Developing Assays for Testing the Effectiveness of a TNF-α Modulating Anti-inflammatory Drug

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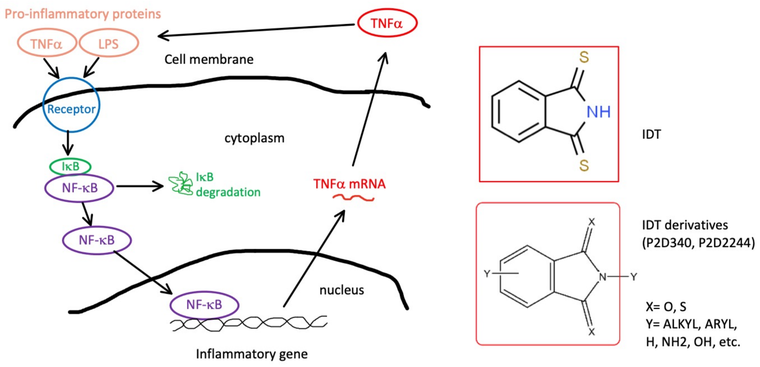
Abstract

Many anti-inflammatory drugs are currently in use to treat neuroinflammation in the brain which can result from Alzheimer's disease, Parkinson's disease, traumatic brain injury, and more. In collaboration with a company, P2Dbiosciences, we are testing drugs that can modulate the function of inflammatory cytokines such as TNFα, with the goal of reducing neuroinflammation and thus benefiting people suffering from the neurodegeneration and cognitive decline associated with neuroinflammation. We hypothesize these drugs work by inhibiting the signaling associated with inflammatory cytokines.

An assay was developed to identify the mechanism of action of these cytokine modulating anti-inflammatory drugs. BV2 cells in culture were used for these assays to model how the drug affects mouse microglial cells (immune cells resident in the brain). The assay uses quantitative RT-PCR (qPCR) to measure changes in TNFα mRNA levels when cells are treated with drug. Levels of TNFα mRNA were also quantified over a period of time following drug treatment to determine whether the degradation time of the TNFα mRNA was affected by treatment.

NF-κB pathway & P2D drug structure

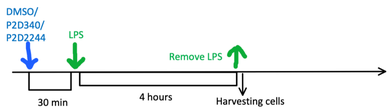


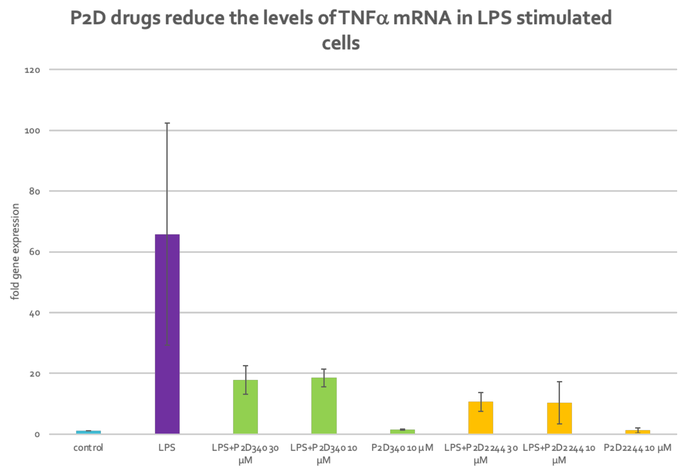
Hypothesis

If a cell with induced inflammation is treated with an inhibitory drug, then the TNFα mRNA levels will be reduced. Additionally, the TNF-α mRNA will be degraded at a faster rate when the inhibitory drug is added.

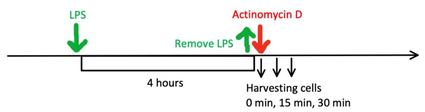
Experimental Setup and Results

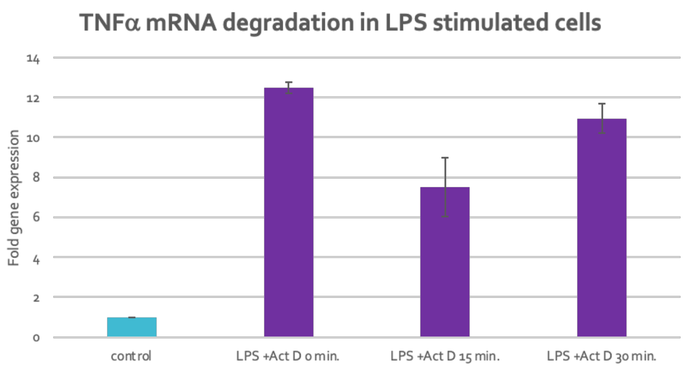
Measuring TNFα mRNA levels



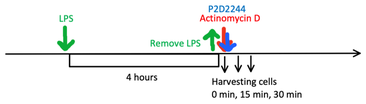


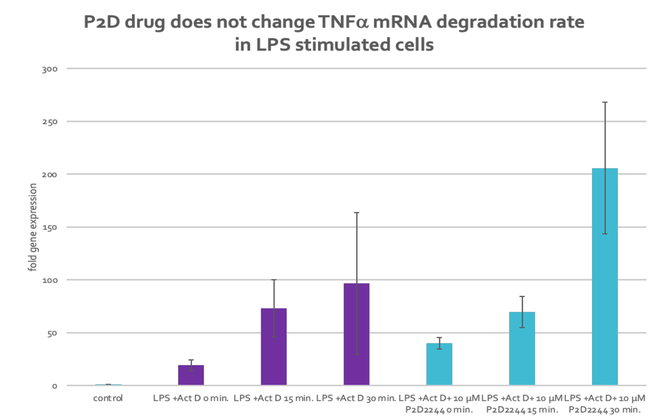
TNFα mRNA degradation





TNFα mRNA degradation with drug





Discussion

The RT-qPCR assay developed by us is meant to observe both the drug's ability to reduce the levels of TNFα mRNA as well as observe the degradation time of the TNFα mRNA. The results indicate that the drug is capable of reducing the TNFα mRNA levels. However, the drug did not seem to reduce the degradation time of the TNFα mRNA. These results suggest that the inhibitory action of the drug takes place before or during the transcription of the TNFα gene, thus affecting the levels of TNFα mRNA being made in the cell. The experiment recording the degradation of the TNFα mRNA was used to observe if the drug acted on this specific step in the NF-κB pathway. From these results it seems that the drug does not act on the stability of the TNFα mRNA, but its action is most likely upstream in the pathway because it still reduces the levels of TNFα mRNA overall. Further studies would look to reproduce and confirm the results shown here, as well as develop assays to study the specific steps upstream of the TNFα mRNA in the NF-κB pathway to see what step the drug acts on.

Acknowledgments

P2D Biosciences provided the drugs tested in these studies.