Characterizing Mitochondrial Inhibitor Derivatives for Pancreatic Cancer

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Introduction:

Known for its aggressive nature and late-stage diagnoses, pancreatic cancer is the tenth most common cancer and third leading cause of cancer-related mortality in the United States [1]. As a result, it is imperative to find treatments for those with pancreatic cancer. In recent decades, Pyrvinium Pamoate has garnered attention for its anticancer properties, especially as a mitochondrial inhibitor; but, more about its mechanism of action and its derivatives MPS56 and BG68 still needed to be investigated [2]. We hypothesized that all three drugs would reduce the expression of mitochondrial protein MT-CO2, and lower the reduced to oxidized glutathione (GSH/GSSG) ratio in affected cells.

Materials and Methods:

The two main procedures utilized in characterizing the novel mitochondrial inhibitors were Western Blotting and the GSH/GSSG Assay. Western blotting is the practice of transferring protein produced by cells onto a membrane, then blotting that membrane with specific antibodies that attach to proteins of interest in order to visualize the expression of those proteins. The GSH/GSSG Assay is a procedure used to measure the ratio of reduced glutathione to oxidized glutathione in the cell and can serve as an indicator of the cells' oxidative stress levels. Using luciferin fluorescence, the amount of reduced glutathione and the amount of oxidized glutathione can be measured.

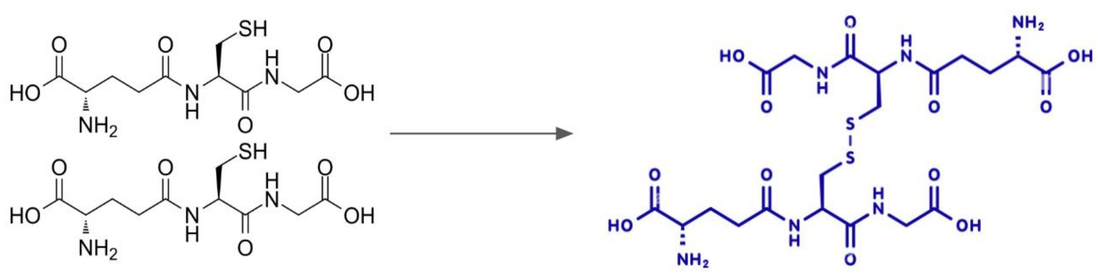

Figure 1: Oxidation of GSH to GSSG under oxidative stress

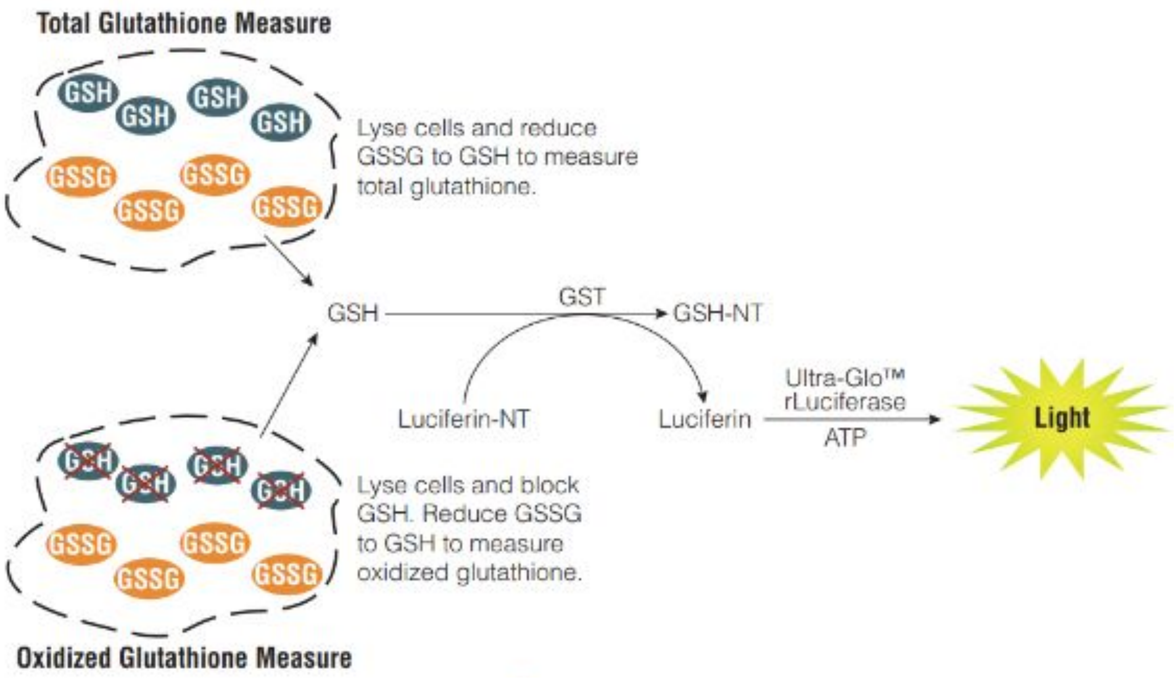

Figure 2: Measurement reduced and oxidized glutathione through GSH/GSSG assay.

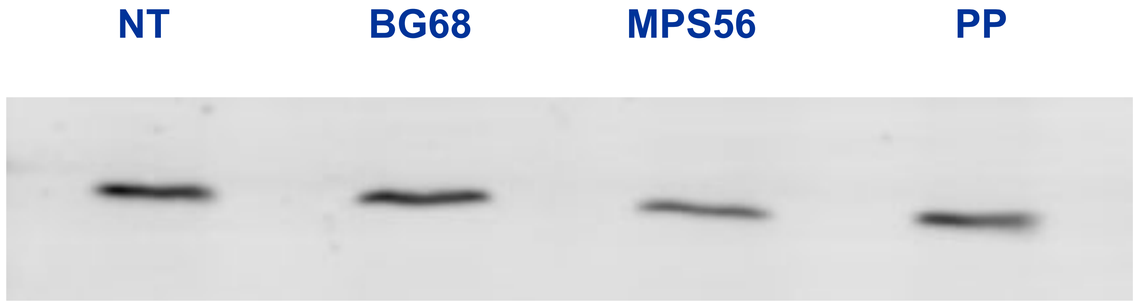

Figure 3: Western Blot exhibiting the expression of mitochondrial protein MT-CO2 and α-Tubulin.

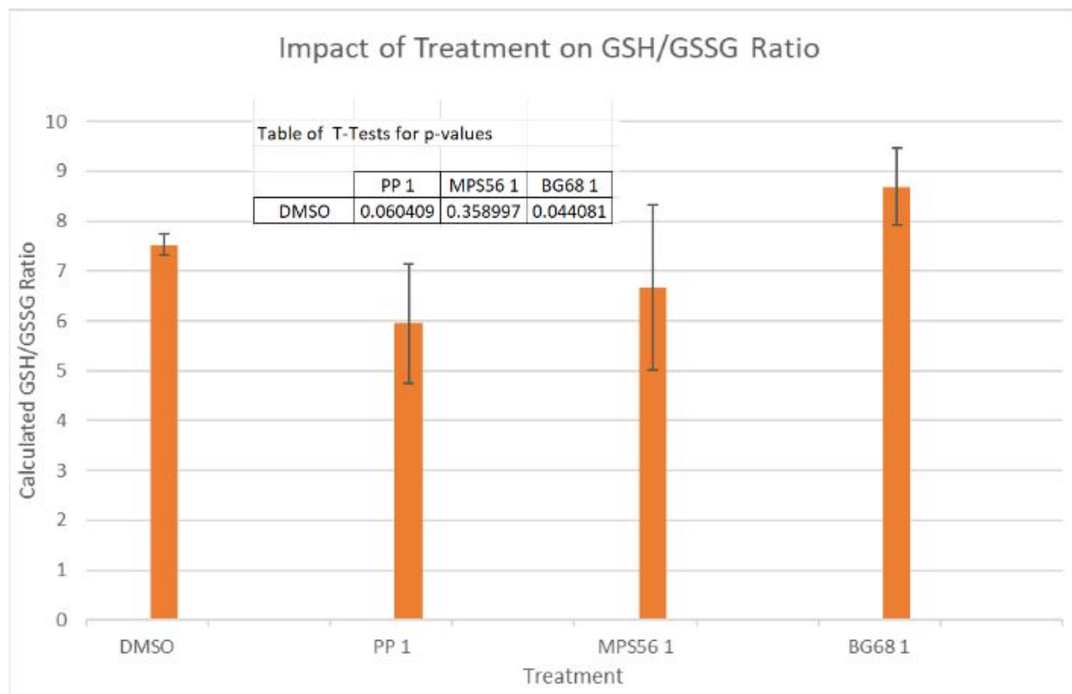

Figure 4: Impact of various treatments on the GSH/GSSG ratio.

Results:

For the western blot, MP2 cells were treated for 72 hours with the following drugs: PP (at 100 nanomolar), MPS56 (at 1 micromolar), and BG68 (at 500 nanomolar). The western blot showed that compared to no treatment, PP and MPS56-treated cells appeared to show less MT-CO2 expression, while BG68-treated cells appeared to show the same amount of MT-CO2 expression. As for the GSH/GSSG assay, we plated 10,000 MP2 cells per well and treated them for 24 hours with 1 micromolar of drug for each treatment (PP, MPS56, and BG68) as well as DMSO for the control. Then, we ran a Picogreen Assay to measure the amount of DNA in the cells, so that we could standardize our GSH and GSSG measurements down the individual cell. Using our standardized GSH/GSSG ratios, we ran a T-Test to measure statistical significance and obtain p-values.

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References:

[1]:https://pancan.org/news/pancreatic-cancer-five-year-survival-rate-increases-to-13/
[2]:https://pubmed.ncbi.nlm.nih.gov/36552005/