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Effect of a Hexylamine Derivative on Cancer Cell Viability

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Abstract

2-[(p-Chlorophenyl)hydroxymethyl]-1-[(methylamino)hexyl]cyclohexanol is a drug produced by hexyl amine and an epoxide through an aldol epoxidation reaction. Motifs of β-amino alcohols and nonpolar R groups in organic compounds have been found to have cytotoxic properties. Past studies in Dr. Hansen's lab has shown that this hexylamine derivative has similar LC_{50} values to other antitumor agents. They also found that the drug was cytotoxic to HL-60 cancer cells. No other cell lines have been tested with this drug. Our study investigates the effect of our compound on varying cell lines to further determine its anticancer properties. Mouse NIH/3T3, Human HEK293, and Human SK-MEL-28 cell lines were cultured and plated into 96-well plates. Varying concentrations of the hexylamine derivative were administered and incubated for 48 hours. MTT assays detected the levels of cell viability. Results showed a significant decrease in HEK293 cells at a 30 µM concentration of our drug. The mouse and cancer cell lines did not produce significant results after statistical ANOVA tests. Future directions include further validation of the current results as well as research on the mechanisms by which this drug causes decreased cell viability. This research includes LDH and wound healing assays in addition to determining which proteins are down- and up-regulated in the process. Our study has found that the compound reduces human embryonic cell viability but does not significantly affect mouse cells or human melanoma cells. Further research is required to determine the methods of the drug and its potential in tumor treatment.

Background

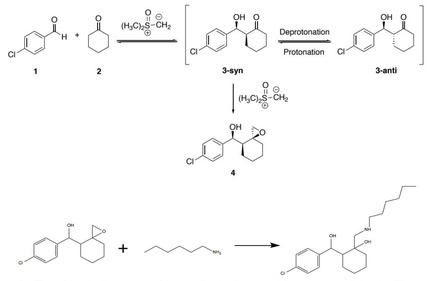

Figure 1. The chemical reaction that results in the epoxide compound (top). The epoxide compound then reacts with hexylamine to create our compound (bottom).

To create our compound, the epoxide ring was broken. The ring was opened by primary and secondary amine nucleophiles that attacked the ring bonds through an Sn2 substitution reaction. This reaction is catalyzed by the acetonitrile through a reflux reaction. The reaction creates a β-amino alcohol component along with the hexylamine chain. This component plays a role in some anticancer agents in compounds such as hapalosin. When tested in Dr. Hansen's lab at DePauw University, this compound had a lethal concentration of LC_{50} = 4.6 uM from a Brine Shrimp Assay.

Materials

Cell Lines: NIH/3T3 (mouse embryonic fibroblasts), SK-MEL-28 (human melanoma cells), HEK-293 (human embryonic kidney cells)
Cell Culture: DMEM with 10% Fetal Bovine Serum (FBS) (NIH/3T3 and HEK-293), EMEM with 10% FBS (SK-MEL-28)
Drug Treatment: 2-[(p-Chlorophenyl)hydroxymethyl]-1-[(methylamino)hexyl]cyclohexanol dissolved in DMSO
MTT Assay: MTT Reagent (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide), Detergent Reagent, spectrophotometer

Methodology

Cell Culture and Drug Treatment: Cells were cultured in media and FBS. Once confluent, cells were split into a 96- well plate at 100k cells per well. The hexylamine derivative was dissolved into DMSO. Cells were assigned to treatment groups of untreated, various hexylamine derivative concentrations, vehicle, or positive control. 0.01% DMSO was used as the vehicle treatment to mimic the DMSO that was included in the drug treatment. 10% DMSO was used as the positive control because it is known to kill cells at higher concentrations. The treated cells were then incubated for 48 hours.

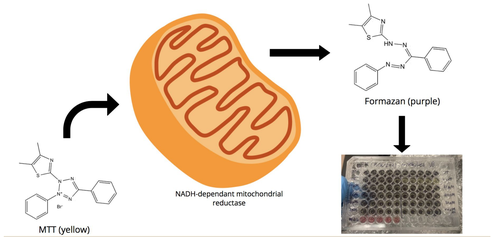

Figure 2. The mechanism by which MTT Assays measure cell viability. Greater amounts of formazin indicates greater cell viability.

MTT Assay: 10 µl of MTT Reagent was added to each well of treated cells. The plate was placed in the dark at room temperature for 4 hours. 100 µl of detergent was added to break open the cell membrane. The plate was stored in the dark at room temperature for another 4 hours. The plate was then removed and read by a spectrophotometer. This measurement provided a quantification of cell viability. We used Prism to run ANOVA statistical tests to determine significance.

Results

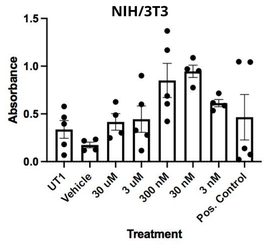

Figure 1. The response of NIH/3T3 cell viability to various concentrations of 2-[(p-Chlorophenyl)hydroxymethyl]-1-[(methylamino)hexyl]cyclohexanol. No significant results.

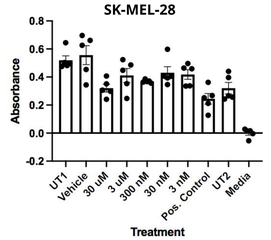

Figure 2. The response of SK-MEL-28 cell viability to various concentrations of 2-[(p-Chlorophenyl)hydroxymethyl]-1-[(methylamino)hexyl]cyclohexanol. Significant results between Untreated and Positive Control and Untreated and Media.

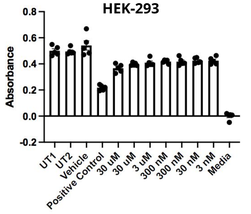

Figure 3. The response of SK-MEL-28 cell viability to various concentrations of 2-[(p-Chlorophenyl)hydroxymethyl]-1-[(methylamino)hexyl]cyclohexanol. Significant results between Untreated and 30 µam, Untreated and Media, and Untreated and Positive Control.

Conclusion

The data shows that cell viability is significantly reduced at a 30 µM concentration of 2-[(p-Chlorophenyl)hydroxymethyl]-1-[(methylamino)hexyl]cyclohexanol. The SK-MEL-28 cells had significant results with the positive control and media treatments, but no significance from the drug treatments. The NIH/3T3 cells had no significant results and the data points were much different from the other two cell lines. Causes for these differences could be that the drug affects mice cells differently from human cells, or that the methods were not run properly.

At this point, this compound does not show great promise as an anti-tumor agent. The data suggests that the drug decreases cell viability in 'healthy' human cells more effectively than in human cancer cells. As a result, it would not be a great option for targeting tumors. However, only melanoma cells were tested. The drug may show stronger effects against other cancer types.

Future Directions

Future studies for this research should look at validating our data results and furthering the tests performed in our lab. Repetition of the treatments and MTT treatments that we executed would confirm our results and their accuracy. Several additional tests could be performed to further study the anti-tumor potential of this hexylamine derivative. For example, an LDH assay would determine if the reduced cell viability was due to cytotoxic properties of the drug or some other mechanism. We also considered performing a wound-healing assay to study the effects of the drug had on cell migration. Future studies could also look into different cancer cell lines to determine if the drug is more effective in decreasing cell viability in other cell lines. Many studies could confirm or refute the results we found in this study.

Acknowledgements

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