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Role of dopamine in regulating microglia inflammatory responses through TLR4-NFκb pathway

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Abstract. Parkinson's disease (PD) is a prevalent neurodegenerative disorder that affects a significant portion of the population. One of its distinguishing features is the gradual loss of dopaminergic cells in a specific region of the brain known as the substantia nigra. In recent years, researchers have uncovered that neuroinflammation facilitates the developmental process of PD. Specifically, studies have shown that the activation of microglia, the brain's immune cells, is closely linked to the levels of dopamine secreted by neurons. However, the influence of dopamine on activated microglia in PD has not been fully explored. In this study, we aimed to explore the impact of dopamine on activated microglia. To establish an activated microglia model, we used BV-2 cell lines and treated them with lipopolysaccharide (LPS) at a concentration of 200 ng/ml. Two separate groups were then exposed to dopamine at concentrations of 2 μM and 10 μM, respectively, to simulate dopamine treatment in the brain. To assess the effects of dopamine, we performed real-time PCR to measure the relative mRNA levels of pro- and anti-inflammatory cytokines, conducted immunofluorescent staining to observe and analyze the cell morphology, carried out a phagocytosis assay to assess the cell's phagocytic ability, and conducted western blotting to identify the specific pathway through which dopamine affects microglia activation. Our findings revealed that dopamine can modulate the activation state of microglia and reduce the cell's inflammatory responses via the TLR4-NFκB pathway. This suggests that dopamine has the potential to alleviate neuroinflammation in PD, opening up new avenues for future treatments and therapies.

Keywords: Parkinson's Disease, Neuroinflammation, Microglia, Dopamine

1. Introduction

1.1. Functions of Microglia in the Central Nervous System During Homeostasis

Microglia, the equivalent of the macrophages in the central nervous system (CNS) [1], play a crucial role as the most prevalent mononuclear phagocytes, making up about 10% of all cells in CNS [2]. Originating from the embryonic yolk sac, microglia migrate into the growing neural tube where they multiply and populate the parenchyma [2]. As they mature, they become integrated among the neurons, effectively becoming brain macrophages [3]. This developmental process establishes microglia as specialized immune cells within the CNS.

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Microglia engage in dynamic interactions with neurons and possess a wide array of important functions that contribute to the overall homeostasis of the brain. [3]. By adjusting the intensity of synaptic transmissions and shaping neuronal synapses, microglia contribute to the formation of brain circuits [2] and engage in the process of neuronal pruning development [3]. Furthermore, microglia, which are ready to trigger programmed cell death and remove the resulting cellular waste, can initiate cell death [3]. Therefore, microglia are essential for the maintenance of brain homeostasis as they are involved in various processes such as neural growth, synapse adjustments, and cell death.

The functions of microglia are highly dependent on their phenotype and activation state. One of the main roles of microglia is phagocytosis, which involves the removal of pathogens, dead cells, protein aggregates, and antigens that can potentially damage the CNS [2]. This process is particularly important following CNS injury. When the homeostasis of the CNS is compromised, microglia can quickly transition from a quiescent or "resting" state to an alert and "activated" state [1]. Recent studies have identified two distinct forms of microglial activation in the central nervous system: the M1 phenotype and the M2 phenotype. The activation of microglia into these phenotypes can result in either cytotoxic or neuroprotective effects, depending on the specific triggers [4]. M1 microglia are associated with "classical activation" and are involved in the production of pro-inflammatory cytokines, which contribute to neuroinflammation [4]. This activation state can further damage the

brain due to the inflammatory response it induces. "M2 microglia" are more often associated with anti-inflammatory responses. These activated microglia possess genes that support tissue healing, extracellular matrix regeneration, and anti-inflammatory activity [4]. Therefore, the activation of M1 microglia leads to neuroinflammation, potentially exacerbating brain damage, whereas M2 activation is associated with phagocytosis, facilitating the clearance of waste products in the brain.

1.2. Microglia Function and Neuroinflammation During Parkinson's Disease

PD is a progressive neurodegenerative disorder that poses a significant global health challenge. It is characterized by impaired motor function and is considered the most common and debilitating movement disorder worldwide. It primarily affects individuals over the age of 60, with an estimated prevalence of 1% among this population [5]. In 2016 alone, approximately 6.1 million people worldwide were affected by Parkinson's disease [6].

Among PD's symptoms and features, three defining characteristics are bradykinesia, stiffness, and rest tremor [5]. PD is associated with the loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies, which are responsible for the characteristic motor symptoms [7]. The primary approach in treating PD is to increase dopamine levels in the nigrostriatal pathway, as patients with PD typically exhibit reduced dopamine levels in the striatum [8]. Therefore, traditional pharmaceutical therapy focuses on dopamine replacement [9]. However, current treatments aim to prolong the patient's functional ability rather than halt the progression of the neurodegenerative process [10]. Consequently, there is an urgent need for the exploration of new treatments for PD.

Central neuroinflammation plays a crucial role in the pathogenesis of PD. The term encompasses inflammatory processes that take place within the neural tissues of the CNS and are mediated by the activation of microglia and astrocytes, leading to the production of various molecules such as chemokines, reactive oxygen species, and secondary messengers [11].

Inflammation precedes the neurodegeneration observed in PD, as the loss of dopamine neurons can be triggered by inflammatory reactions from non-neuronal cells alone [11]. Dysregulation of microglia, the resident immune cells in the central nervous system, has been implicated in PD [12]. Neuroinflammation plays a crucial role in the development and progression of PD. Over the years, researchers have extensively studied various cellular and animal models to gain a deeper understanding of the underlying mechanisms. Their efforts have led to the discovery of neuroinflammation-based biomarkers that can aid in the diagnosis of PD [12]. One significant finding is that the inhibition of specific metabolic pathways in M1 microglia or the transformation of M1 cells into the M2 phenotype can effectively reduce neuroinflammation and protect dopaminergic neurons from degeneration [13]. These studies provide strong evidence for the interplay between microglia, neuroinflammation, and PD.

1.3. Scientific questions about dopamine and microglia in our project

As discussed above, patients with PD experience a depletion of dopaminergic neurons in the substantia nigra, leading to motor abnormalities [7]. This suggests that there is a deficiency of dopamine in the brains of PD patients. Notably, dopamine is closely related to glial and immunological cells, which possess the capability to synthesize, deactivate, and transport dopamine [11]. All of the components necessary for the production, metabolization, and storage of dopamine are expressed by microglia, along with functioning dopamine receptors (DRs) that regulate immune cell activity [11]. Since the catechol-O-methyltransferase (COMT) metabolic pathways regulate dopamine, and microglia are the main source of COMT [11], microglia can induce dopamine inactivation.

All five DRs cause an immune response in human microglia [11]. Dopamine receptors are discovered to be abnormally expressed in all inflammatory cell types in post-mortem brains and PD patients [11]. Importantly, it has been hypothesized that particular DR subtypes can affect the activation of microglia at either the M1 or M2 stage. This, in turn, determines whether a pro-inflammatory or an anti-inflammatory response occurs. [11]. The effects of dopamine on microglia and the inflammatory response have become an area of considerable interest in current research. Recent studies have shown that dopamine receptors play a crucial role in the activation and phenotype change of microglia, leading to changes in dopamine signal pathways [14]. However, the exact mechanisms through which these pathways are altered remain unclear [15, 16]. Moreover, the impact of dopamine on microglia activation, cytokine release, and cell function following microglia activation induced by LPS is debatable. Additionally, the role of dopamine in microglial phagocytosis remains poorly understood, with no definitive conclusions available regarding the pathways that mediate dopamine's effects on microglia function.

We aimed to identify the relationship between Dopamine levels and the activation of BV-2 microglial cells. To attain this goal, we conducted an experiment to activate the BV-2 microglial cell line with LPS and treat the M1-activated microglial cells with different concentrations of dopamine. The findings of this study have unveiled a potential therapeutic target for the clinical management of neuroinflammation. Given the significance of neuroinflammation and microglia in the development and treatment of PD, our results have shed light on a promising avenue for the alleviation of PD symptoms and mitigation of the adverse effects of neuroinflammation.

2. Method

2.1. Cell culture

BV2 cell line generated from raf/myc-immortalised murine neonatal microglia was chosen for this study, as it was the most often alternative for primary microglia and widely used in pharmaceutical research, phagocytosis investigations, research on neurodegeneration, etc. [17]. The BV2 cell line used in this study was obtained from Shanghai Anwei Biotechnology Co., Ltd. (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 100U/ml penicillin, 100 mg/ml streptomycin, and 100 mg/ml amphotericin in a 37°C, 5% CO2/95% air incubator. The cell medium was replaced every two days to ensure optimal cell growth and viability.

2.2. Dopamine treatment and cell viability assay

BV2 cells were seeded in 96-well plates with a seeding density of 1 × 104 cells per well. The plates were then incubated at 37 °C in an atmosphere with 5% CO2. Both LPS and dopamine were dissolved in sterile water. To create an M1-activated model, the cells were treated with 200 ng/ml of lipopolysaccharide (LPS), which was dissolved in sterile water. The experimental groups consisted of a control group, a group treated with LPS, a group treated with a medium level of dopamine (2 μM) in addition to LPS, and a group treated with a high level of dopamine (10 μM) in addition to LPS. LPS and dopamine were also dissolved in sterile water before being added to the cell cultures. After the treatment with dopamine, the cell viability of all groups was evaluated after 48 hours using the Cell Counting Kit-

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8 (CCK-8) assay (Dojindo, Japan). The optical density (OD) at a wavelength of 450nm following a 30-minute incubation period was detected.

2.3. Immunofluorescent staining

The poly-L-lysine coated coverslips were incubated at room temperature for 1 hour to ensure proper adherence. The coverslips were then rinsed thoroughly three times with sterile H2O, with each rinse lasting for 1 hour. After rinsing, the coverslips were left to air dry completely before undergoing sterilization under UV light for 4 hours. BV2 cells were seeded at a density of 1 × 105 cells per well in 12-well plates. The plates were then placed in an incubator set at 37 °C with 5% CO2 for cell growth and maintenance.

Following treatment with dopamine and/or LPS, the cells were briefly rinsed in phosphate-buffered saline (PBS) to remove any extraneous substances. Next, the cells were incubated in a 4% paraformaldehyde (PFA) solution for 10 minutes at room temperature to fix them. Subsequently, the cells were washed three times with ice-cold PBS to remove any excess PFA. To stain the actin filaments, the samples were incubated in PBS containing Actin-Tracker Green-488 for 30 minutes.

Following the incubation, the cells were washed three times for 5 minutes each with PBS. To visualize the cell nuclei, the cells were then exposed to DAPI (DNA stain) for 1 minute. The cells were rinsed with PBS to remove any unbound DAPI. A drop of mounting medium was placed on the coverslip, on which the cells were mounted. Nail polish was applied to seal the coverslip, preventing drying and movement during microscope examination. Subsequently, the cells were stored in a dark environment at -20°C to maintain sample integrity and stability. The captured images were obtained using the Echo Revolve microscope.

2.4. RNA extraction and Real-Time PCR

The cell was lysed in 350 µL lysis buffer. Then, 350 µL of 70% methanol was added to bind the total RNA. The RNA was washed with 700 µL wash buffer 1 and centrifuged at 8000 rpm for 30 seconds. Subsequently, the RNA was washed with 500 µL wash buffer 2 and centrifuged at 8000 rpm for 30 seconds. Finally, the RNA was washed again with 500 µL wash buffer 2 at 8000 rpm for 2 minutes.

The RNA was eluted with 50 µL RNA elution buffer at 8000 rpm for 1 minute. Polymerase and dNTP were added to reverse transcribe the RNA into cDNA. Primers, dNTPs, Taq polymerase, mix buffer, and DNA dye were added to the mixture. The PCR processes were repeated: pre-denaturation at 95°C for 5 minutes, followed by 40 cyclic reactions at 95°C for 30 seconds, and then at 60°C for 30 seconds.

The relative expression level was analyzed using the $2^{-\Delta\Delta ct}$ method.

Table 1. Primers used in this study

Primers	5’-3’
Tgfb1-FP	TGATACGCCTGAGTGGCTGTCT
Tgfb1-RP	CACAAGAGCAGTGAGCGCTGAA
Il10 -FP	CGGGAAGACAATAACTGCACCC
Il10 -RP	CGGTTAGCAGTATGTTGTCCAGC
CD206-FP	GTTCACCTGGAGTGATGGTTCTC
CD206-RP	AGGACA TGCCAGGGTCACCTTT
TNF-a-FP	GGTGCCTATGTCTCAGCCTCTT
TNF-a-RP	GCCATAGAACTGATGAGAGGGAG
Il1b -FP	TGGACCTTCCAGGA TGAGGACA
Il1b -RP	GTTCATCTCGGAGCCTGTAGTG
Cd86-FP	ACGTATTGGAAGGAGATTACAGCT

Table 1. (continued)

Cd86-RP	TCTGTCAGCGTTACTATCCCGC
iNos -FP	GAGACAGGGAAGTCTGAAGCAC
iNos -RP	CCAGCAGTAGTTGCTCCTCTTC
Arg1-FP	CATTGGCTTGCGAGACGTAGAC
Arg1-RP	GCTGAAGGTCTCTTCCATCACC

2.5. Phagocytosis assay

BV2 cells were seeded at 1 × 105 cells/well in 12-well plates and incubated at 37 °C in an atmosphere with 5% CO2. Two hours after plating, pre-treatment with dopamine and LPS stimulation was applied. In the 37 °C cell culture incubator, the cell media was changed to DMEM alone, and the cells were allowed to incubate for 30 min. Following this, the media were changed to DMEM supplemented with 5% FBS under varied dopamine and LPS stimulus treatment settings. Fluorescent latex beads (1 M, L2778 Sigma) were pre-opsonized in 50% FBS and PBS. The pre-opsonized beads were added to the cells at concentrations of 50 BV2 cells per bead and 100 cells per bead, respectively, and incubated at 37 °C for 2 and 3 hours. After incubation, the leftover beads were carefully removed from the cells, and the cells were then fixed at room temperature with 4% PFA. After that, cells were stained for 15 minutes at room temperature with DAPI (4’, 6-diamidino-2-phenylindole) (1 μg/mL). Finally, the images were captured by an Echo Revolve microscope.

2.6. Western Blotting

The cell sample was placed on ice and the dish was washed with ice-cold PBS. The PBS was aspirated, and ice-cold lysis buffer (1 mL per 107 cells/100 mm dish/150 cm2 flask; 0.5 mL per 5x106 cells/60 mm dish/75 cm2 flask) was added. The adherent cells were scraped off the dish using a cold plastic cell scraper, and the cell suspension was gently transferred into a pre-cooled microcentrifuge tube. The cell suspension was maintained constant agitation for 30 minutes at 4°C.

Afterwards, the microcentrifuge tube was centrifuged in a microcentrifuge at 4°C for 20 minutes at 12,000 rpm. The tubes were gently removed from the centrifuge and placed on ice, and the supernatant was aspirated and transferred into a fresh tube kept on ice. The pellet (cell debris) was discarded. The protein concentration was determined using a BCA kit and the samples were prepared for a protein quantification assay.

The cell lysates were boiled in a sample buffer (4% SDS, 10% 2-mercaptoethanol, 20% glycerol, 0.004% bromophenol blue, and 0.125M Tris-HCl) at 100°C for 5 minutes. The lysates were then aliquoted and stored at -20°C for future use. Equal amounts of protein were loaded into the wells of the SDS-PAGE gel, along with a molecular weight marker. 20–30 μg of total protein from cell lysate or tissue homogenate was loaded. The gel was run for 1–2 hours at 100 V.

PVDF membrane was activated with methanol for 1 minute and rinsed with transfer buffer. The protein was then transferred to the membrane. The membrane was blocked for 1 hour at room temperature or overnight at 4°C using a blocking buffer (5% BSA in TBST). The membrane was incubated with appropriate dilutions of primary antibody in blocking buffer at 4°C overnight.

The membrane was washed in three washes of TBST, 5 minutes each. Then, the membrane was incubated with the recommended dilution of conjugated secondary antibody in blocking buffer at room temperature for 1 hour. The membrane was washed again in three washes of TBST, 5 minutes each. Finally, images were acquired using darkroom development techniques for chemiluminescence.

2.7. Statistical analyses

Statistical analyses were performed using Prism 7.0 (Graph-Pad Software, Inc., La Jolla, CA, USA). Quantitative data were expressed as mean ± standard errors of the mean (S.E.M.) and analyzed using

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one-way ANOVA followed by post hoc comparison using Tukey's test. The difference was considered to be statistically significant when $p< 0.05$.

3. Results

3.1. Dopamine did not show any significant impact on the reduction of cell viability caused by LPS exposure

Levodopa is the first choice for medical treatment in PD [18]. PD is characterized by neuroinflammation, which worsens symptoms and contributes to its development [19]. However, the exact mechanism by which dopamine affects microglia, especially pro-inflammatory (M1) microglia, is still unclear. Endotoxins called lipopolysaccharides (LPS), a type of endotoxin found in the outer membrane of gram-negative bacteria and known to be a potent microglial activator, have been identified as one of the most effective stimuli for microglial activation [20]. To answer the question of how dopamine functions on activated microglia during neuroinflammation, we employed LPS stimulation to activate microglia, creating an M1 microglia model. Subsequently, we exposed the cells to varying concentrations of dopamine, specifically 2 μM (middle concentration) and 10 μM (high concentration), and assessed cell viability as well as morphological changes.

We divided our subjects into four groups for the experiment, with one control group that was not given any substances, one group with 200 ng/mL LPS, one group with LPS and middle concentration (2 μM) of dopamine, and one group with LPS and high concentration (10 μM) of dopamine. After 48 hours, we assessed cell viability using CCK-8. The findings revealed a significant decrease in cell viability between the control group and the LPS groups. This indicates that the LPS modeling was successful. Interestingly, the three dosing groups demonstrated similar cell viability levels, hovering around 40%. Additionally, the differences between the LPS, LPS + 2 μM dopamine group, and the LPS + 10 μM dopamine group are insignificant (Fig. 1). The result demonstrated that dopamine does not have a significant effect on LPS-activated microglia's cell viability, and is not able to reverse or enhance the decreased cell viability induced by LPS.

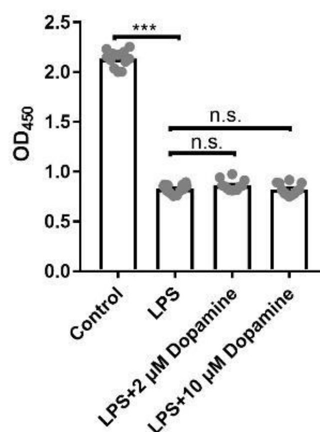

Figure 1. Comparison between microglia cell viability in different groups by CCK-8

ANOVA test, n.s., no significance, ***p<0.001.

3.2. Dopamine reduced the global activation level of microglia

Microglial activation has historically been linked to an amoeboid-like shape that promotes microglia movement and phagocytic function. This particular shape is a feature of M1-activated microglia [21]. In our study, we used Actin-Tracker Green-488 as a fluorescent dye and performed immunofluorescent

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staining on the cell cytoskeleton to investigate the changes in morphology and overall cell activation level of microglia after dopamine treatment.

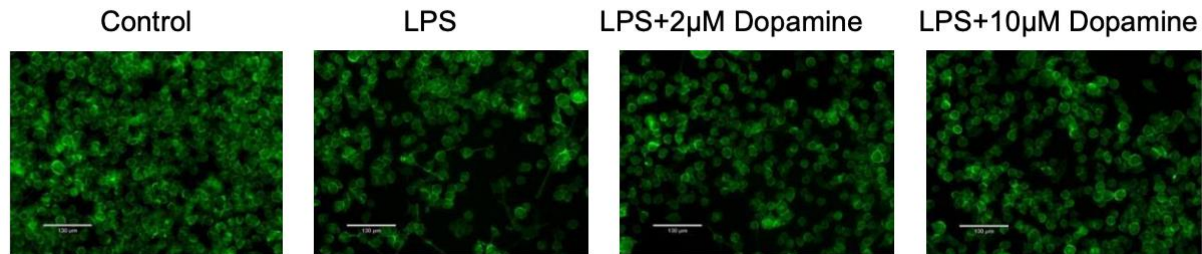

Figure 2. F-actin/phalloidin-FTIC immunofluorescent staining of BV2 cell line in LPS, LPS + 2 μM dopamine and LPS + 10 μM dopamine groups (bar = 130 μm)

We observed a decrease in cell density in the groups treated with LPS compared to the control group (Fig. 2), which was consistent with the results obtained from the CCK-8 assay (Fig. 1). Interestingly, the LPS group exhibited an increased number of cells with larger cell areas and longer neurites, along with some cells displaying an ameboid appearance. Calculations revealed that approximately 5.8% of cells in the field had neurites (calculated by number of cells with neurites divided by the number of total cells in the field). However, when comparing the LPS group to the LPS + 2 μM dopamine group and the LPS + 10 μM dopamine group, we noted a reduction in the number of cells with an ameboid morphology. Specifically, the LPS + 2 μM dopamine group had approximately 1.3% of cells with neurites, while the LPS + 10 μM dopamine group had only 0.64% (Fig. 2).

The results indicated that LPS successfully activated the microglia and changed into its M1 phenotype, and dopamine would decrease the percentage of the activated microglia. Therefore, dopamine exhibited the potential to lower the overall cell activation caused by LPS.

3.3. Dopamine decreased LPS-induced pro-inflammatory microglia activation

Typically, M1 microglia are known to exhibit pro-inflammatory behavior, promoting inflammation in the brain [22]. On the other hand, M2 microglia display an anti-inflammatory response, inhibiting inflammation and facilitating wound healing [22]. However, distinguishing the activation type of microglia solely based on their morphology is not sufficient. As different activation stages p result in the production of different cytokines and variations in cellular metabolic pathways, it is essential to identify their activation state by examining the cytokines they produce and their related metabolic enzymes. In our study, we employed real-time PCR to test the specific activation types of cytokines and metabolism-related enzymes.

The above results of our study revealed significant differences in the mRNA levels of TGF-β1, IL-10, and CD206 between the control group and the LPS group (Fig. 3). Specifically, the LPS group showed an increase in the mRNA levels of these markers, suggesting that LPS stimulation promotes M2 activation of microglia. Interestingly, no notable changes were observed in the Arg1 group, indicating that LPS does not affect Arg1 metabolism. Furthermore, the addition of dopamine only resulted in a decrease in the mRNA level of TGF-β1. This effect was more pronounced with higher concentrations of dopamine. These findings suggest that dopamine has a suppressive effect on the mRNA expression of TGF-β1. Overall, these results provide further evidence for LPS-induced M2 polarization of microglia.

All cytokines and enzymes related to metabolism for M1 microglia increased after LPS injection, which implied that the LPS model was constructed successfully. However, when comparing the LPS group to the LPS + 2 μM dopamine group, there was only a slight downward trend in results, which was not statistically significant. In contrast, treatment with 10 μM dopamine resulted in a significant decrease in the mRNA level for all cytokines and enzymes associated with M1 microglia metabolism. This observation indicates that dopamine has the potential to reduce the activation of pro-inflammatory

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microglia. In addition, the varying effects observed between the two dopamine concentrations (2 μM and 10 μM) suggest that the effects of dopamine treatment may be concentration-dependent.

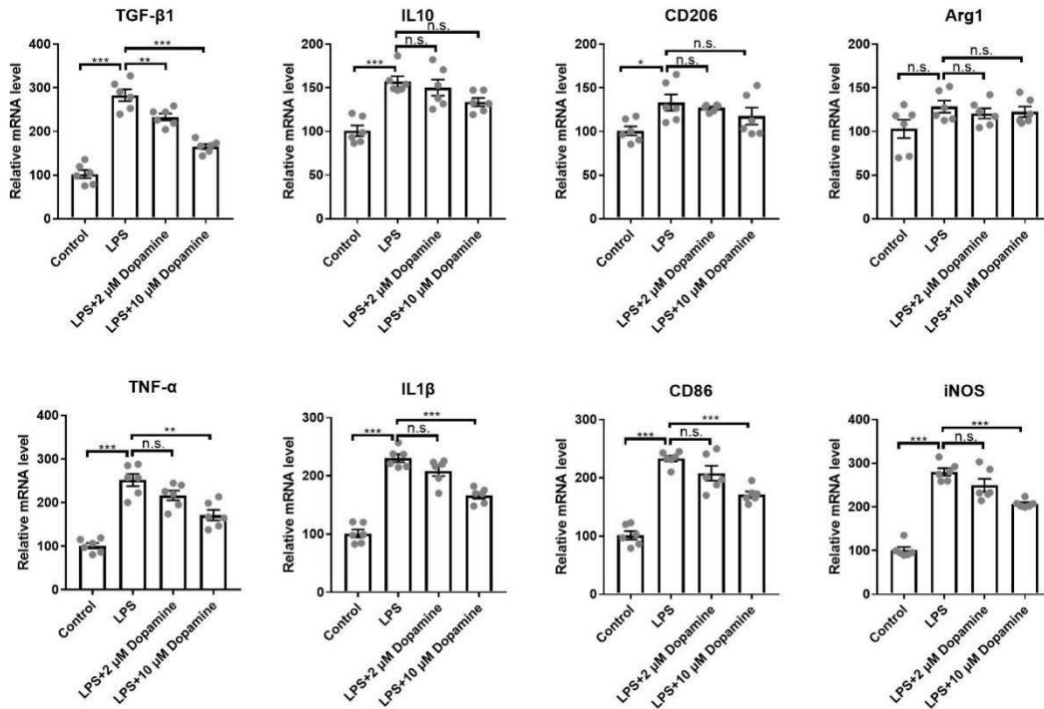

Figure 3. Relative mRNA level of pro-inflammatory (TNF-α, IL1β, CD86, iNOS) and anti-inflammatory (TGF-β1, IL-10, CD206, Arg1) cytokines in BV2 cells detected using Real-time PCR.

ANOVA test n.s. not significant, **p*<0.05 ***p*<0.01 ****p*<0.001

3.4. Dopamine improved the phagocytosis ability of microglia

PD is characterized by the constant presence of the Lewy body, a characteristic neuronal inclusion with α-Synuclein aggregation [23]. Microglia, the primary phagocytes of the brain, can clear the Lewy body by phagocytosis to protect the brain [24, 25].

Our initial findings highlighted the ability of dopamine to alleviate the pro-inflammatory activation of microglia induced by LPS (Fig. 2 and Fig. 3). To further investigate the impact of dopamine on the phagocytosis ability of microglia, we conducted an experiment involving the administration of fluorescent latex beads to the cells, followed by cell staining and observation. Figure 4 displays the results obtained from this experiment. In the control group, approximately 47.2% of cells in the view exhibited positive staining for latex beads. Conversely, in the LPS group, only 9.6% of cells displayed positive staining. Notably, the LPS + 2 μM group showed a substantial increase in the percentage of cells with positive staining (39.6%), which was further enhanced in the LPS + 10 μM group (45.7%). These results indicate that microglia in the LPS group exhibited a decreased efficiency in the process of phagocytosis compared to the control group. Furthermore, this effect was found to be concentration-dependent, with higher concentrations of dopamine resulting in greater efficiency in phagocytosis.

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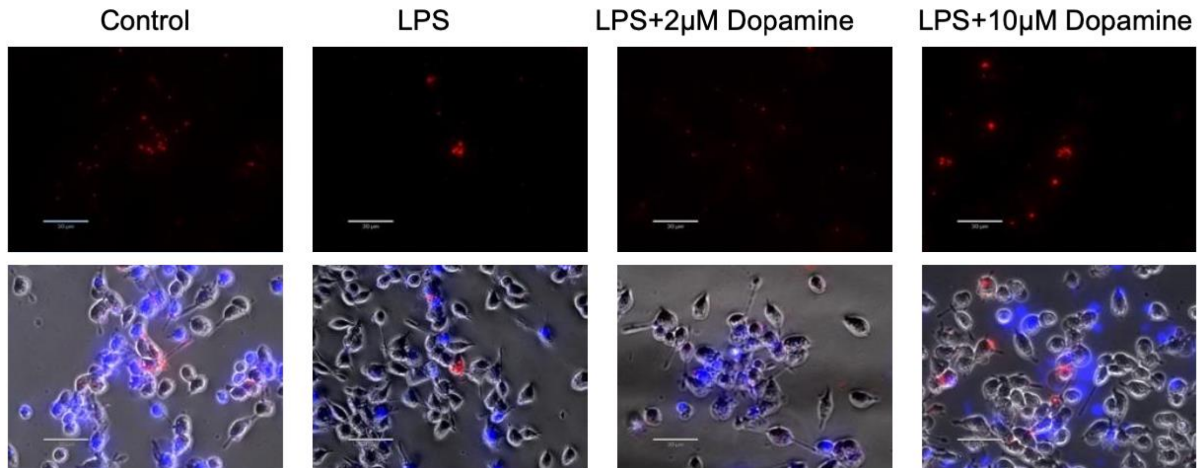

Figure 4. Cells with fluorescent latex beads in phagocytosis experiments. Red latex beads, and blue DAPI nucleus

3.5. Dopamine might be involved in M1 microglia activation by affecting LPS receptor levels and nucleoplasmic levels of downstream transcription factors

Our recent results showed that dopamine can relieve the M1 activation induced by LPS, and improve the phagocytosis ability of cells (Fig. 3 and Fig. 4). LPS binds to a type of receptor called the Toll-Like Receptor 4 (TLR4) and then activates the main transcription factor of inflammation, NF-κB [26]. NF-κB is a transcriptional factor that plays an essential role in the expression of proinflammatory cytokines in microglial cells [27].

Activated NF-κB performs its function in the nucleus and up-regulated the pro-inflammatory cytokines produced in M1-activated microglia. When exposed to LPS, an increase in TLR4 expression is expected, and as a result, the level of NF-κB in the cytoplasm should decrease as it moves to the nucleus to fulfill its task (Fig. 5). In our study, we observed a significant increase in the expression level of TLR4 in the LPS group compared to the control group. However, there was no significant difference in TLR4 expression between the dopamine groups and the LPS group, although a decreasing trend was noticed. Notably, the NF-κB level in the cytoplasm decreased in the LPS group, but adding dopamine increased its level, yet no statistical significance was observed between the groups. The NF-κB level in the nucleus experienced the same situation as the NF-κB in the cytoplasm did, with an increase in the LPS group compared to the control group, and a decrease in the dopamine groups, but again, no statistical significance was observed. These results indicate that dopamine may mitigate M1-activated microglia by blocking LPS binding with TLR4, which is responsible for triggering the transcription and production of pro-inflammatory cytokines. Furthermore, dopamine decreased the NF-κB level that entered the nucleus to play its role in transcribing the cytokines. However, the effects were not significant. Furthermore, our results indicate that the regulation of TLR4 and NF-κB levels by dopamine is concentration-dependent, with the 10 μM dopamine treatment showing a more pronounced effect in alleviating the LPS-induced response.

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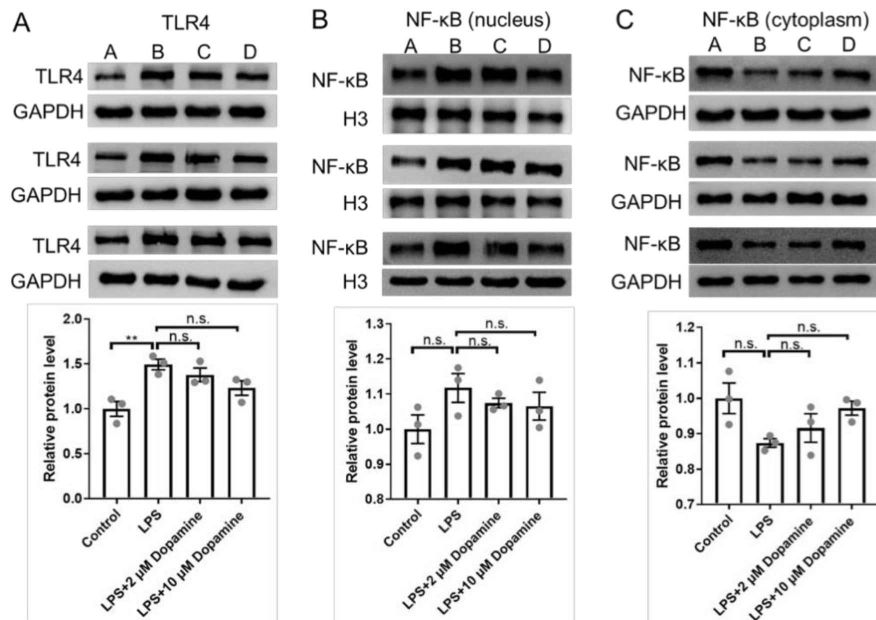

Figure 5. Dopamine regulated the molecular pathway of LPS-induced microglial inflammation. (A) TLR4 electrophoretic result and relative TLR4 level measured with confidential reference GAPDH. (B) Electrophoretic result of NF-κB in the nucleus measured with confidential reference histone protein 3 (H3). (C) Electrophoretic result of NF-κb in the cytoplasm and relative NF-κb (cytoplasm) level measured with confidential reference GAPDH.

In each section, A is the control group, B is the LPS group, C is the LPS + 2μM dopamine group, and D is the LPS + 10μM dopamine group. ANOVA test, n.s., no significance; ***p*<0.01

4. Conclusion

Based on our thorough analysis and experimentation, we have reached a conclusive finding indicating that dopamine has a favorable impact on the activation of microglia activation in PD. Although its effect on cell viability was not statistically significant, dopamine exhibited a notable reduction in the pro-inflammatory levels of microglia. Moreover, dopamine was found to enhance the phagocytic capabilities of microglia, which play a crucial role in eliminating cytotoxic substances associated with PD. Additionally, our research revealed that dopamine has the potential to modulate the TLR4-NFκB pathway, resulting in a decrease in TLR4 expression and the transcription of NF-κB. This modulation could serve as a mechanism through which dopamine mitigates the release of pro-inflammatory cytokines in microglia.

In recent scientific studies, researchers have made significant discoveries regarding the impact of specific DR subtypes on microglial activation in different stages, known as M1 or M2. These stages ultimately determine whether a pro-inflammatory or anti-inflammatory response occurs. On the one hand, dopamine has been found to suppress the production of the pro-inflammatory cytokine interleukin-12 (IL-12), which is typically associated with the M1 stage of microglia. On the other hand, dopamine increases the expression of the anti-inflammatory cytokine interleukin-10 (IL-10), which is commonly produced during the M2 stage of microglia [14]. In studies with LPS, Gaskill et al. [15] found that dopamine dramatically reduced the release of TNF-α in monocytes exposed to LPS, Therefore, recent studies have concluded that dopamine can promote the expression of the M2 gene in microglia, induce the phenotype switch of M1 to M2, and reduce the production of pro-inflammatory cytokines while increasing the production of anti-inflammatory cytokines. These results align with the findings from our own experiments in the current study. In our research, we have made further advancements by discovering that dopamine not only influences the inflammatory response of microglia but also enhances

their ability to phagocytose. Furthermore, we have identified the underlying mechanism behind this process, which suggests a new avenue for future investigations into neuroinflammation in PD.

Our findings have provided initial evidence supporting the role of dopamine in blocking the binding of LPS to TLR4, which then activates the transcription factor NF-κB and reduces the NF-κB level in the nucleus. However, the impact of dopamine appears to be insignificant based on our results. To explain this, we propose the following potential reasons.

Firstly, it is possible that our experiments lacked sufficient repetition, resulting in widely dispersed results. Therefore, conducting further repetitions of the experiments is necessary to validate and reinforce our findings.

Secondly, based on the trends seen in our results, we hypothesize that the effect of dopamine may be concentration-dependent. Consequently, the concentration of dopamine employed in our western blotting assays could potentially be a factor contributing to the observed insignificance.

Therefore, further studies should be conducted to address the limitations of this research. It is essential to include more groups with higher concentrations of dopamine to obtain a more comprehensive understanding of its effects. Additionally, repeating the experiments more than three times will enhance the credibility and reliability of the results.

Furthermore, we found that dopamine can improve the phagocytosis ability of microglia. However, the underlying reasons and mechanisms behind this phenomenon remain unknown. Further studies are required to determine the mechanisms of the improvements of phagocytosis induced by dopamine. One approach is to identify a specific pathway that cytotoxic substances activate to trigger the process of phagocytosis in microglia. Subsequently, we can investigate the expression levels of relevant receptors and proteins involved in this pathway using western blotting analysis.

Despite its limitations, our research has identified a new target for treating neuroinflammation in PD. Specifically, we have discovered that dopamine has the ability to reduce inflammation responses in PD, thereby alleviating PD symptoms. This is significant because inflammation is a key influential factor for α-Synuclein aggregation in PD patients. Furthermore, considering that dopamine plays a big role in the pathophysiology of depression, and PD shares some similar symptoms with depression [28], our findings indicate that dopamine may also have a role in neuroinflammation that is related to depression and the activation of microglia in the brains of depression patients. This implies that dopamine can also be a potential treatment for patients suffering from depression.

In summary, our study demonstrated that dopamine has the ability to decrease the presence of M1 microglia and reduce inflammation responses, ultimately enhancing the microglia’s phagocytosis capability. These functions of dopamine are achieved by regulating the TLR4-TNκ b pathways. Ultimately, our findings not only provide a new target for PD treatments by tackling neuroinflammation, but they also suggest a new approach to treating neuroinflammation associated with depression, as individuals with depression often experience dopamine deficiency in the brain.

Overall, these findings have significant implications for improving the understanding and treatment of neuroinflammation in PD and depression, potentially leading to reduced pain and enhanced quality of life for affected individuals.

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