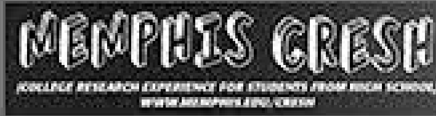
Zinc Cytotoxicity on Glioblastoma Cells



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Background and Goal of the Study

Glioblastoma is a highly aggressive type of brain tumor for which available therapy is of limited efficacy.

One of the primary therapies used clinically to treat these tumors is *temozolomide* (TMZ), an alkylating cytotoxic agent. Recent tissue culture studies have shown that when TMZ is supplemented with zinc, astrocytoma death is increased when compared to cells treated with TMZ alone [1]. However, these studies have not addressed the toxicity of zinc itself nor have they evaluated where in the cell the zinc exerts its action. To follow up on this work, we have evaluated the following:

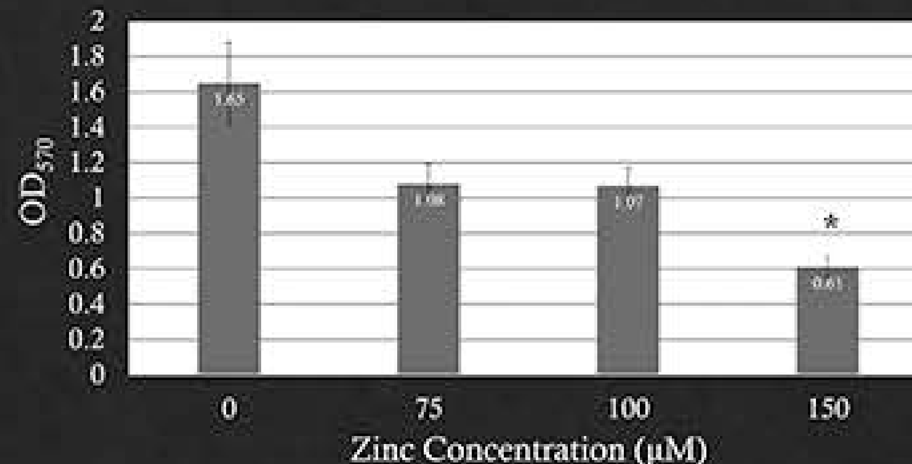
1. The toxicity of zinc by treating U87 human glioblastoma cells with increasing doses of zinc chloride ($ZnCl_2$)
2. Where in the cell the zinc can be mapped (cytoplasm, nucleus, or both) using EDS

Energy Dispersive Spectroscopy (EDS) is used to determine the elemental composition of a sample by measuring the number and energy of the x-rays emitted from the sample after electron excitation. This can be done with a scanning electron microscope (SEM), by using the SEM electron beam to generate both the image and x-rays that produce elemental information [2].

Zn Toxicity Analysis by Crystal Violet Assay

Method: A 24-well plate was seeded with U87 human glioblastoma cells following standard tissue culture procedure. To each row of wells, we added the following concentrations of $ZnCl_2$ solution: 0 µM, 75 µM, 100 µM, and 150 µM. The cells were incubated for three days. They were then stained with a 0.5% crystal violet solution in methanol for 20 minutes. The dye was removed and the wells were washed with water. The dye was then eluded with 100% methanol, and the optical density of the eluate was measured using a spectrophotometer. Statistical significance was then determined using a t-test.

Results:



Qualitatively, there is a color change in the culture medium for 150 µM of $ZnCl_2$. Cells release lactic acid, so the more cells present, the more acidic, or yellower, the medium is.

According to the t-test performed comparing the control to each Zn concentration, the difference between the control and 150 µM was considered to be very statistically significant ($p = 0.0019$), supporting the qualitative data.

Conclusion: Zn appears to have some cytotoxicity, but this is only statistically significant at the highest concentration.

Cell Preparation for SEM/EDS

1. Plate U87 glioblastoma cells on plastic coverslips
2. Fix cells in 2.5% glutaraldehyde, 2.5% formaldehyde phosphate buffered solution for 3 days
3. Fix cells in 2% osmium tetroxide phosphate buffered solution for 30 minutes
4. Dehydrate cells with increasing concentration of ethanol (10%, 25%, 50%, 75%, 95%, 100%)
5. Critical Point Drying: a method of drying samples for SEM that preserves the surface structure
6. Sputter coat cells with 5 nm of Au/Pd (density = 16.40 g/cm³) metal alloy

4.

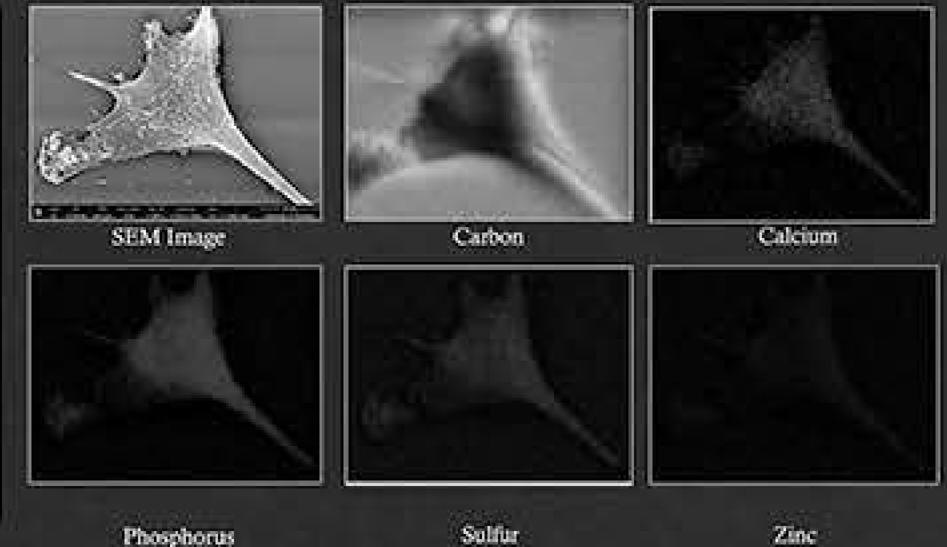
5.

6.

EDS Scans

0 µM Zn

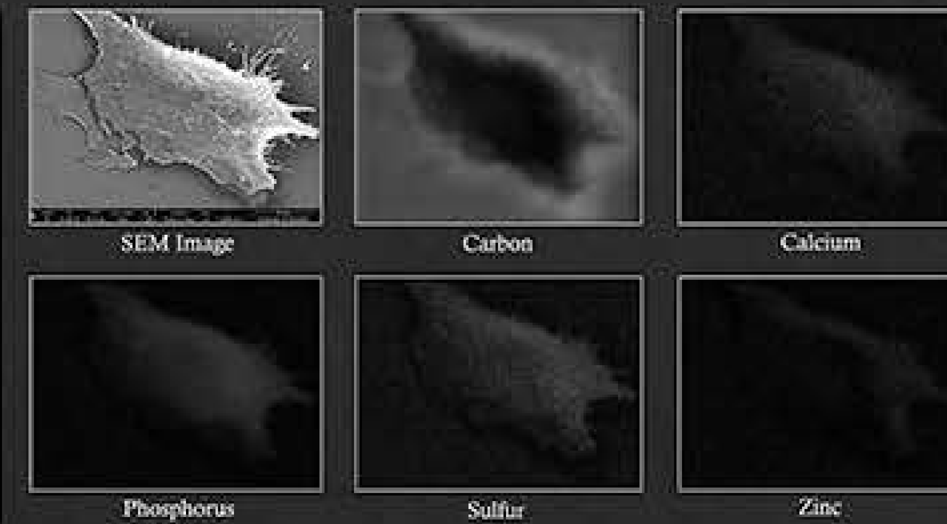
	% Wt
C	85.7
O	13.9
S	0.2
P	0.1
Ca	0.1
Zn	0.0
Fe	0.0
Cu	0.0
N	0.0



Results: The element with the highest percent abundance was carbon, which showed a negative image because the specimen was mounted on a carbon substrate. No zinc was detected, as expected, since these were the control cells.

150 µM Zn

	% Wt
C	84.9
O	14.2
S	0.3
P	0.2
Ca	0.1
Zn	0.1
Fe	0.0
Cu	0.0
N	0.0



Results: The Zn map scan is very faint, but the quantitative analysis reveals that the cells contain about 0.1% of Zn.

Conclusion

- The crystal violet assay suggested that Zn itself has some cytotoxic effect on glioblastoma cells, which may explain why zinc increased the cytotoxicity effect of TMZ in the study of Toren et al. where the two were used in combination. [1]
- By combining SEM and EDS, Zn can be detected in treated, but not in control cells. However, mapping of Zn could not be achieved at this point. This could be due to: either 1) Zn amount was too low to be mapped with precision or 2) Zn was not at the surface of the cell, but instead is concentrated at the interior rather than at the surface of the cell.

Future Work

- Determine whether the amount of Zn increases in the cell in the presence of TMZ using EDS
- Try to map Zn inside the cell by disrupting the plasma membrane using physical methods like detergent, Scotch tape, or freeze sectioning

References

[1] Toren, Amos et. al. "Zinc enhances temozolomide cytotoxicity in glioblastoma multiforme model systems" (2016) *Oncotarget*. [2] Dey, Sangeeta. Choudhury, Manabendra D. "Sublethal effects of pulp and paper mill effluent on two commonly cultured carps: a SEM- and EDS-based hematological biomarker analysis" (2016) *Fish Physiology and Biochemistry*. [3] Feoktistova, Maria et. al. "Crystal Violet Assay for Determining Viability of Cultured Cells." (2016) *Cold Spring Harbor Protocols*. Pala, Eva M. "Microscopy and Microanalysis of Blood in a Snake Head Fish, Channa gachua Exposed to Environmental Pollution" (2016) *Microscopy and Microanalysis*.

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