World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Time-window analysis of lipopolysaccharide-induced activation and M1 polarization in BV2 microglial cells

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World Journal of Biology Pharmacy and Health Sciences, 2026, 25(03), 001-005

Publication history: Received on 10 January 2026; revised on 26 February 2026; accepted on 28 February 2026

Article DOI: https://doi.org/10.30574/wjbphs.2026.25.3.0133

Abstract

Objective: This study aimed to systematically investigate lipopolysaccharide (LPS)-induced activation of BV2 microglial cells and to evaluate the effects of different exposure durations on cellular activation, cytokine release, and polarization status, thereby clarifying the role of LPS in microglial activation and defining an optimal stimulation window for model establishment.

Methods: BV2 microglial cells treated with LPS for different time intervals were analyzed using ELISA kits, quantitative PCR (qPCR), and Western blotting. Specifically, morphological changes were documented by inverted microscopy by recording cell size, the number of processes, and cytoplasmic granularity; the expression levels of pro-inflammatory cytokines (IL-6, IL-1β) and anti-inflammatory cytokines (IL-10) were measured; and the mRNA and protein expression of the polarization-related marker cyclooxygenase-2 (COX-2) were determined.

Results: LPS induced time-dependent microglial activation with coordinated shifts in polarization markers and cytokine profiles. After 6 h, the anti-inflammatory marker CD206 was significantly downregulated ($P < 0.05$), whereas the M1-associated marker COX-2 was markedly upregulated ($P < 0.01$), accompanied by increased pro-inflammatory cytokines including IL-6 and IL-1β ($P < 0.01$) and a reduction in IL-10. These changes became more pronounced at 12 and 24 h, with further elevations of pro-inflammatory indices and continued decreases of anti-inflammatory indicators, consistent with a progressive shift toward an M1-like phenotype under sustained stimulation.

Conclusion: LPS effectively induces inflammatory activation of BV2 microglia and drives a shift toward an M1-like pro-inflammatory phenotype. Integrating morphological changes, cytokine profiles, and polarization marker expression, 12 h appears to represent a robust and relatively stable time window for model establishment.

Keywords: Lipopolysaccharide-Induced Activation; M1 Polarization; BV2 Microglial Cells; CD206; COX-2

1. Introduction

Mechanistic studies of spinal cord and central nervous system (CNS)–related disorders have long been a major focus in medicine and neuroscience. Among these, microglia, the resident immune cells of the CNS, have attracted widespread attention for their roles in maintaining homeostasis and mediating pathological responses. Microglia play critical roles under both physiological and pathological conditions by maintaining CNS homeostasis, regulating neuronal network activity, and participating in post-injury regeneration and repair[1, 2]. Meanwhile, microglia exert important regulatory effects in neuroinflammatory responses and in the onset and progression of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease[3, 4]. Therefore, systematically elucidating the biological characteristics of

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microglia and their regulatory mechanisms is important for understanding CNS homeostasis and the development and progression of related diseases.

In recent years, the lipopolysaccharide (LPS)-induced microglial activation model has been widely used in neuroinflammation-related research. As a classic inflammatory stimulus, LPS can trigger pro-inflammatory responses by activating microglia and thereby shape the immune microenvironment of the CNS[5]. Previous studies have shown that LPS promotes the release of inflammatory mediators from microglia and activates multiple signaling pathways, thereby altering neuronal function and synaptic plasticity and ultimately affecting CNS homeostasis and disease progression[6]. Accordingly, dissecting the molecular mechanisms underlying LPS-induced microglial activation and polarization will help clarify the pathological basis of neuroinflammation-related diseases and provide a theoretical foundation for identifying potential therapeutic targets. However, key experimental parameters—particularly the stimulation duration required to achieve stable and reproducible activation—remain insufficiently standardized across studies. Defining an optimal time window for LPS-induced BV2 microglial activation is therefore essential for improving model reliability and for enabling mechanistic and interventional investigations.

2. Materials and Methods

2.1. Main materials, reagents, and instruments

Cells: BV2 microglial cells. Main reagents: DMEM medium, fetal bovine serum, penicillin–streptomycin, PBS, and 0.25% trypsin–EDTA; lipopolysaccharide (LPS) was used as the inflammatory stimulant. ELISA kits were used to measure IL-6, IL-1β, and IL-10. Reagents for qPCR included TriQuick Reagent, a reverse transcription kit, and SYBR Green qPCR Mix. Major instruments included a CO_2 incubator, an inverted microscope, a microplate reader, a real-time fluorescence quantitative PCR system (Agilent, AriaMx), and a chemiluminescence imaging system.

2.2. Cell culture, seeding, and grouping/treatments

BV2 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. After trypsinization and counting, cells were seeded into 6-well plates (5×10^5 cells/well, 2 mL/well) and allowed to adhere overnight; subsequent treatments were performed after cells entered the logarithmic growth phase. The groups included a control group and LPS stimulation groups for 6 h, 12 h, and 24 h. LPS (final concentration, 100 ng/mL) was added to the treatment groups for 6, 12, or 24 h, whereas the control group received an equal volume of vehicle. All experiments were performed in ≥3 independent replicates.

2.3. Morphological observation

At different time points, morphological changes (cell body size, number and length of processes, cytoplasmic granularity, etc.) were observed and recorded under an inverted microscope, and representative field images were captured for presentation.

2.4. Measurement of inflammatory cytokines in culture supernatants (ELISA)

After treatment, culture supernatants were collected and centrifuged at 2000×g for 5 min to remove cell debris; the supernatants were used for assays or aliquoted and stored at −80 °C, avoiding repeated freeze–thaw cycles. Standard curves were generated according to the kit instructions to measure IL-6, IL-1β, IL-10, and Arg-1 levels, and absorbance at 450 nm was read to calculate concentrations.

2.5. Real-time fluorescence quantitative PCR (qPCR)

After treatment, the medium was removed, total RNA was extracted using TriQuick lysis, and RNA concentration and purity were assessed (samples with A260/A280 of 1.7–2.1 were included). Equal amounts of RNA were reverse-transcribed to obtain cDNA. qPCR amplification was performed using the SYBR Green system with Actb as the internal reference; melting curves were generated to evaluate specificity, and relative expression was calculated using the 2^-ΔΔCt method. The genes analyzed were CD206 and COX-2. All primers used for qPCR in this study were mouse-derived, and the amplified targets included COX-2, CD206, and the reference gene Actb.

2.6. Statistical analysis

Statistical analyses were performed using SPSS 25.0 and GraphPad Prism 8. Quantitative data are presented as mean ± standard deviation (mean ± SD). Comparisons among multiple groups were performed using one-way analysis of

variance (one-way ANOVA), followed by Tukey's test for post hoc pairwise comparisons; $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of LPS on BV2 polarization and inflammatory cytokine expression

Effects of LPS on BV2 polarization and inflammatory cytokine expression As shown in Figure 1, the phenotype and function of BV2 microglial cells changed markedly following LPS treatment at different time points. Compared with the control group, LPS treatment significantly altered the activation state of BV2 microglia. In the control group, cells largely maintained an M2-like anti-inflammatory phenotype, characterized by high expression of the M2 marker CD206, low expression of the M1 marker (COX-2) and pro-inflammatory cytokines (e.g., IL-6 and IL-1β), and relatively high expression of the anti-inflammatory cytokine IL-10, indicating a resting or anti-inflammatory state. However, as the duration of LPS exposure increased, BV2 microglia gradually shifted toward an M1-like pro-inflammatory phenotype. At 6 h, CD206 expression was significantly reduced ($P < 0.05$), suggesting that anti-inflammatory features began to be suppressed; in contrast, expression of the M1 activation marker COX-2 was significantly increased ($P < 0.01$), along with a marked elevation in pro-inflammatory cytokines (IL-6 and IL-1β; $P < 0.01$). This shift became more evident at 12 h and 24 h, when pro-inflammatory markers further increased and anti-inflammatory markers decreased, consistent with a progressive polarization toward an M1-like state. Ultimately, 24 h of LPS exposure produced the strongest pro-inflammatory phenotype, as evidenced by peak expression of pro-inflammatory cytokines and M1 markers accompanied by a further reduction in anti-inflammatory cytokines. Overall, LPS induced a stepwise shift of BV2 microglia from an M2-like anti-inflammatory phenotype toward an M1-like pro-inflammatory phenotype, which deepened over time and ultimately resulted in a marked enhancement of the pro-inflammatory response.

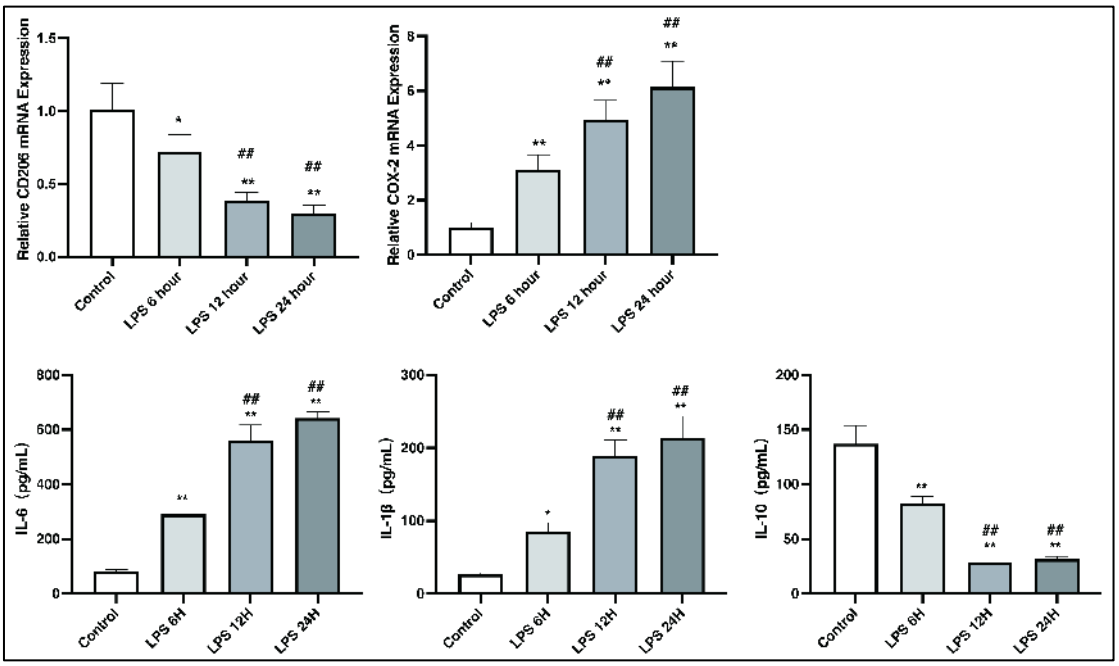

Figure 1 Changes in pro-inflammatory polarization and inflammatory cytokine expression in BV2 microglia induced by LPS

4. Discussion

This study demonstrates that LPS (100 ng/mL) effectively induces canonical inflammatory activation of BV2 microglia in vitro, with a progressively strengthened response as stimulation time increases. These time-resolved data provide an evidence-based basis for selecting stimulation durations that yield stable and reproducible pro-inflammatory activation in vitro.

Consistent with established microglial biology, a shift toward an M1-like pro-inflammatory phenotype is typically accompanied by activation of multiple inflammatory signaling pathways and upregulation of pro-inflammatory

molecules and cytokines[7]. In this study, LPS stimulation for 6 h was sufficient to increase pro-inflammatory markers such as COX-2, accompanied by marked elevations of pro-inflammatory cytokines including IL-6 and IL-1β, indicating that inflammatory programs were initiated early after stimulation. Upregulation of COX-2 suggests activation of the prostaglandin biosynthesis pathway, an important component of the pro-inflammatory cascade[8]. Meanwhile, anti-inflammatory indicators, including CD206 and IL-10, showed a downward trend, suggesting suppression of anti-inflammatory regulation and an overall bias toward a pro-inflammatory state[9, 10]. Together, these coordinated changes support an early transition from a relatively anti-inflammatory baseline toward M1-like activation.

Building on the early changes at 6 h, stimulation extended to 12 h further intensified pro-inflammatory activation of BV2 cells: morphologically, somata enlarged and processes became more distinct and slender; pro-inflammatory cytokine release continued to rise; and M1-associated marker proteins were markedly increased, suggesting that the inflammatory response had reached a robust and comparatively stable phase. Notably, we found that some pro-inflammatory factors approached a plateau after 12 h, whereas other molecules (e.g., COX-2) continued to increase at 24 h, highlighting marker-specific response kinetics. This phenomenon may relate to differences in induction kinetics among genes/proteins, post-transcriptional regulation, and feedback-inhibitory mechanisms, and therefore the choice of an "optimal stimulation window" should be guided by the specific study objective and endpoint readouts.

From the perspective of experimental design and model construction, 12 h provided a favorable balance between "efficiency" and "intensity" under our conditions: most cytokines and polarization markers showed significant changes and approached a plateau at this time point, facilitating a stable and reproducible pro-inflammatory model. In addition, compared with 24 h, a 12-h exposure may reduce confounding from nonspecific stress or culture-condition drift associated with prolonged stimulation. Therefore, if the goal is to establish a stable BV2 pro-inflammatory activation model for mechanistic studies or candidate-intervention screening, 12 h may be preferred; however, if maximizing COX-2 induction or cumulative inflammatory burden is required, extending stimulation to 24 h may be warranted. In summary, this study provides experimental evidence for selecting the stimulation time window in an LPS-induced BV2 microglial inflammatory activation model, supporting more consistent model implementation in downstream neuroinflammation studies and in vitro evaluation workflows.

5. Conclusion

Under the conditions of this study (LPS, 100 ng/mL), LPS reliably induced inflammatory activation of BV2 microglia, with significant increases in pro-inflammatory cytokines such as IL-1β and IL-6; it also drove a shift toward an M1-like pro-inflammatory phenotype, as reflected by upregulation of pro-inflammatory markers including COX-2 and downregulation of anti-inflammatory indicators such as CD206 and IL-10, indicating a transition from a relatively quiescent/anti-inflammatory state to a pro-inflammatory activated state. Time-course analyses further showed that pro-inflammatory activation was detectable by 6 h, whereas cytokine and polarization-marker changes were stronger and largely stabilized by 12 h. Accordingly, a 12-h stimulation window may provide a robust and reproducible condition for establishing an LPS-induced BV2 pro-inflammatory activation model, thereby facilitating downstream mechanistic studies and in vitro evaluation.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no competing interests.

Author contribution

Yaling Jin wrote the paper; Jiuzhou Lin and Xiaoshuang Jiang collected and analyzed the data; Weiting Chen and Min Tang conceived the study. All authors read and approved the final manuscript.

Funding

This research was supported by Linhai Science and Technology Plan Project under Grant No (2025YW06).

Data Availability

Data are available from the corresponding author upon reasonable request.

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