The Effects of Novel Anti-Inflammatory Drugs on LPS-Induced Cytokine Gene Expression in BV2 Cells

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ABSTRACT

Alzheimer's Disease (AD), the most common form of dementia, currently impacts almost seven million people in the United States over the age of 65. It is predicted that by 2060, over 13 million people in the United States will be affected by AD, which is why there is a growing demand for treatments. Amyloid β plaques and phosphorylated tau proteins are both associated with the progression of the AD pathology since they play a role in the disruption of neuronal integrity. These aggregated proteins, along with other molecules such as lipopolysaccharide (LPS), lead to increased inflammation by activating the NFκB pathway. The NFκB pathway regulates the production of pro-inflammatory cytokines, such as Interleukin-1 Beta (IL-1β) and tumor necrosis factor-alpha (TNFα); however, if it is overactive, it can lead to harmful inflammation. The company P2D Biosciences provides novel compounds designed to reduce inflammation, but the exact mode of action of these compounds is unknown. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) can be utilized to measure cytokine mRNA from BV2 cells that have been pretreated with the drugs and then with LPS. In this project we screened multiple compounds (PD340 and PD2244) provided by P2D Biosciences to evaluate their use as anti-inflammatory agents to treat AD. It was concluded that PD340 reduced IL-1β and TNFα gene expression while PD2244 reduced IL-1β gene expression. Further testing needs to be conducted to find the exact mechanism of the compounds.

BACKGROUND

The progression of neurodegeneration and impaired cognitive abilities is broadly referred to as dementia. Underneath the umbrella term of dementia, there are four distinct variations isolated by different pathologies: Alzheimer's, frontotemporal, Lewy body, and vascular. Alzheimer's prevails as the most common form of dementia, impacting almost over seven million people in the United States over the age of 65, and by 2060 that number is expected to be 14 million (Mayo Clinic). Early-onset, late-onset, and familial represent the three different classifications of Alzheimer's, with late-onset being the most common. Distinct from the other variations of dementia, the main hallmarks of Alzheimer's are the presence of neurofibrillary tau tangles and amyloid β plaques interspersed between healthy neurons. Under normal conditions, soluble amyloid β contributes to synaptic plasticity and neurogenesis, but the oligomerization of soluble fragments leads to the clustering of plaques (Sehar et al., 2022). Furthermore, tau proteins help regulate microtubule stability in healthy neurons, but hyperphosphorylation of tau can result in the disruption of microtubules.

To understand the development of amyloid β, exploring the roles of BACE1, APP, and RCAN1 are crucial. BACE1 (β-secretase enzyme/beta site amyloid precursor protein cleavage enzyme) cleaves APP (amyloid precursor protein) into amyloid β, and RCAN1 (regulator of calcineurin) is hypothesized to contribute to the phosphorylation and accumulation of tau proteins (Ermak et al., 2011). Under standard conditions, α-secretase cleaves APP, but an abundance of cytokines and ROS induces BACE1, which incorrectly cleaves APP and leads to plaque formation. In addition, studies have shown that both Ubiquitin Specific Peptidase 16 (USP16) and RCAN1 located on chromosome 21, heavily contribute to the early development of Alzheimer's in individuals with Down syndrome. USP16 and chronic RCAN1 expression contribute to AD by negatively impacting neural precursor cells and facilitating neuronal apoptosis (Fu and Wu, 2017). The trisomy of Chromosome 21 in individuals with Down syndrome has been identified as a prominent factor for inducing Alzheimer's because of the specific genes and their relationships with NFκB. Researchers have previously attempted to reduce BACE1 as a solution for the accumulation of amyloid β plaques, but a study conducted by Hampel et al. (2021) provided a deeper insight into the complexities of Alzheimer's pathology. The authors found that significantly decreasing BACE1 activity can cause sensorimotor impairments, seizures, schizophrenia-like phenotypes, and retinal pathology (Hampel et al., 2021). The findings from both studies work in conjunction with each other as they accentuate the complexities surrounding the development of treatments for Alzheimer's.

The NFκB pathway is a proinflammatory signaling pathway that regulates the production of cytokines such as Interleukin-1Beta (IL-1β) and tumor necrosis factor (TNF-α); however, disrupted regulation can lead to the overexpression of cytokines. While numerous genes and transcription factors influence Alzheimer's, NFκB has remained a consistent subject in this field of research because of its pro-inflammatory effects. Under normal conditions, cytokines assist with regulating inflammation, but overexpression or dysregulation of proinflammatory cytokines (IL-1β and TNF-α) contributes to an expedited timeline of AD symptoms. IL-1β is an example of a proinflammatory cytokine that contributes to Alzheimer's when overexpressed through microglial cell activation due to the production of neurotoxic substances and can eventually lead to neuronal death (Chen et al., 2024). In addition, TNF-α advances neuronal necroptosis when binding to the tumor necrosis factor receptor 1 (TNFR1) and activating the NFκB pathway. After describing the known functions of TNF-α, Chen et al. (2024) express that more research needs to be conducted to reveal how the TNF-α signal reaches the brain.

Regarding current treatments, acetylcholinesterase inhibitors and N-methyl-D-aspartic acid receptor antagonists have been found to temporarily relieve symptoms of AD. Both treatments have shown potential, but they are accompanied by effects ranging from dizziness to nausea (Wang et al., 2024). Alternative treatments with the extracts of medicinal plants have demonstrated the ability to reduce the degenerative processes associated with Alzheimer's (Alhazmi & Albratty, 2022). In a recent study, twelve botanical herbs were blended to make a Shenghui Yizhi Decoction (SHYZD) which originates from traditional Chinese medicine. It was concluded that SHYZD reduces protein β1-42, inflammatory factors, and NF-κB/NLRP3 signaling pathway proteins (Wang et al., 2024). Finally, the authors suggested that more research needs to be conducted with SHYZD since it impacted regulation of the NFκB pathway.

To activate the NFκB pathway, lipopolysaccharide (LPS), found in the walls of gram-negative bacteria, binds to leukocyte and microglial toll-like receptors (TLR4- CD14/TLR2) to induce the production of cytokines (Zhan et al., 2018). In accordance with a study conducted by Zhan et al. (2018), TLR4, MyD88, and NF-κB activity levels were elevated in BV2 cells that were treated for 24 hours with 10, 20, or 30 μg/ml LPS (Dai et al., 2015). BV2 cells are mouse microglial cells that are utilized as experimental models for AD research due to their receptor diversity and resemblance to human microglial cells. Beyond the scope of LPS which binds to toll-like receptor 4 (TLR4), IL-1β and TNF-α are common examples of cytokines that bind to interleukin-1 receptors (IL-1Rs) and tumor necrosis factor receptors (TNFRs) to stimulate the pathway. To assess which part of the pathway a drug or compound is interacting with in cell

cultures, a variety of assays can be utilized: RT-qPCR, ELISA, luciferase, western blot, and immunostaining.

As for current research, there remains a gap in knowledge surrounding the exact mechanism of Alzheimer's pathology, which has encouraged pharmaceutical companies to synthesize novel compounds. In collaboration with P2D Biosciences, we have tested their novel anti-inflammatory compounds (PD340 and PD2244) to identify where in the NFκB pathway the drugs are acting. As previously described, the NFκB pathway remains a prominent research target because of its dual ability to initiate controlled and irregular pro-inflammatory cytokine production. Furthermore, cytokines such as IL-1β possess qualities of interest because of their ability to bind to the IL-1β receptors and create a positive feedback loop, which induces cytokine production. Hypothetically, inhibition of IL-1β maturation could lead to a drastic reduction in the constant cycle of cytokine production; however, there needs to be a balance as cytokines are critical for a proper immune response.

To observe the sensitive activation and disruption of the NFκB pathway, one of the most efficient assays is the reverse transcription polymerase chain reaction (RT-qPCR) since the mRNA concentrations of cytokines in a sample can be measured. One notable event that showcased the value of this assay was the SARS-COV2 pandemic which resulted in COVID-19. The RT-qPCR assay served as a reliable reporter of SARS-CoV-2 infection due to the qualities of being fast, sensitive, and reproducible (Dutta et al., 2022).

My current research involves testing novel anti-inflammatory compounds from a company (P2D Biosciences) which has gathered data from animal trials to support the efficacy of their drugs in improving behavior and cognition. While the impacts of the drugs have been demonstrated by behavioral tests, the exact location of the cellular impacts are unknown. Measuring mRNA levels of pro-inflammatory cytokines through RT-qPCR has the potential to identify the specific point in the NFκB pathway these novel drugs are acting. Identifying the exact mechanisms of these drugs could potentially provide P2D the insight they need to chemically modify or adapt their drugs to be more effective. Based on the preliminary studies, we hypothesized that novel compounds (PD2244 and PD340) from P2D Biosciences reduce LPS-induced cytokine gene expression in BV2 through NFκB regulation.

METHODS

Cell Culture

BV2 mouse microglial cells and HEK293 human embryonic kidney cells were used in this study. They were grown in DMEM (Dulbecco's Modified Eagle Medium) along with 10% FBS (fetal bovine serum), 1% L-Glutamine, 1% amino acids, and 1% Penicillin-Streptomycin to ensure cell viability. Cells were grown in 37℃ in an atmosphere of 5% CO_2 and 95% air.

Plating and Treating

Cells were plated at a density of 50,000 cells per well in a twelve-well plate, and then the plate was transferred to the incubator for twenty-four hours. After twenty-four hours, the cells were treated with the indicated concentrations of drug. After one hour, the cells were treated with either 10 μg/mL LPS (BV2) or 10 ng/mL TNF-α (HEK293) to induce NFκB activation. The cells were incubated for a further 24 hours before RNA extraction.

RNA Extraction

The medium was aspirated, and cells were washed three times with 1x PBS. The cells were then scraped off the bottom of the well with a rubber scraper and centrifuged at 10,000 rpm for 4 minutes. All remaining PBS was removed, and the pellet was stored in the -80℃ freezer until the next step. RNA was extracted using the Macherey-Nagel Nucleospin RNA Extraction Kit following the manufacturer's instructions.

Reverse Transcription (RNA → cDNA)

A nano-drop spectrophotometer was used to measure the RNA concentration of each sample to ensure proportional cDNA synthesis and elimination of variability between samples. After the samples were diluted, QuantaBio cDNA Super Mix was added to each sample, and then the thermal cycler converted the RNA to cDNA (Table 1).

RT-qPCR

RT-qPCR was performed on identical sample volumes from the cDNA reaction (1.33 μL) using primers specific to the genes of interest. Levels of β-Actin mRNA, a house keeping gene, were measured as a control for loading. The RT-qPCR provided a set of data for each sample which displays the cycle threshold (CT).

To evaluate the efficacy of the novel compounds, the CTs of the cytokine groups were directly compared to the CTs of the β-Actin. For each specific test/sample group, the CTs were compared to provide the ΔCT, which is then set against the ΔCT of the control groups. This produces a ΔΔCT which provides the fold change in gene expression of the sample when plugged into this equation: 2^-ΔΔCT.

Stage	**Conditions – number of cycles**
Stage 1	25℃ for 5 minutes
Stage 2	42℃ for 30 minutes
Stage 3	85℃ for 5 minutes
Stage 4	4℃ indefinitely

Table 1

cDNA Stages (BIO RAD T100 Thermal Cycler)

Genes	**Sequences**
β-Actin	Forward: GTTTCCGAAGTGGACATCGCA Reverse: CTGCACAGGTTGTTCTCAGC
IL1-β (BV2)	Forward: TGGACCTTCCAGGATGAGGACA Reverse: GTTCATCTCGGAGCCTGTAGTG
TNFα (BV2)	Forward: GGTGCCTATGTCTCAGCCTCTT Reverse: GCCATAGAACTGATGAGAGGGAG
IL1-β (HEK293)	Forward: CTCTTCTGCCTGCTGCACTTTG Reverse: ATGGGCTACAGGCTTGTCACTC
TNFα (HEK293)	Forward: CCACAGACCTTCCAGGAGAATG Reverse: GTGCAGTTCAGTGATCGTACAGG

Table 2

Primer Sequences (BV2 and HEK293)

Stage	**Conditions**
Stage 1 - Activation	50℃ for 2 minutes
Stage 2 - Pre-soak	95℃ for 10 minutes
Stage 3 - Denaturation & Annealing	95℃ for 15 seconds, 60℃ for 1 minute
Stage 4 - Melting Curve	95℃ for 15 seconds, 60℃ for 15 seconds, 95℃ for 15 seconds

Table 3

RT-qPCR Stages (CFX OPUS 96 RT-qPCR Machine), (40 cycles)

RESULTS

NFκB impacts the innate immune response, inflammation, and apoptosis through the regulation of chemokines and cytokines. Determining ways in which to pharmacologically regulate the activity of the NFκB pathway remains difficult due to the presence of feedback loops. The negative feedback loop arises because of newly produced IκB shuttling NFκB from the nucleus back to the cytosol (Mitchell et. al, 2016). In contrast, IL-1β production elicits a positive feedback response when it binds to the IL-1 Receptor and induces the movement of NFκB into the nucleus. As a result, the NFκB pathway must be closely regulated to prevent uncontrolled expression of proinflammatory cytokines.

BV2 and HEK293 are two cell lines which serve as useful models to elucidate the mechanism of action of the drugs being tested in this study because of their different receptors. BV2 cells are murine microglial cells which mirror the properties of human microglia and have TLR4, TNFR, and IL-1R. Generally, microglial cells serve as resident immunocompetent cells of the CNS (Li et. al, 2023); however, they are unique because they possess the potential for both neuroprotective and neurotoxic impacts (Dai et. al, 2015). To produce a cytokine-driven response in BV2 cells, inflammatory stimuli such as LPS or cytokines bind to TLR4 or IL-1R to promote the release of NFκB by degradation of IκB (Figure 1). For this project, LPS was utilized to stimulate the pathway in BV2 cells because of its larger relevance to Alzheimer's pathology. Studies have found that blood LPS levels in AD patients are threefold the levels greater than that of the control cells (Zhan et. al, 2018). The NFκB is also activated in HEK293 cells by the TNFR to the initiate the movement of NFκB into the nucleus. The rationale for using two different cell types lies within receptor diversity.

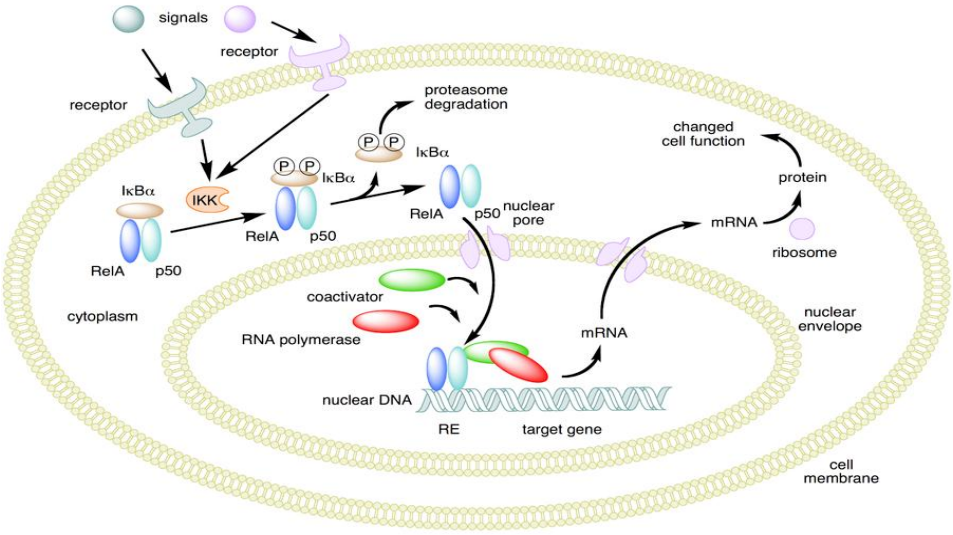


Figure 1

NFκB Pathway

Effectively measuring a decrease or increase in cytokine mRNA requires sensitive and meticulous testing, which the RT-qPCR provides. PD340 was designed as an analogue of isoindolinedione because of its known ability to inhibit acetylcholinesterase (AChE). Inhibiting AChE results in sufficient levels of acetylcholine to maintain synaptic plasticity (Figure 2).

Lower concentrations of PD2244 were used when treating BV2 cells because this compound expressed higher levels of cytotoxicity. The PD2244 structure (Figure 7) was designed as a benztropine analogue even though benztropine models are more commonly associated with Parkinson's Disease treatments rather than Alzheimer's.

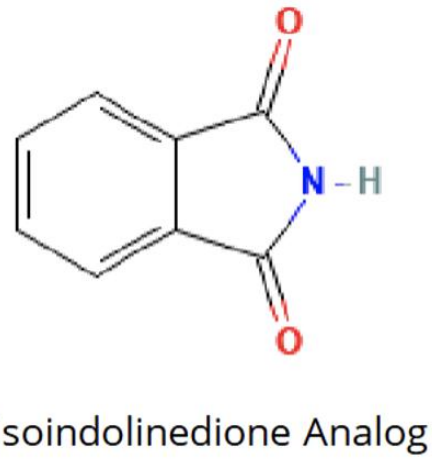


Figure 2

PD340 Backbone

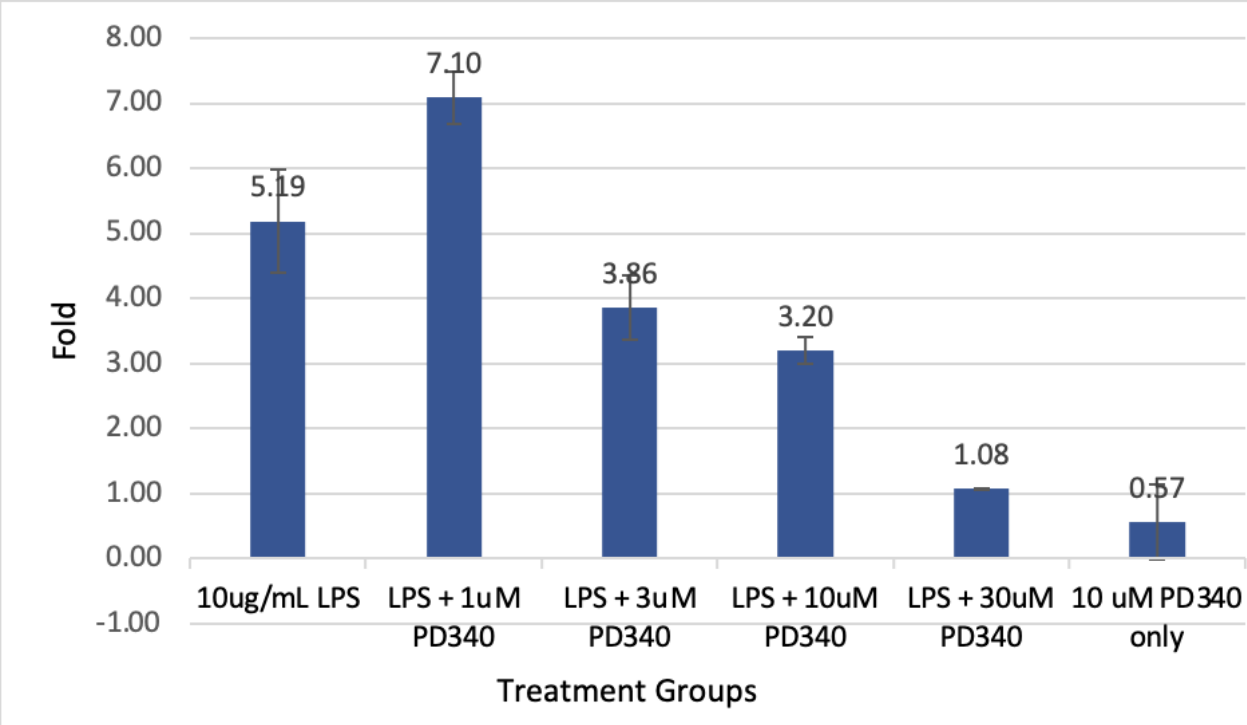


Figure 3

TNF-α Gene Expression in BV2 Cells Pretreated with PD340 Followed by LPS (10 µg/mL)

First, we measured the effects of PD340 on TNF-α gene expression in LPS-induced BV2 cells. Treatment with 1μM PD340 + LPS resulted in a 27% increase in TNF-α gene expression when compared to LPS alone followed by decreases in gene expression by 25.7% at 3μM PD340 + LPS, 38.4% at 10μM PD340+ LPS, 79.2% at 30 μM PD340 + LPS, and 95.3% at 10 μM PD340 alone.

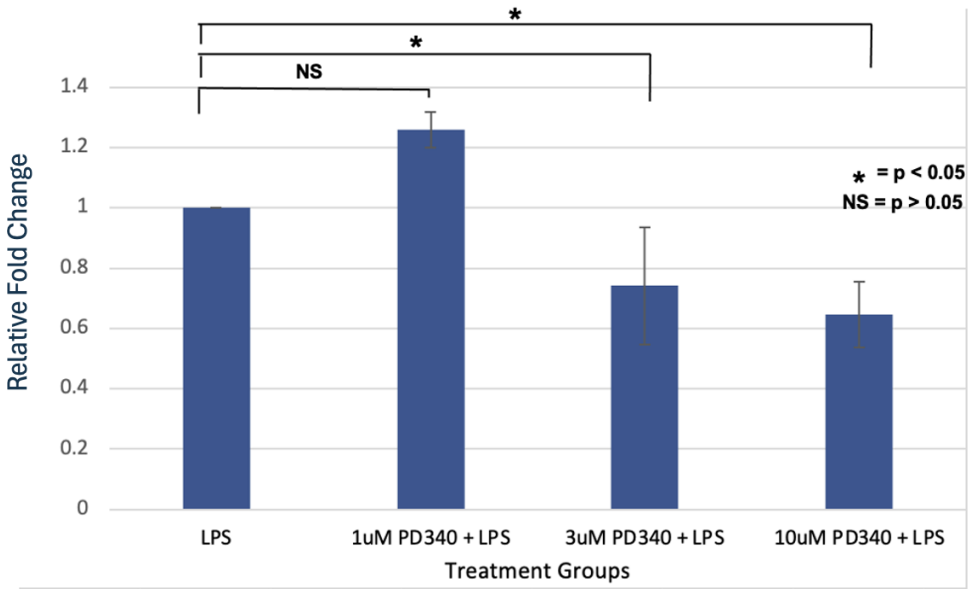


Figure 4:

Aggregate TNF-α Gene Expression Levels Relative to LPS in BV2 Cells Pretreated with PD340 Followed by LPS (10 μg/mL)

We aggregated the data from three PD340 experiments that measured TNF-α gene expression in LPS-induced BV2 cells to see if we could get consistent results and record statistical significance. Treatment with 1μM PD340 + LPS increased TNF-α gene expression by 18% compared to LPS alone. In contrast, treatment with 3μM PD340 + LPS resulted in a 19% decrease in TNF-α gene expression and at 10 μM PD340 + LPS there was a 35% decrease. There was statistical significance at the higher concentrations.

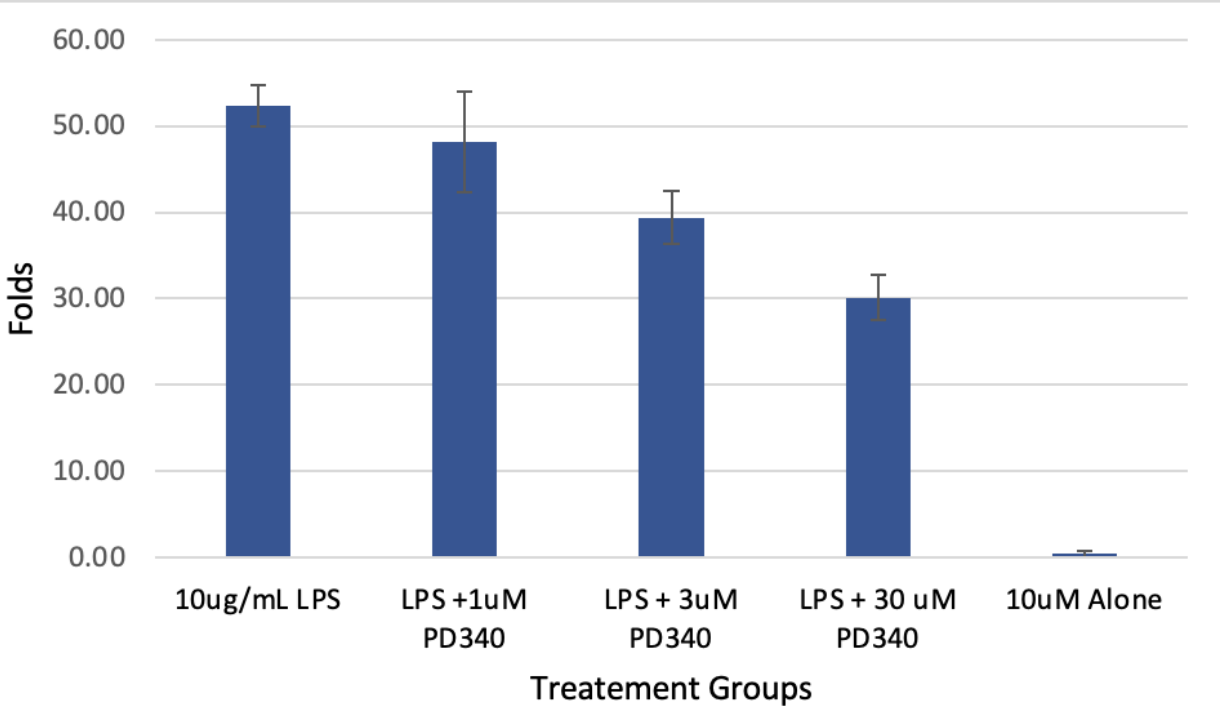


Figure 5

IL-1β Gene Expression in BV2 Cells Pretreated with PD340 Followed by LPS (10 μg/mL)

We measured the effects of PD340 on IL-1β gene expression in LPS-induced BV2 cells. When compared to LPS alone, treatment with 1μM PD340 + LPS resulted in an 8% reduction in gene expression followed by a 24.7% (3 μM PD340 + LPS), 42.5% (30 μM PD340 + LPS), and then a 99.2% (10 μM PD340) decrease in IL-1β gene expression.

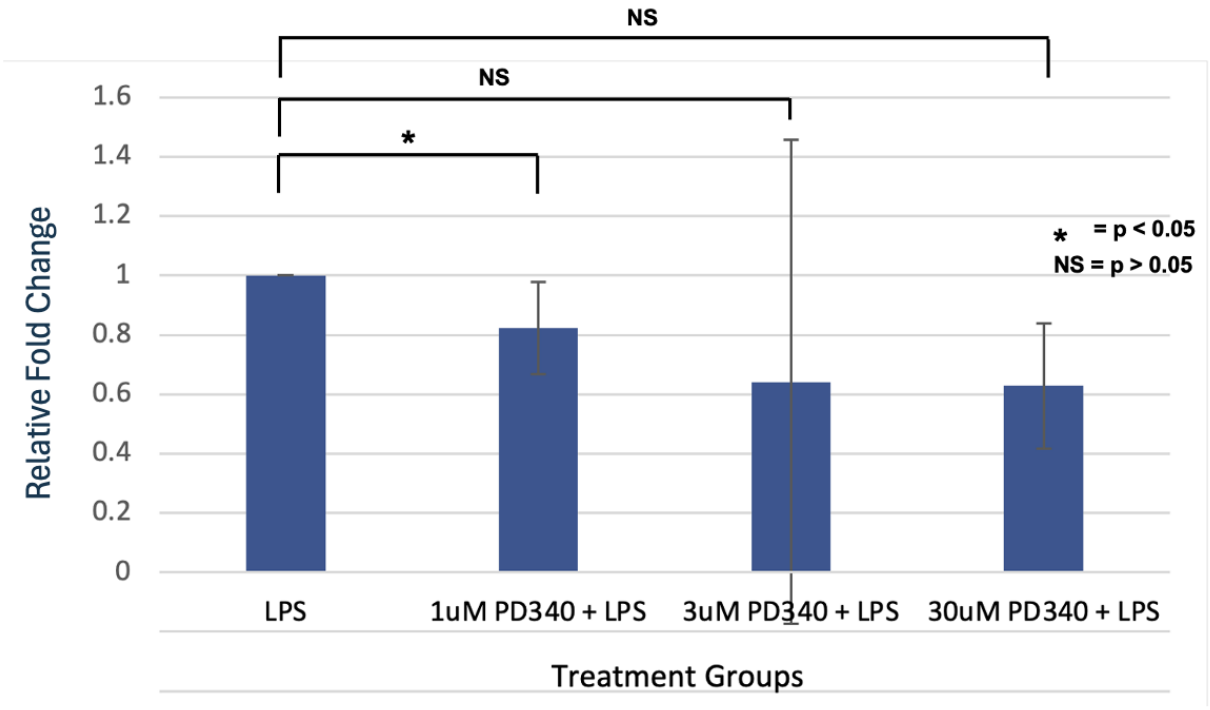
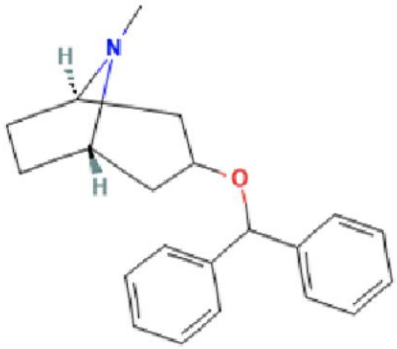


Figure 6

Aggregate IL-1β Gene Expression Levels Relative to LPS in BV2 Cells Pretreated with PD340 Followed by LPS (10 µg/mL)

We aggregated data from three PD340 experiments that measured IL-1β gene expression in LPS-induced BV2 cells to see if we could get consistent results and record statistical significance. Treatment at 1.0 µM PD340 + LPS resulted in an 18% decrease followed by a 36% (3 µM PD340 + LPS) and then a 37% (30 µM PD340 + LPS) reduction in IL-1β gene expression compared to LPS alone. There was no statistical significance associated with the highest concentrations.



Benztropine Analog

Figure 7

PD2244 Backbone

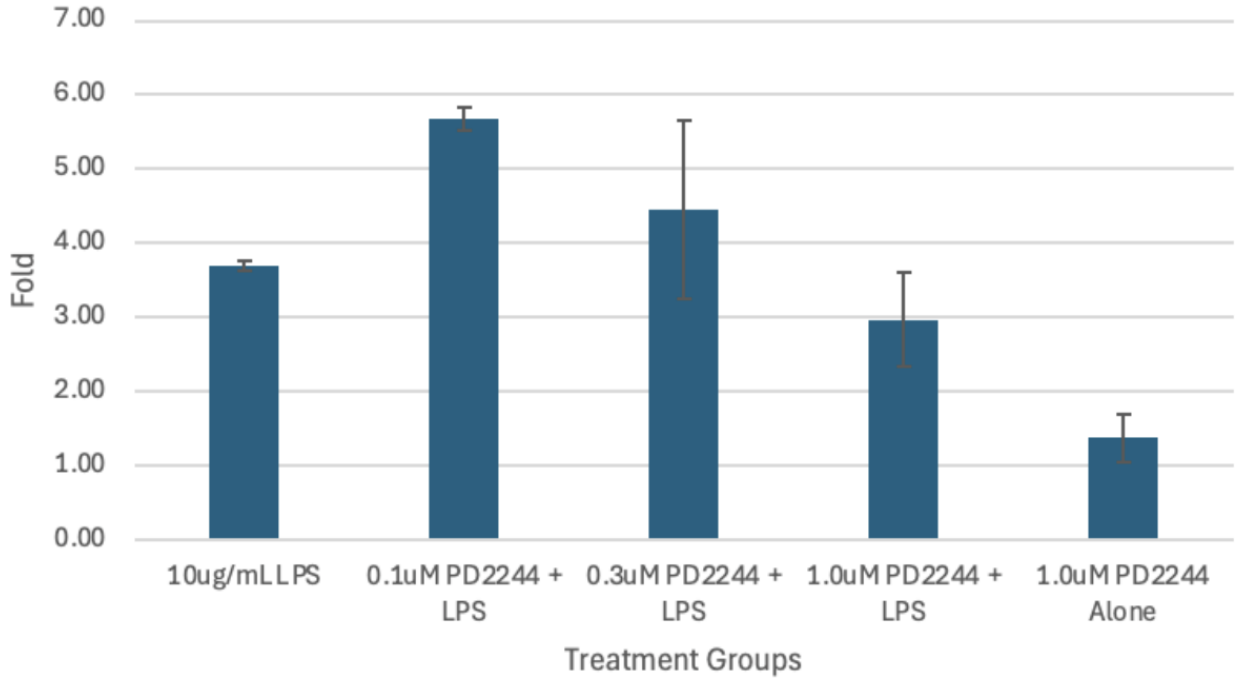


Figure 8:

TNF-α Gene Expression in BV2 Cells Pretreated with PD2244 Followed by LPS (10 µg/mL)

The next novel compound we tested was PD2244 (benztropine analogue). We measured the effects of PD2244 on TNF-α gene expression in LPS-Induced BV2 cells. Treatment with 0.1 µM PD2244 + LPS resulted in a 35% increase in TNF-α gene expression, while at 0.3 µM PD2244+ LPS there was a 17.1 % increase compared to LPS alone. At 1.0 µM PD2244 + LPS, there was a 21% decrease in TNF-α gene expression, and the drug had a minimal effect on inducing and inflammatory response.

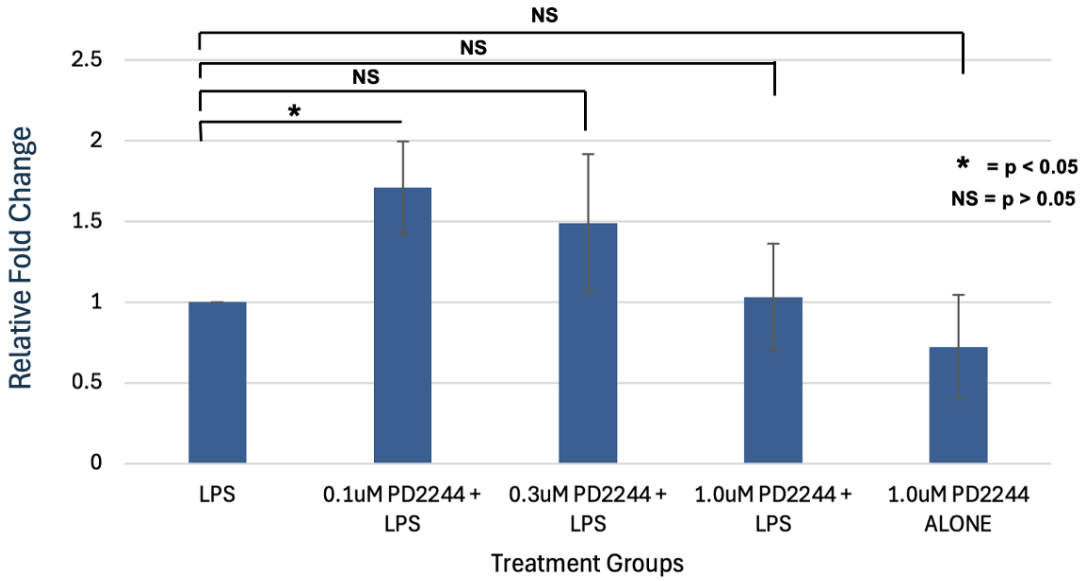


Figure 9

Aggregate TNF-α Gene Expression Levels Relative to LPS in BV2 Cells Pretreated with PD2244 Followed by LPS (10 µg/mL)

We aggregated data from three PD2244 experiments that measured TNF-α gene expression in LPS-induced BV2 cells to see if we could get consistent results and record statistical significance. In order of the treatment groups, there was a 70% (0.1 µM PD2244 + LPS), 50% (0.3 µM PD2244 + LPS), and 0% (1.0 µM PD2244 + LPS) increase in gene expression compared to LPS alone. The 1.0 µM PD2244 alone samples expressed a 25% reduction in gene expression. Overall, there was no statistical significance associated with increasing concentrations of the drug.

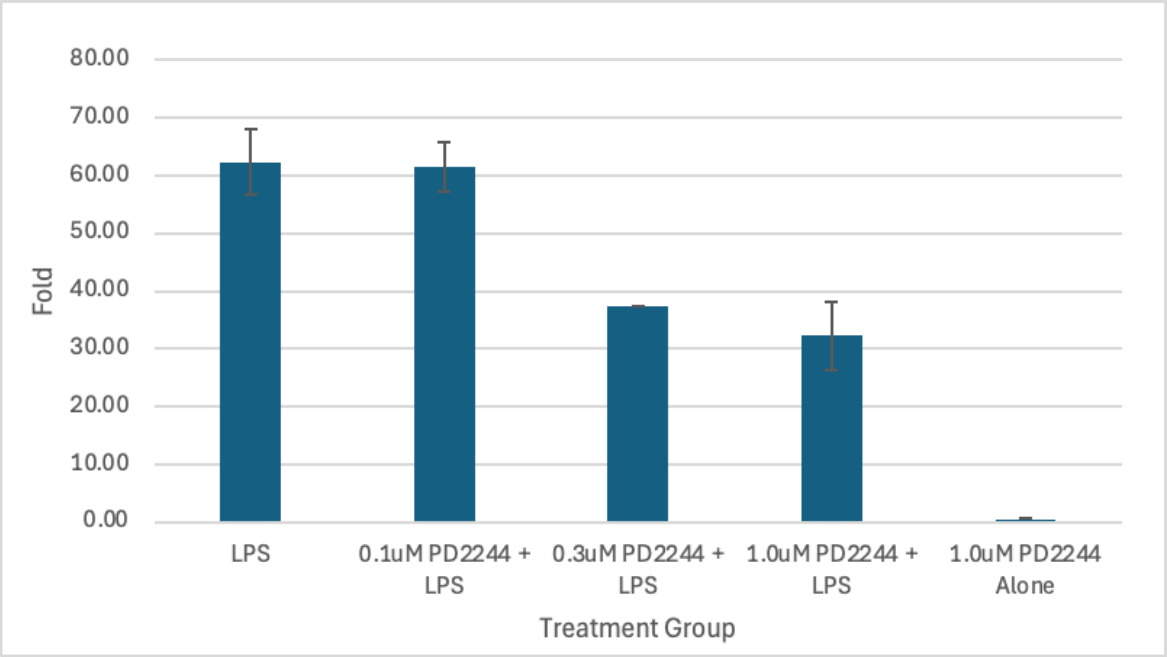


Figure 10

IL-1β Gene Expression in BV2 Cells Pretreated with PD2244 Followed by LPS (10 µg/mL)

We measured the effects of PD2244 on IL-1β gene expression in LPS-induced BV2 cells. Treatment with 0.1µM PD2244 + LPS decreased IL-1β gene expression by 1.5%, followed by a 40% reduction when treated with 0.3 µM PD2244 + LPS when compared to LPS alone. At 1.0 µM PD2244 + LPS there was a 48% reduction in gene expression. The drug alone had no effect on gene expression.

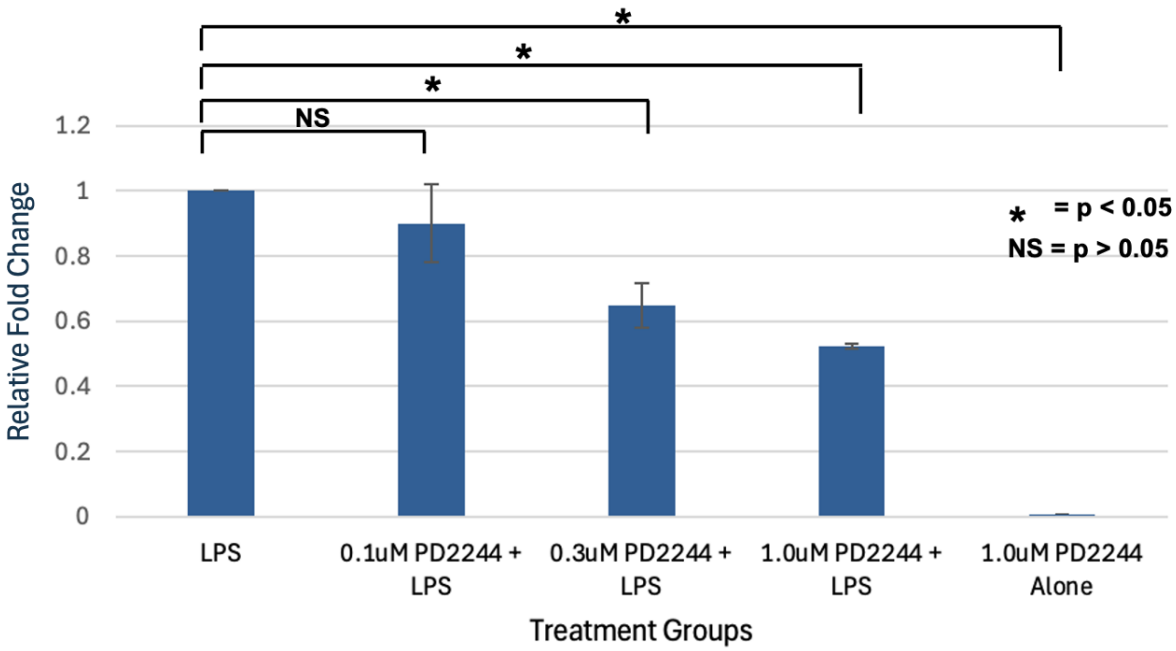


Figure 11

Aggregate IL-1β Gene Expression Levels Relative to LPS in BV2 Cells Pretreated with PD2244 Followed by LPS (10 µg/mL)

We aggregated the data from three PD2244 experiments that measured IL-1β gene expression in LPS-induced BV2 cells to see if we could get consistent results and record statistical significance. In order of the treatment groups, there was a 10% (0.1 µM PD2244 + LPS), 35% (0.3 µM PD2244 + LPS), and then 48% (1.0 µM PD2244 + LPS) decrease in IL-1β gene expression compared to LPS alone. The samples that were only treated with 1.0 µM PD2244 did not display an effect on gene expression. Overall, there was statistical significance associated with increasing concentrations of PD2244.

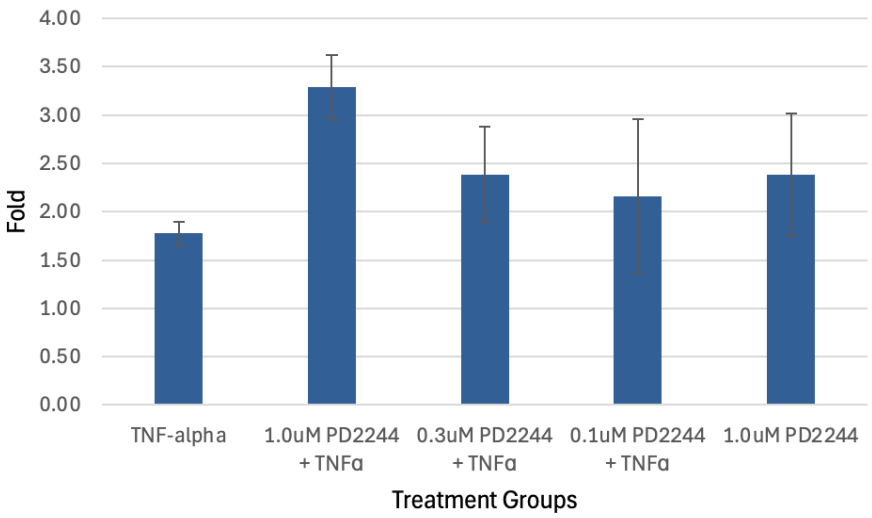


Figure 12

IL-1β Gene Expression in HEK293 Cells Pretreated with PD2244 Followed by TNFα (20 ng/mL)

Finally, we measured the effects of PD2244 on IL-1β Gene Expression in TNFα-induced HEK293 cells. Since we did not measure an effect of PD2244 on TNFα gene expression in BV2 cells, we decided to test HEK293 since this would provide insight into whether PD2244 is receptor specific. We chose to use HEK293 in addition to BV2 cells since they only express the TNFR and not the TLR4 or IL-1R that BV2 cells have. The TNFα treatment group did not reach our threshold of a 2-fold increase in gene expression. Treatment with drug alone increased gene expression compared to TNFα alone. These results are representative of a single experiment and need to be replicated because the control (TNFα) did not sufficiently activate the pathway.

DISCUSSION

Effect of PD340 on LPS-Induced Cytokine Gene Expression in BV2 Cells

After measuring TNF-α and IL-1β mRNA in LPS-induced BV2 microglial cells, we concluded that PD340 decreased cytokine gene expression in a concentration-dependent manner (Figure 13). Although there was a not a statistically significant decrease in IL-1β gene expression, more research needs to be done to determine whether PD340 is interfering with the NFκB pathway at this point in the pathway. Regarding TNF-α gene expression, there was a statistically significant decrease in LPS-induced gene expression at 3 μM PD340 and 10 μM PD340. As previously mentioned, PD340 impacted cytokine gene expression; however, the differences were not statistically significant. These results are still beneficial for P2D Biosciences and Dr. Akkaraju's lab since they can guide further testing to look at the preceding and following steps in the pathway in which these drugs could be working. This opens opportunities to conduct other assays to determine if PD340 is impacting IL-1β maturation and other cytokines through later steps in the NFκB pathway.

Effect of PD2244 on LPS-Induced Cytokine Gene Expression in BV2 Cells

Overall, PD2244 did not impact IL-1β and TNF-α cytokine gene expression in LPS-induced BV2 cells in the same manner. There was a measurable reduction in IL-1β gene expression in the samples treated with PD2244 when compared to LPS alone. In juxtaposition to the IL-1β data, there was an increase in TNF-α gene expression that was not statistically significant, and conclusions could be drawn. Furthermore, there was a significant decrease of gene expression in the 1.0 μM PD2244 group alone for IL-1β which was not measured for TNF-α. These results re-emphasize and illuminate the specificity of PD2244 for reducing IL-1β gene expression. The differences between the two cytokine results suggest that the drug is potentially more effective at inhibiting IL-1β expression rather than TNF-α (Figure 13). This trend, although not seen in most studies, has been recorded by other researchers when testing novel drugs (Lee et al., 2020). Further studies can look at steps before and after RNA production to further pinpoint the mode of action of this compound.

PD340 and PD2244 Comparison

In comparison to PD340, the PD2244 results for IL-1β and TNF-α mRNA were more consistent and reproducible, which was reflected in the levels of statistical significance. This could be due to a conformational advantage since the two drugs differ physically (isoindoline

dione -340 vs benztropine-2244). The most effective concentration measured was 1.0uM of PD2244, deeming it the most impactful concentration in reducing cytokine mRNA. The conclusions from the data prompted the testing of PD2244 in HEK293 cells since they activate the NFκB pathway through the TNFR rather than TLR4 or IL-1R.

Effect of PD2244 on LPS-Induced Cytokine Gene Expression Pre-Treated HEK293 Cells

After measuring IL-1β gene expression, it was evident that the TNF-α control group did not sufficiently stimulate the HEK293 cells to produce measurable amounts of cytokine mRNA. Although the drug groups (including the drug alone group) showed an increase in gene expression, it cannot be concluded that PD2244 induced inflammation since the control was not significant. Further experiments will be conducted to determine if there is an ideal treatment time with TNF-α to induce gene expression.

	PD340	PD2244
IL-1β	↓	↓
TNFα	↓	↑

Figure 13

Effect of PD340 and PD2244 on Cytokine Gene Expression in LPS-Induced BV2 Cells

FUTURE DIRECTIONS

Regarding future directions for PD340 and PD2244, more testing needs to be conducted to identify the exact mechanism of how the compounds are reducing cytokine gene expression. HT22 cells (mouse hippocampal neuronal cells) pre-treated with PD2244 and then subsequently treated with amyloid beta (20 μg/mL) would simulate more realistic conditions of Alzheimer's and provide significant insight. It would also be beneficial to look at the effects of expressing TLR4 in HEK293 cells to determine whether PD2244 has a stronger effect on TLR4 or TNFR. Beyond the scope of RT-qPCR, a Western Blot can be used to identify the phosphorylation state of IκB, the activation state of pro-IL-1β, or phosphorylated tau levels. Furthermore, since the current body of literature has divided opinions on how long cells should be treated with a stimulus before collection, more experiments should be done to compare the effects of different treatment periods with LPS and TNF-α on RT-qPCR results. If results among the previously mentioned cell lines are consistent, it would be valuable to test the drug with THP-1 cells since they are human monocytes that respond to amyloid β as well. In summary, PD340 reduced TNF-α in a statistically significant manner, and PD2244 reduced IL-1β in a statistically significant manner. Therefore, these novel compounds are promising candidates for future testing and potential treatments for patients with Alzheimer's.

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