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

Niusha Mariana Alvarez, Ali Senejani
Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516

INTRODUCTION

Chlorine Dioxide (ClO₂) is a synthetic, green-yellowish gas with a chlorine-like, irritating odor that was discovered in 1848 by Sir Humphrey Davy. ClO₂ is a water-soluble and a safely ingested molecule at the right dosage currently approved and administered on the treatment of drinking water in several countries. Compared to Ozone and Oxygen. ClO₂ has the lowest oxidation strength, a fact potentially indicating that just like Oxygen, Chlorine Dioxide, at a suitable concentration, might be as well tolerated by the body.

OZONE
OXYGEN
CHLORINE DIOXIDE

OXIDATION STRENGTH
ClO₂ : 1.30 VOLTS
O₂ : 0.95 VOLTS

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MATERIALS AND EXPERIMENTAL PLAN

Preparation of Solution:
The Chlorine Dioxide was produced from a reaction of 22.4% sodium chloride (NaClO₂) solution and citric acid. They were each first diluted to 50% and mixed with a ratio of 1:1. The preparation of the Chlorine Dioxide solution should be done on ice and immediately be added into pre-chilled flask containing cells, then allowed to act for 5 minutes on ice, preferably in dark conditions due to the light sensitivity of the reagents.

Cell Culture and Cell Survival Studies:
The effect of chlorine dioxide was tested on two different cell lines, Human Embryonic Kidney cells (HEK293) and Mouse Embryonic Fibroblast (MEF) wild type and mutant, cultured in a DMEM high glucose medium supplemented with 10% Fetal Bovine Serum . The cells were left 24 hours at 37°C for recovery, then the MTT Assay was performed in order to calculate the percentage of viable cells with active metabolism.

The Comet Assay:
Alkaline and neutral comet assays were executed in order to respectively observe the presence of single and double stranded DNA breaks on HEK293 cells in response to Chlorine Dioxide treatment. The comet assay is a sensitive and rapid technique for quantifying and analyzing DNA damage in individual cells.

RESULTS

Figure 1. Chlorine Dioxide reduces HEK293 survival. HEK293 were treated with varying amounts of chlorine dioxide for 30 minutes, allowed to recover for 24 hours and assessed via an MTT assay. Line chart showing error bars with Standard deviation.

Figure 2. Polβ deficient mouse embryonic fibroblast cells are more susceptible to ClO₂ exposure compared to wild type cells. The cells were treated for 30 minutes, recovered for 24 hours, and assessed via MTT assay. Line chart showing error bars with Standard deviation.

Figure 3. Chlorine Dioxide induces DNA damage and single stranded breaks. HEK293 Samples were treated for 30 minutes and assessed via comet analysis.

CONCLUSIONS

- Chlorine Dioxide is a green-yellow gas that can decompose rapidly in air. Because it is a hazardous gas, Chlorine Dioxide is always made freshly at the location where it is used.
- Chlorine Dioxide is used in public water-treatment facilities to kill bacteria, microorganisms and to deactivate viruses.
- Human HEK293 cells exposed to 250 mM levels of chlorine dioxide exhibit rapid cell death.
- MEF cells lacking the key DNA repair gene DNA polymerase beta display an increased level of sensitivity to Chlorine Dioxide indicating its use as a potential chemotherapy approach for cancer cells harboring defective DNA repair genes.

Results from the alkaline comet assay indicate HEK293 cells treated with a low level of Chlorine Dioxide carry high levels of DNA damage and single stranded breaks.

ACKNOWLEDGEMENTS

A special note of gratitude to the SURF support team at the University of New Haven, particularly Mrs. Carol Wilkins. I also want to thank Dr. Ali Senejani for helping me design the whole model for this research and guiding me with useful strategies. I find myself absolutely grateful for all his time, patience and 100% availability.

Lastly, I would like to acknowledge graduate students Beatrice Dosebert, Joseph Magrino, George Tsai, who helped me unconditionally with my many inquiries during the research.

REFERENCES