
- The results suggest that ClO_2 can be used as a possible treatment for cancer cells that are deficient in DNA repair genes.
- The next step is to repeat all assays multiple times and verify the data using a cancer cell line.

REFERENCES

1. Ma, Jui-Wen et al. "Efficacy and Safety Evaluation of a Chlorine Dioxide Solution." Ed. Miklas Scholz. *International Journal of Environmental Research and Public Health* 14.3 (2017): 329. *PMC*. Web. 7 Sept. 2017.
2. Alvarez, Niuska. *Evaluating Cell Survival and DNA Damage after Exposure to Various Amounts of Disinfectant "Chlorine Dioxide" and Exploring Its Use as a Potential Cancer Chemotherapy Agent. Evaluating Cell Survival and DNA Damage after Exposure to Various Amounts of Disinfectant "Chlorine Dioxide" and Exploring Its Use as a Potential Cancer Chemotherapy Agent*.
3. "Water Treatability Database." *EPA*, Environmental Protection Agency, 5 Feb. 2007.
4. "Antibodies, Proteins, ELISA Kits." *Abbexa*, Abbexa.com.

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