Preliminary Study Shows: Hepatocellular GCLC/GCLM Production is Not Significantly Different with Zinc Treatment

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Introduction

Zinc is essential to cellular function in all organisms. It plays an important role in stimulating the activity of almost 100 different enzymes and acts as a component of certain proteins [1]. Recent research has also shown that zinc may be hepatoprotective in toxic conditions by preventing cellular apoptosis induced by tumor necrosis factor-α (TNF- α)[2, 3].

The tripeptide glutathione (GSH), more formally called L-gamma-glutamyl-L-cysteinylglycine is also a prominent player in protection of the cell from TNF- α as well as environmental toxicants. Glutamate cysteine ligase (GCL) is the rate-limiting enzyme in GSH synthesis and is made up of catalytic (GCLC) and modifier (GCLM) subunits[4].

Hepatocytes (liver cells) naturally produce these proteins, GCLC and GCLM, but with the use of transformed hepatocyes this production can be increased. The Hepa-1 cells used in the present study are parental cells. Hepa-V cells have been transfected with a metallothione (zinc responsive-binding protein) promoter. CR17 cells have been transfected with the promoter and the GCLC/GCLM expression vectors (Fig 1.).

Materials and Methods

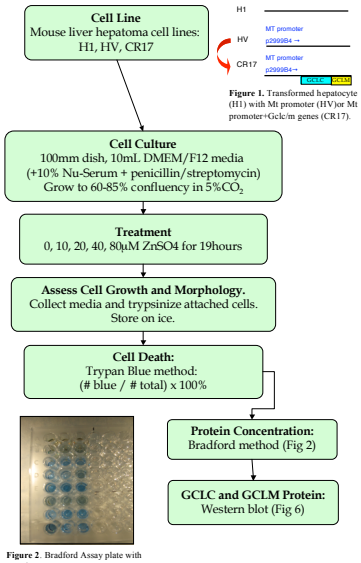

Figure 1. Transformed hepatocyte (H1) with Mt promoter (HV)or Mt promoter+Gclc/m genes (CR17).

Figure 2. Bradford Assay plate with samples

Rationale

Previous research in our lab [2] shows an increased presence of both the GCLC and GCLM subunits of GCL in Hepa-V and CR17 cells on exposure to 55μM zinc sulfate for 16hrs. The objective of the present study was to determine the optimum zinc sulfate treatment for GCL expression and to characterize possible morphological and health benefits of Zn to Hepa-1, Hepa-V and CR17 cells. We anticipate that treatments of 10, 20, 40 μM Zn will increase GCLC/M expression in CR17 cells with cell death and significant morphological changes occurring in all cells at 80 μM Zn. CR17 cells should produce the most GCLC/M.

Results

Morphology

Observation of cell Set #1 and #2 showed slight rounding HV and CR17 cell types after treatment with Zn (data not shown).

As shown in **Figure 3.**, no morphological differences were seen in CR17 cells in the 0, 20, or 80μM zinc sulfate treatments in Set #3; nor were they detected in the 10 and 40 μM treatments. No difference was seen in HV or H1 cells

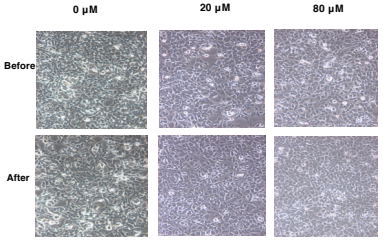

Figure 3. CR17 cells before and after 0, 10, and 20 μM Zn++ treatments. (A) Before and after 0 μM Zn++ treatment. (B) Before and after 10 μM Zn++ treatment. (C) Before and after 20 treatment.

Results (cont'd)

Cell Growth

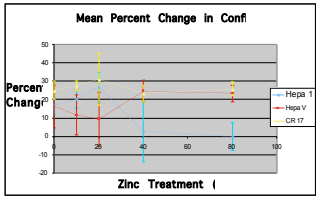

Figure 4. Average (±SEM) of percent change in confluency across all three data sets for each cell line.

Cell Death

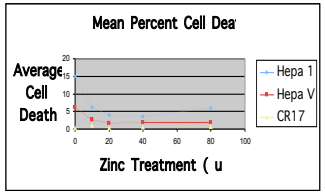

Figure 5. Average percent cell death in all three data sets across all three cell lines.

GCLC/GCLM Protein Expression

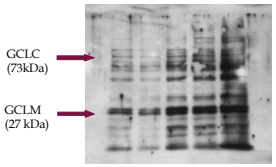

Figure 6. Western Blot of GCLC/M in CR17 cells from data set #3. Data sets #1 and #2 showed no difference in GCLC/M presence with treatment.

Discussion

With this study we hoped to see an up-regulation of GCLC and GCLM subunits in CR17 cells. Three trials of this experiment were completed, with variable results, therefore no conclusions can be made. However, the preliminary results of this study suggest that:

- H1 cells experience may experience no significant morphological changes as a result of Zn treatment (at all doses)
- HV and CR17 cells may experience morphological changes after Zn treatments (all doses). This may be caused by the Mt (zinc-binding) promoters with which they are transfected.
- There may be no significant difference in cell death trend between cell types. Which could show that no one cell type is significantly more sensitive to zinc treatments. The descending order of cell death between the cell types (Figure 5) is proportional to each cell type's rate of growth.
- Zinc may inhibit growth of Hepa-1 cell. There is an indication that at 80 μM Zn treatment there is a significant difference between the Hepa-1 cells and both Hepa-V and CR17 cells.
- No conclusion about the up-regulation of GCLC and GCLM can be made because of the variability in the data.
- Further trials of this experiment are needed to make any real conclusions. The variability in the this small data set is too great. However, this study may have identified some interesting trends.

Citations

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[2] Botta D, et.al. (2004). Glutatmate-cysteine ligase attenuates TNF-induced mitochondrial injury and apoptosis. *Free Radical Biology and Medicine*. 37(5): 632-42.

[3] Zhou Z, et.al. (2007). Preservation of hepatocyte nuclear factor-4α is associated with zinc protection against TNF-α hepatoxicity in mice. *Exp Biol Med (Maywood)*. 232(5):622-8.

[4] "Glutathione." PDR Health. 2002 http://www.pdrhealth.com/drug_info

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