The Effect of Novel Drug, P2D340, on Inflammatory Pathways Involved in Alzheimer's Disease and Traumatic Brain Injuries

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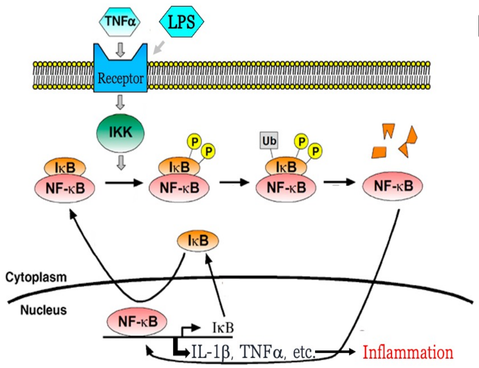
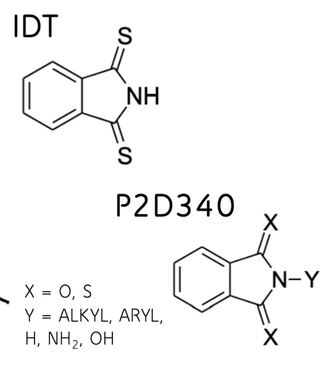
INTRODUCTION

Alzheimer's Disease (AD) and Traumatic Brain Injury (TBI) are global societal problems affecting millions of people and costing billions of dollars every year. There is currently no cure for AD, nor is there an effective treatment for chronic inflammation caused by TBI. In collaboration with biotech company, P2D Bioscience®, we are testing a series of drugs for their ability to target inflammation in a BV-2 microglial cell culture model with LPS-induced inflammation. To understand the cellular mechanism of these novel drugs, we used SDS-PAGE electrophoresis and Western Blot analysis to investigate protein levels involved in the activation of the NFκB signaling pathway, which modulates inflammation. We specifically measured the levels of the inhibitor of NFκB, IκBα, to determine whether the drug was blocking the phosphorylation and degradation of IκBα and subsequently blocking the activation NFκB.

NFκB PATHWAY



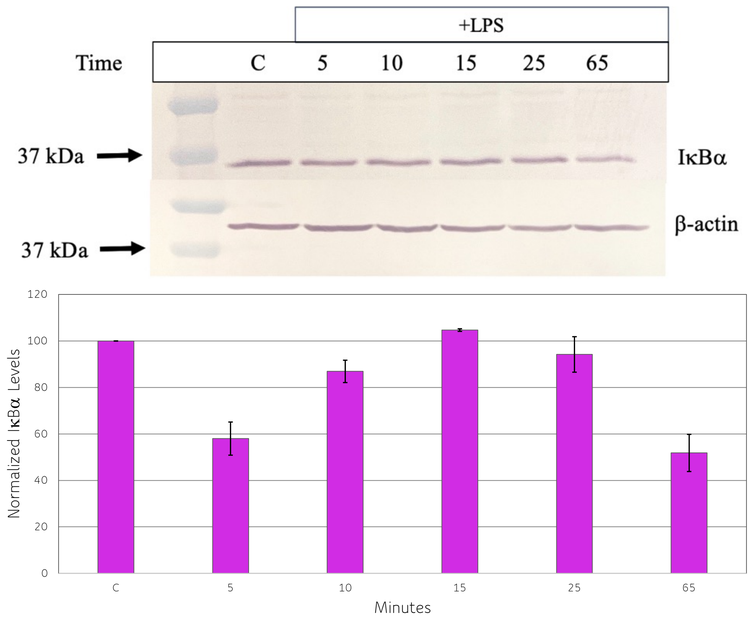
P2D340



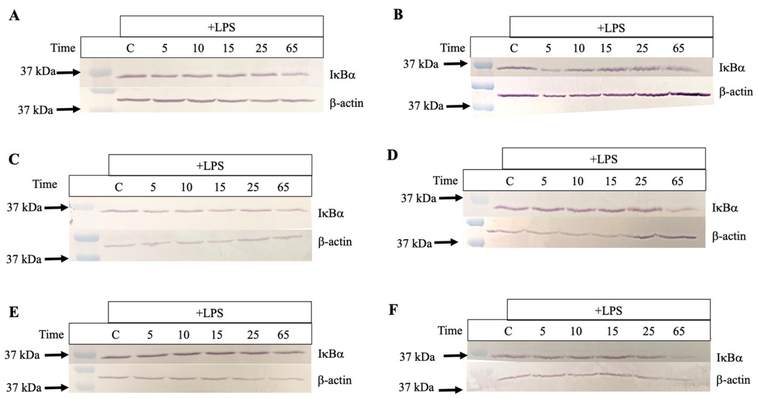
HYPOTHESIS

We investigated the levels of IκBα in cells treated with P2D340 and LPS, an inflammatory stimulus, to determine whether or not P2D340 was blocking the degradation of IκBα and thus, subsequently blocking the translocation of NFκB into the nucleus. If IκBα degradation is blocked, then NFκB cannot enter the nucleus and activate the expression of pro-inflammatory genes.

NORMAL BEHAVIOR OF IκBα POST-LPS STIMULATION



EFFECT OF P2D340 ON LPS-STIMULATED IκBα DEGRADATION



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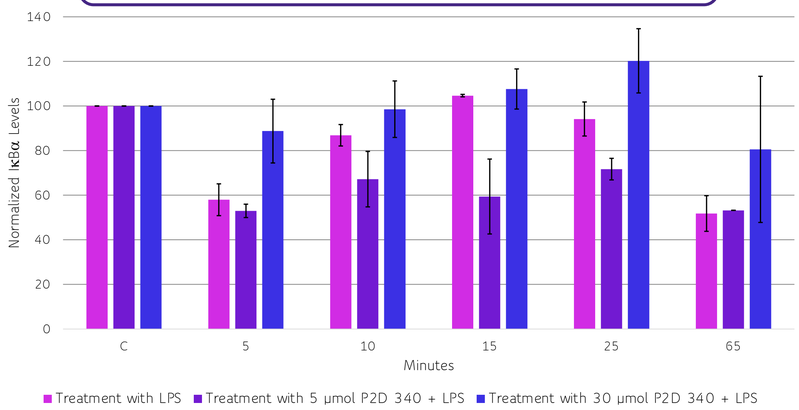

DISCUSSION

The elevated IκBα levels indicate that NFκB is not translocating into the nucleus and activating pro-inflammatory genes after treatment with P2D340 at a 30 μmol concentration. Since IκBα is the inhibitory protein of NFκB, the sustained presence of IκBα would indicate a continuous inhibition of NFκB. Multiple cellular events lead to the removal of IκBα from the cell, including phosphorylation by IKK, ubiquitination, and degradation by the proteasome. It is unclear which of these processes is being affected by P2D340. Future studies are required to understand further the role P2D340 plays in cell signaling upstream of IκBα degradation.

CONCLUSION

P2D340 is an anti-inflammatory drug that, at concentrations of 30 mmol, blocks the degradation of IkBa, thus maintaining the inhibition of NFkB, blocking its ability to translocate into the nucleus and activate pro-inflammatory genes.

ACKNOWLEDGMENTS

Thank you to P2D Bioscience® for the synthesis of P2D340