Toxicity of Antipyrine and N1-Desmethylantipyrine in A549 Cells

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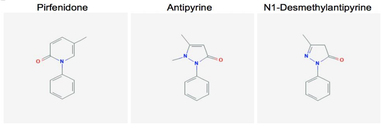
Background Information

- **Idiopathic pulmonary Fibrosis (IPF)** is a disease that impaires lung function by depositing copious amounts of collagen fibers around the alveolar sacs of the lungs making it hard for a patient to breathe
- **Increased collagen fibers** in the lungs is due to overactive fibroblasts and increased levels of transforming growth factor-β (TGF-β)[1,2]
- Currently about **13-20 people in every 100,000** are affected by this disease with only a 3-5-year life expectancy after being diagnosed
- There is **currently no cure for IPF** but two drugs that reduce the production of fibroblasts are Pirfenidone and Nintedanib, both are FDA approved but they are expensive and have low efficacies [3]
- Antipyrine and therefore its analogs are **structurally similar drugs** to Pirfenidone [4, 5, 6]



- Our research question **asks if Antipyrine and N1-Desmethylantipyrine are toxic to cells at specific concentrations**

H_o: There will be no significant difference in the toxicities after a certain point of the varying concentrations of antipyrine and N1-Desmethylantipyrine.

H_a: There will be significant differences in the toxicities after a certain point of the varying concentrations of antipyrine and N1-Desmethylantipyrine.

- These findings will be useful because **Antipyrine and its analogs** are cheaper to produce than Pirfenidone and **may be a cheaper and more viable option for IPF patients**

Methods

Subculture a 96-well plate at 3200 cells/well from healthy cells until 70% confluency is reached

Make **10X solutions** of both Antipyrine and N1-Desmethylantipyrine

Add drugs and straight DMSO to cells in a 1:2 dilution. Incubate for 24 hours.

Incubate for 4 hours

Add 12-nM MTT solution to each well (Includes a control of just media and MTT solution)

Remove old media and add new media to each well

Remove media and add DMSO to each well, making sure to mix gently

Incubate for 10 minutes

Gently mix again and **read plate at 540 nm**

Well Plate Layout

Key for well plates
1= 160μM Antipyrine
2= 80μM Antipyrine
3= 40μM Antipyrine
4= 20μM Antipyrine
5= 10μM Antipyrine
6= 5μM Antipyrine
7=2.5μM Antipyrine
8= 1.25μM Antipyrine
9= 160μM N1-Desmethylantipyrine
10= 80μM N1-Desmethylantipyrine
11= 40μM N1-Desmethylantipyrine
12= 20μM N1-Desmethylantipyrine
13= 10μM N1-Desmethylantipyrine
14= 5μM N1-Desmethylantipyrine
15= 2.5μM N1-Desmethylantipyrine
16= 1.25μM N1-Desmethylantipyrine
17=160μM DMSO
18= 80μM DMSO
19= 40μM DMSO
20= 20μM DMSO
21= 10μM DMSO
22= 5μM DMSO
23= 2.5μM DMSO
24= 1.25μMDMSO
25= Cells Only
26= DMSO Only
27= Antipyrine Only
28= N1-Desmethylantipyrine Only

Results

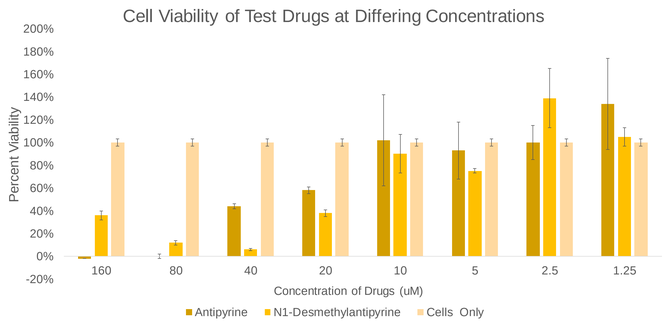


Figure 1. Cell Viability of Test Drugs at Differing Concentrations. The mean (±SEM) cell viability for each concentration is shown for Antipyrine, N1-Desmethylantipyrine and no drugs.

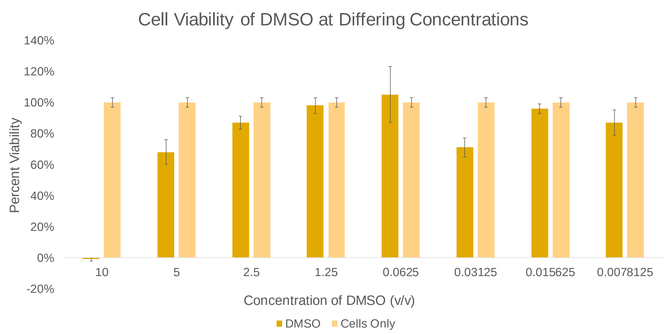


Figure 2. Cell Viability of DMSO at Differing Concentrations. The mean (±SEM) cell viability for each concentration is shown for both DMSO and only cells.

- There was **no significant difference in toxicity after the 10 uM concentrations** of both Antipyrine and N1-Desmethylantipyrine compared to the no drug control
- Using a **single factor ANOVA** statistical analysis showed that there was no significant difference throughout the whole data set **(p>0.05)**
- The DMSO data showed **no significant difference in toxicity after the 0.0625 v/v** reading, excluding the 0.03125 v/v.
- Using a **t-test**, it was shown that there was no significant difference between DMSO toxicity and the no drug toxicity with the whole data set **(p>0.05)**
- **we reject our null hypothesis on both counts** due to significant differences in toxicity at higher concentrations

Discussion & Conclusion

IPF is a serious disease that renders its victims breathless and produces a short life expectancy after diagnosis. It was found that at concentration of 10uM and under the drugs were not toxic to the A549 cells. This supports the alternative hypothesis and in turn we can reject our null hypothesis.

Due to the results of this preliminary study, future research can be done with fibroblast cells and monitoring TGF-β concentrations at varying time scales utilizing both drugs. The next steps for the experiment will determine if these two drugs will be able to replace Pirfenidone and Nintedanib in treating idiopathic pulmonary fibrosis.

References

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