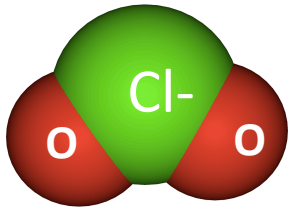
Evaluating Cell Survival and DNA Damage after Exposure to Various Amounts of Disinfectant "Chlorine Dioxide" and Exploring Its Use as a Potential Cancer Chemotherapy Agent



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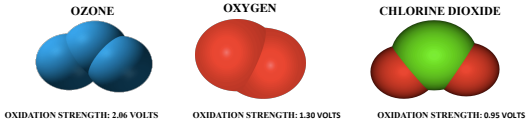
INTRODUCTION

Chlorine Dioxide (ClO_2) is a synthetic, green-yellowish gas with a chlorine-like, irritating odor that was discovered in 1814 by Sir Humphrey Davy [1]. ClO_2 is water-soluble and a safely ingested molecule at the right dosage currently approved and administered on the treatment of drinking water in several countries. When 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[2, 3].



Although there is not enough evidence collected about the specific mechanisms of reaction of Chlorine Dioxide against pathogens, many biological tests have been performed in order to determine its effect especially in the treatment of drinking water. There are more than 495 water treatment plants in Europe applying the usage of chlorine dioxide as the primary disinfectant agent against bacteria, especially in highly developed countries such as Germany, Austria, Switzerland and France, which remarkably states the safety of chlorine dioxide ingestion in humans under the proper tolerable conditions. In the United States the Safe Water Drinking Act has been making improvements and trying to make adjustments towards the exchange from chlorine usage into Chlorine Dioxide, which is most commonly used as a regulator of taste, odor and color of water [4].

Many pathogenic bacteria undergo anaerobic respiration cycles, therefore lacking the ability to produce enzymes such as superoxide dismutase and catalase that are needed for survival in the presence of oxygen[4]. Given a powerful oxidation capacity at low dosage and its possible achievement on acting without causing any considerable damage to the body or secondary effects manifested by its low oxidation strength of 0.95 volts, ClO_2 can be used as a disinfectant, since It is a very strong oxidizer and it effectively kills and deactivates pathogenic microorganisms such as fungi, bacteria and viruses. It also prevents and removes bio film which establishes its potential use as an alternative to antibiotics in case of resistance. Similarly, cancer cells lacking certain DNA repair genes can be more sensitive to such oxidation indicating that the right dose of ClO_2 can substantially be applied as a chemotherapy agent..

The aim of this study was to determine what dose of **Chlorine Dioxide** is tolerable to normal mammalian cells and to DNA repair mutant cells, by evaluating cell survival, the level of DNA damage, and more importantly, to explore its use a potential chemotherapy agent for cancer.

MATERIALS AND EXPERIMENTAL PLAN

Preparation of Solution:

The Chlorine Dioxide was produced from a reaction of 22.4% sodium chlorite ($NaClO_2$) 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 metabolisms [5, 6].

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 [7].

RESULTS

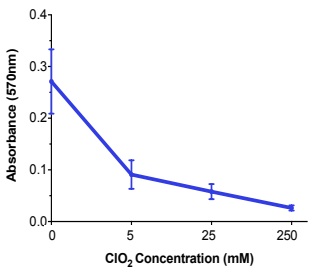


Figure 1. Chlorine Dioxide reduces HEK293 survival. HEK293 were treated with varying amounts of chlorine dioxide for 30 minutes, allowed to recover for 24 hours and assessed via an MTT assay. Line chart showing error bars with Standard deviation.

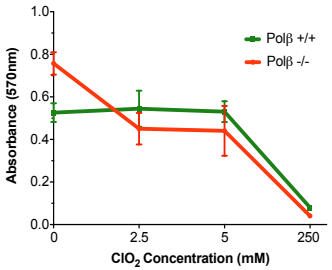


Figure 2. Polβ deficient mouse embryonic fibroblast cells are more susceptible to ClO2 exposure compared to wild type cells. The cells were treated for 30 minutes, recovered for 24 hours, and assessed via MTT assay. Line chart showing error bars with Standard deviation.

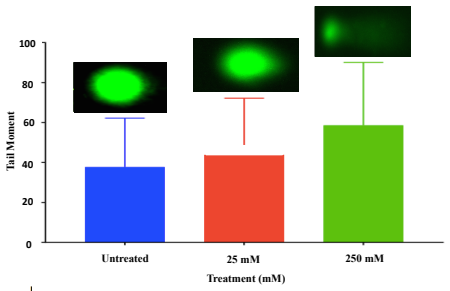


Figure 3. Chlorine Dioxide induces DNA damage and single stranded breaks. HEK293 Samples were treated for 30 minutes and assessed via comet analysis.

Chlorine Dioxide appears to induce elevated cytotoxic repercussions on HEK293 cells at doses lower than 5 mM, whereas wild type MEF cells seem to be resistant to doses lower than 5 mM ClO_2. Additionally, Polβ deficient MEF present a higher sensitivity at the same conditions in comparison to the wild type.

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.

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ACKNOWLEDGEMENTS

A special note of gratitude to the SURF support team at the University of New Haven, particularly Mrs. Carol Withers. 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 Doubert, Joseph Magrino, George Tsai, who helped me unconditionally with my many inquires during the research.