Determining Anti-Cancer Properties of Novel Tris-Indolyl Amines

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Abstract

Cancer still remains as a prevalent disease today. This disease may manifest itself in many different ways and affect a variety of tissues. Treatment can be complicated since there is not a "one size fits all" medicine that can treat the disease. With this need for medicine, research is warranted into potential cancer treatments and drugs. A novel tris-indolyl iron-chelating drug was synthesized and tested for its potential anti-cancer properties. MTS and apoptosis assays were carried out with the PC3 cell line and preliminary results show a decrease in cell viability. Furthermore, the compound was shown to be toxic within a specific concentration range which may be of interest for the potential of new chemotherapy agent.

Background

The novel tripodl indolyl amine synthesized, referred to as "Compound B," belongs to a class of molecules called iron chelators. Iron chelators already have practical application in medicine as a means to remove excess iron in patients.

Here we investigate potential use of Compound B in cancer treatment. An important aspect of Compound B is that it is only toxic to cells in a very narrow range of concentrations. If the concentration increases or decreases, Compound B actually increases cell viability. This shows potential for cancer treatment since the concentration of Compound B would decrease as it diffuses through the body, thus making the treatment more bearable for the patient. This unique property of Compound B warrants continued investigation.

Synthesis of Compound B

Indole was weighed (2.5 g) and placed in a round-bottom flask. To the indole, (40 mL) of diethyl ether was added and stirred in an ice bath until the indole was dissolved. Oxalyl chloride (2.78 g) was added dropwise over 15 min and the reaction mixture allowed to stir for 30 min. An amine mixture consisting of triethylamine (TEA) (2.2 g), tris(2-aminoethyl)amine (TREN) (0.87 g), and THF was made and allowed to chill in an ice bath for 30 min. After the 30 min the reaction mixture was filtered and the solid collected was placed into another round-bottom flask. The chilled amine solution was added dropwise to the solid and then stirred for 1 hour at 0°C. After stirring, the mixture was filtered. The aqueous solution collected was then recrystallized with methanol, and filtered again to yield a beige solid.

Methods

Apoptosis Assay:

Cells were plated into a 96 well plate at a concentration of 7,500 cells per well and incubated for 24 hours at 37°C. Mitochondrial membrane were labeled via incubation with Mito-tracker red for 30 minutes at 37°C. The cells were then washed and resuspended in cell culture media. Control cells were incubated in the normal cell culture media. Other cells were treated with either 1% DMSO, 2 mM staurosporine, or 74.8 µM Compound B. Three samples were taken from each experimental condition at varying time points. Acridine Orange was used to fluorescently label cells previously stained with Mito-tracker red. Cells were also fluorescently labeled with either Caspase 3, 7 green detection reagent and Sytox Advanced. A BD Accuri C6 flow cytometer was used for cytometric analysis. Images were captured using a confocal imaging microscope.

Results

Figure 1. MitoTracker Red stained PC3 cells at 12 hour timepoint. PC3 cells were treated with Compound B and labeled with the fluorescent indicator MitoTracker Red. This stains intact mitochondrial membrane. At the 12 hour time point, the PC3 cells were fixed. Z-stacked images were collected using a Zeiss confocal microscope.

Figure 2. Hoechst 422 stained PC3 cells at 12 hour timepoint. PC3 cells were treated with Compound B and labeled with the fluorescent indicator Hoechst 422. Hoechst 422 is a DNA intercalator that fluoresces when bound to DNA. At the 12 hour time point, the PC3 cells were fixed. Z-stacked images were collected using a Zeiss confocal microscope.

Figure 5. PC3 cell treated with Compound B at 12 hour timepoint. PC3 cells were treated with Compound B fluorescently labeled with Hoechst 422 stain and MitoTracker Red. At the 12 hour time point, the PC3 cells were fixed. Z-stacked images were collected using a Zeiss confocal microscope. The figure shows fluorescent activity from both fluorescent indicators of the Hoechst 422 (green) and MitoTracker Red (red) stains.

Figure 6. Compound B treated PC3 cells with overlapping of fluorescent indicators at 12 hours. Multiple PC3 cells shown with both indicators of Hoechst 422 and MitoTracker Red indicator present. Overlapping of the red MitoTracker Red indicator and the green Hoechst 422 stain show where both are present with an orange/yellow color. This indicates that the mitochondrial membrane is breaking down and the Hoechst 422 stain is able to go inside the mitochondria and bind to the mitochondrial DNA.

Figure 3. Relative fluorescent intensity of Mito-tracker red across varying time points. Cells were fluorescently labeled with Mito-tracker red which fluoresces red when bound to intact mitochondrial membranes. For the Stauro treatment group, the relative fluorescent intensity remains fairly constant over the first two time points, but begins to decrease at 8 hours and decrease further at 24 hours. Com B treated cells show constant levels of relative fluorescent intensity at time points of 2 and 4 hours. However, the intensity drops at 8 hours and remains almost relatively constant at 24 hours. The drop in relative fluorescent intensity indicates that the mitochondrial membrane is breaking up. The degradation of the mitochondrial membrane provides evidence that apoptosis is the type of cell death occurring in the Stauro and Com B treated cells.

Figure 4. Relative fluorescent intensity of Caspase 3, 7 across varying time points. Cells were incubated with CellEvent Caspase 3, 7 green detection reagent, a four-amino acid peptide (DEVY) conjugated to a nucleic acid-binding dye. Across time points, the control displays a baseline level of caspase-3,7 activity. In contrast, cells treated with staurosporine (Stauro) and Compound B (Com B) show a spike in caspase levels at the 2 hour time point. These levels decrease as time continues. These results provide evidence that apoptosis is induced in cells treated with either Stauro or Com B.

Conclusion and Discussion

These data provide evidence that Compound B induces apoptosis in PC3 cells within two hours of treatment, leading to increased levels of cell death between 8 and 24 hours. Figures 3 and 4 show that the type of cell death is apoptosis. The results shown indicate that there is Caspase-3,7 activity and that the mitochondrial membrane is breaking down, both of which are events in apoptosis. Figures 5 and 6 also suggest that apoptosis is happening since the only way to obtain the yellow coloring in both figures is if Hoechst 422 and MitoTracker Red were occupying the same location. This then means that holes are appearing in the mitochondria, signaling break down, and the DNA intercalator Hoechst 422 is able to access the mitochondrial DNA.

Future Research

- These experiments must be repeated with more time points to gain a more accurate measure of apoptotic events.
- These experiments should be repeated using other cancer cell lines to determine if compound B's effect on other types of cancer cells.
- It will be necessary to repeat these experiments on primary cells to gain insight into the effect Compound B may have on healthy cells and inform future development into a full anti-cancer drug.
- The mechanism by which Compound B induces apoptosis needs to be elucidated as well as the metabolic pathway.
- Finally, other derivatives of Compound B may be synthesized to see if those possess any anti-cancer properties.

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