

UDC 615.37:616-002:612.112.3

DOI <https://doi.org/10.32782/2226-2008-2026-1-2>**Batuhan Yurtseven¹** <https://orcid.org/0009-0003-5047-3340>**Esra Aydemir²** <https://orcid.org/0000-0002-6965-2838>**Furkan Ayaz^{2,3}** <https://orcid.org/0000-0003-0271-0594>**INVESTIGATION OF THE IMMUNOMODULATORY EFFECTS OF GABAPENTIN ON MAMMALIAN MACROPHAGE CELLS**¹ Biruni University, Istanbul, Türkiye² Istinye University, Istanbul, Türkiye³ Odesa National Medical University, Odesa, Ukraine

UDC 615.37:616-002:612.112.3

Batuhan Yurtseven¹, Esra Aydemir², Furkan Ayaz^{2,3}**INVESTIGATION OF THE IMMUNOMODULATORY EFFECTS OF GABAPENTIN ON MAMMALIAN MACROPHAGE CELLS**¹ *Biruni University, Istanbul, Türkiye*² *Istinye University, Istanbul, Türkiye*³ *Odesa National Medical University, Odesa, Ukraine*

Background. Recent research has increasingly highlighted the intricate crosstalk between the immune and nervous systems, particularly in the context of neurological pathogenesis. Gabapentin, a structural analogue of γ -aminobutyric acid (GABA) conventionally prescribed for epilepsy and neuropathic pain, is now gaining attention for its potential immunomodulatory properties. While it is well established that the nervous, endocrine, and immune systems coordinate through a complex network of shared signaling molecules, the specific impact of gabapentin on macrophages – the primary effectors of innate immunity that also orchestrate adaptive responses via antigen presentation – remains poorly understood.

Methods. To elucidate gabapentin's immunomodulatory capabilities, the murine macrophage cell line J774.2 was exposed to varying concentrations of the drug in the presence or absence of lipopolysaccharide (LPS). Following treatment, the secretion profiles of major inflammatory cytokines, specifically IL-6, TNF- α , IL-12p40, and GM-CSF, were quantified utilizing enzyme-linked immunosorbent assays (ELISA).

Results. Our analyses revealed that gabapentin exerted a significant, dose-dependent suppressive effect on the production of IL-6, TNF- α , and IL-12p40 in LPS-stimulated macrophages. Conversely, the secretion of GM-CSF remained largely unaffected. Importantly, none of the tested concentrations induced cytotoxicity, demonstrating that gabapentin effectively dampens the release of pro-inflammatory cytokines without compromising overall macrophage viability.

Conclusion. In summary, this study provides compelling evidence for the selective anti-inflammatory and immunomodulatory actions of gabapentin on macrophages. These findings broaden our understanding of gabapentin's pharmacological profile beyond its classical neurological applications, underscoring its potential therapeutic value in the clinical management of neuroinflammatory and immune-mediated conditions.

Keywords: inflammation, immunomodulator, gabapentin, cytokine, neuroimmune interaction.

УДК 615.37:616-002:612.112.3

Батухан Юртсеєв¹, Есра Айдемір², Фуркан Аяз^{2,3}**ДОСЛІДЖЕННЯ ІМУНОМОДУЛЮЮЧИХ ЕФЕКТІВ ГАБАПЕНТИНУ НА КЛІТИНИ МАКРОФАГІВ ССАВЦІВ**¹ *Університет Біруні, Стамбул, Туреччина*² *Університет Істінє, Стамбул, Туреччина*³ *Одеський національний медичний університет, Одеса, Україна*

Останнім часом зростає інтерес до взаємодії імунної та нервової систем. Габапентин – структурний аналог γ -аміномасляної кислоти, що широко застосовується для лікування нейропатичного болю та епілепсії, – розглядається як потенційний імуномодулюючий агент. Водночас його вплив на клітини вродженого імунітету, зокрема макрофаги, залишається недостатньо вивченим. у цьому дослідженні макрофаги миші лінії J774.2 обробляли різними концентраціями габапентину за наявності або відсутності ліпополісахариду. Рівні прозапальних цитокінів (IL-6, TNF- α , IL-12p40, GM-CSF) визначали методом імуноферментного аналізу. Встановлено, що габапентин дозозалежно знижує продукцію IL-6, TNF- α та IL-12p40 у LPS-стимульованих макрофагах, не впливаючи на рівень GM-CSF і життєздатність клітин. Отримані результати свідчать про протизапальні та імуномодулюючі властивості габапентину й розширюють уявлення про можливості його застосування за імунозалежних і нейрозапальних станів.

Ключові слова: запалення, імуномодулятор, габапентин, цитокіни, нейроімунна взаємодія.

© Batuhan Yurtseven, Esra Aydemir, Furkan Ayaz, 2026

Стаття поширюється на умовах ліцензії



Introduction

Gabapentin is a structural analogue of a structural analogue of γ -aminobutyric acid (GABA) and is primarily prescribed for the treatment of neuropathic pain, partial seizures, and postherpetic neuralgia [1]. Although its mechanism of action is not fully understood, gabapentin binds to the $\alpha 2\delta$ subunit of voltage-gated calcium channels in the central nervous system, thereby modulating neurotransmitter release [2]. Despite its favorable efficacy in various neurological disorders, gabapentin has been associated with adverse effects such as dizziness, fatigue, and more recently, immunological alterations [3]. There is growing clinical and experimental interest regarding its impact on immune cells, as some reports suggest gabapentin may suppress cytokine production or modulate inflammatory responses [4].

Our study aims to investigate the potential immunomodulatory effects of gabapentin on macrophage cells, which play a central role in both innate and adaptive immunity. Macrophages, which are differentiated from circulating monocytes, are essential in host defense through their roles in antigen presentation, phagocytosis, and cytokine secretion [5–7]. Their interaction with pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), initiates signaling cascades leading to the release of pro-inflammatory cytokines including IL-6, TNF- α , and IL-12 [8; 9]. Modulation of these cytokines may reflect the drug's influence on immune activation or suppression.

Since gabapentin's direct effects on immune cells, particularly macrophages, remain poorly understood, our study addresses a crucial knowledge gap. We hypothesize that gabapentin, upon exposure to activated macrophage cells, may alter cytokine production levels in response to LPS stimulation. Considering the role of macrophages in inflammatory and neuroimmune interactions, understanding these effects may provide insights into gabapentin's broader impact on the immune system and potentially inform its long-term safety profile in vulnerable patient populations.

Materials and Methods

2.1. Cell culture and drug treatment

Gabapentin (P4666, Sigma-Aldrich, USA) was obtained from Sigma-Aldrich, and murine macrophage cell line J774.2 were sourced from the American Type Culture Collection (ATCC). All procedures were carried out under BSL2 aseptic conditions to prevent contamination. The compound was dissolved in sterile distilled water to a final concentration of 10 mg/mL. J774 macrophage cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640; 11875093, Thermo Scientific, USA) supplemented with 10 % fetal bovine serum (FBS; A5209501, Thermo Scientific, USA) and 1 % antibiotic solution (100 μ g/mL streptomycin and 100 μ g/mL penicillin; 15140122, Thermo Scientific, USA) [10; 11]. The cells were plated in 24-well plates at a density of 10^6 cells per well and allowed to adhere for 24 hours at 37 °C in a humidified 5 % CO₂ incubator. Subsequently, the cells were treated with 1, 5, and 10 μ g/mL concentrations of gabapentin, either alone or in combination with 1 μ g/mL lipopolysaccharide (LPS; L5293, Sigma-Aldrich, USA). The negative control group consisted of cells maintained in culture medium without any treatment.

2.2. Cell Viability Assay

Cell viability was assessed using Trypan Blue exclusion staining. For this, an equal volume of Trypan Blue solution was mixed with the cell suspension. The mixture was then loaded onto a hemocytometer, and both stained (non-viable) and unstained (viable) cells were counted. Viability was calculated by subtracting the number of blue-stained cells from the total cell count, dividing the result by the total, and multiplying by 100 to obtain a percentage. All experiments were carried out in triplicate, including three biological replicates. Statistical significance was analyzed using the Student's t-test in GraphPad Prism version V.

2.3. The determination of immunomodulatory activity

After a 24-hour incubation with different gabapentin concentrations, cell culture supernatants were collected. Cytokine quantification was performed using ELISA kits from BD Biosciences (CA, USA), specifically IL-12p40 (Cat. No. 555220), GM-CSF (Cat. No. 555126), TNF- α (Cat. No. 555212), and IL-6 (Cat. No. 555183). In brief, ELISA plates were pre-coated with specific monoclonal antibodies for each cytokine and incubated overnight. After incubation, the collected supernatants were transferred into the antibody-coated wells and further incubated for 24 hours. Following this, the wells were emptied, and 100 μ L of TMB substrate (555214, BD Biosciences, USA, USA) was added for 1 hour. To terminate the colorimetric reaction, 1 M sulfuric acid (339741, Sigma-Aldrich, USA) was applied. Absorbance was then read at 450 nm using an ELISA microplate reader (BioTek® 800 TS Absorbance Reader, USA). Cytokine concentrations were calculated by comparing the absorbance values with a standard curve. For statistical analysis, the Student's t-test was performed using GraphPad Prism Version 10.0 [10; 11].

Ethical considerations

This study was conducted using established murine macrophage cell lines (J774.2) obtained from a certified cell repository. No human participants or experimental animals were involved in this research. Therefore, approval from an institutional ethics committee was not required. All animal experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986) and applicable ethical guidelines.

Research results and their discussion

3.1. Gabapentin was used at non-cytotoxic concentrations

Trypan Blue staining was performed to assess the viability of J774.2 macrophages following exposure to varying concentrations of gabapentin, with or without LPS stimulation (Fig. 1). No significant differences in cell viability were observed between treated and untreated groups at concentrations ranging from 1 to 10 μ g/mL. Therefore, these doses were deemed non-cytotoxic for the cells.

3.2. Gabapentin had anti-inflammatory effects on J774.2 cells

Immunomodulation refers to the ability of a compound to alter immune cell activity by enhancing or suppressing the production of pro- or anti-inflammatory cytokines. To assess the immunomodulatory potential of Gabapentin on macrophages, cytokine levels of IL-6 (Fig. 2), TNF- α (Fig. 3), IL-12p40 (Fig. 4), and GM-CSF (Fig. 5) were measured in J774.2 cells. Upon LPS stimulation, the levels

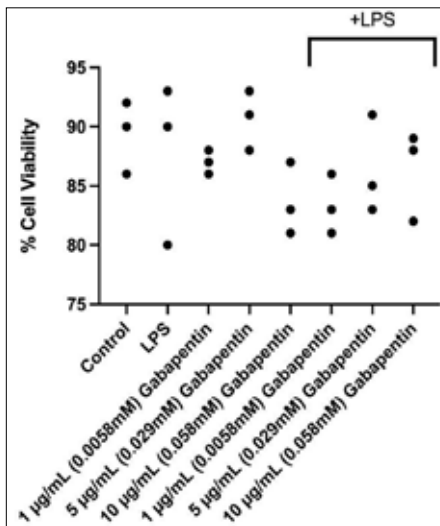


Fig. 1. The cell viability results upon incubation with Gabapentin

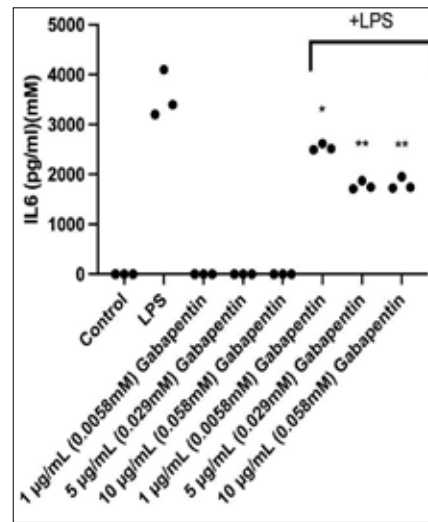


Fig. 2. The production levels of IL-6 in J774.2 cells in the presence of in the presence or absence of LPS (* – $p < 0.001$, ** – $p < 0.0005$, $N = 3$)

of IL-6, TNF- α , and IL-12p40 showed a marked increase, confirming the activation of the inflammatory response. However, Gabapentin treatment – especially at higher concentrations – resulted in a significant, dose-dependent decrease in cytokine production. Notably, cells treated with Gabapentin alone did not show any significant cytokine elevation, indicating that the drug itself does not provoke inflammation. These findings suggest that Gabapentin can attenuate LPS-induced inflammatory signaling in macrophages, supporting its potential role as an anti-inflammatory agent with immunomodulatory properties.

Gabapentin, initially developed for neuropathic pain and seizure control, is gaining increasing attention for its potential immunomodulatory effects beyond the central nervous system [12; 13]. As neuroinflammation has been identified as a key contributor to many neuropsychiatric and neuropathic disorders, exploring how gabapentin influences immune cell function is crucial to understanding its broader therapeutic utility.

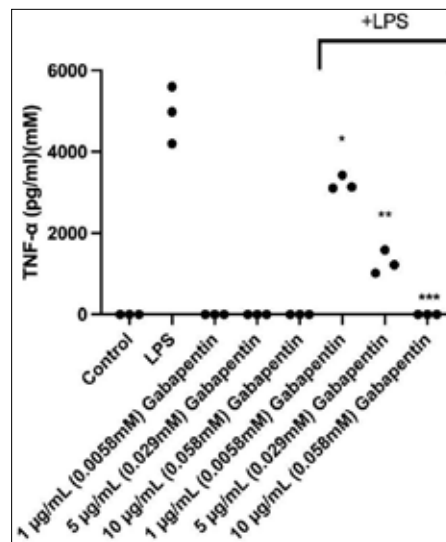


Fig. 3. The production levels of TNF- α in J774.2 cells in the presence of in the presence or absence of LPS (* – $p < 0.001$, ** – $p < 0.0005$, *** – $p < 0.0001$, $N = 3$)

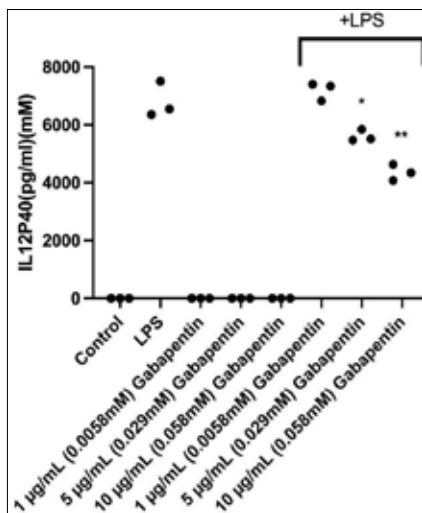


Fig. 4. The production levels of IL-12p40 in J774.2 cells in the presence of in the presence or absence of LPS (* – $p < 0.001$, ** – $p < 0.0005$, $N = 3$)

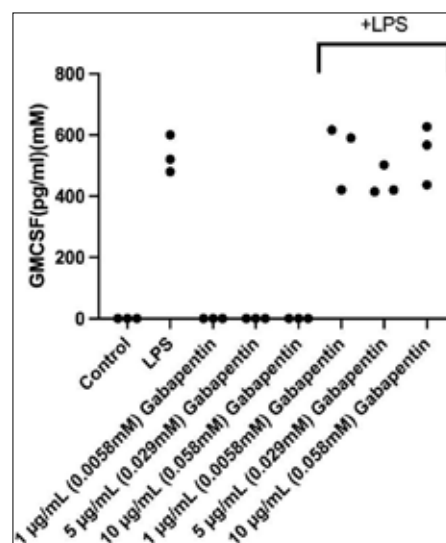


Fig. 5. The production levels of GM-CSF in J774.2 cells

in the presence of in the presence or absence of LPS

In this study, we investigated the effect of gabapentin on LPS-induced cytokine production in the murine macrophage cell line J774.2. Using ELISA, we quantified the levels of IL-6, TNF- α , IL-12p40, and GM-CSF. As expected, LPS stimulation led to a marked increase in IL-6, TNF- α , and IL-12p40 levels, confirming macrophage activation. Co-treatment with gabapentin resulted in a dose-dependent suppression of these cytokines, with the most significant inhibition observed at 10 μ g/mL. In contrast, GM-CSF levels remained unchanged across all conditions, suggesting a selective modulation of inflammatory pathways.

IL-6, a key mediator of the acute phase response and chronic inflammation [9], was significantly reduced at the highest gabapentin dose, while lower concentrations (1 and 5 μ g/mL) showed more moderate effects. These results support earlier findings that gabapentin may downregulate IL-6 via glial and macrophage pathways [14].

Similarly, TNF- α , which plays a central role in initiating inflammatory cascades [15], showed a significant dose-dependent decline, with 10 μ g/mL gabapentin producing the most pronounced reduction. This is consistent with previous in vitro studies demonstrating gabapentin's ability to suppress TNF- α in activated macrophages [15].

Gabapentin also suppressed IL-12p40, a subunit of IL-12 and IL-23 involved in Th1 immune responses and autoimmune pathogenesis [16]. The downregulation of IL-12p40 suggests that gabapentin may attenuate Th1-driven inflammation, further supporting its potential in modulating cellular immunity.

In contrast, GM-CSF, a critical hematopoietic growth factor and cytokine essential for macrophage differentiation and survival, did not exhibit significant changes upon gabapentin treatment. The consistent GM-CSF levels across all doses indicate that gabapentin does not interfere with macrophage proliferation or viability.

Not applicable.

and its immunomodulatory effects are likely limited to inflammatory signaling rather than affecting cellular maintenance pathways [8].

Conclusions

In summary, our findings reveal that gabapentin can selectively inhibit pro-inflammatory cytokines such as IL-6, TNF- α , and IL-12p40 in LPS-stimulated macrophages, while leaving homeostatic cytokines like GM-CSF unaffected. These data highlight gabapentin's potential as an anti-inflammatory agent in immune-mediated or neuroinflammatory conditions. Future work should explore the underlying molecular mechanisms – particularly involving NF- κ B or MAPK pathways – to further clarify gabapentin's role in immune regulation.

Statements and Declarations

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

BY, EA, and FA conceptualized the study. BY, EA, and FA conducted the experiments, FA supplied the drug molecules. BY, EA, and FA analyzed the data, wrote the manuscript, read and approved the final version of the manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

BIBLIOGRAPHY

1. Kaye AD, Armistead G, Amedio LS, et al. Evolving treatment strategies for neuropathic pain: a narrative review. *Medicina (Kaunas)*. 2025;61(6):1063. DOI: 10.3390/medicina61061063.
2. Muttalib MAMA. Neuropathic pain pharmacotherapy: mechanisms, anti-inflammatory actions, and clinical perspectives. *Pharma Innovation*. 2025;14(4):12–18. DOI: 10.22271/tpi.2025.v14.i4a.26070.
3. Meaadi J, Obara I, Eldabe S, Nazar H. The safety and efficacy of gabapentinoids in the management of neuropathic pain: a systematic review with meta-analysis of randomised controlled trials. *Int J Clin Pharm*. 2023;45(3):556–65. DOI: 10.1007/s11096-022-01528-y.
4. Rosa AS, Freitas MF, Rocha IRC, Chacur M. Gabapentin decreases microglial cells and reverses bilateral hyperalgesia and allodynia in rats with chronic myositis. *Eur J Pharmacol*. 2017;799:111–7. DOI: 10.1016/j.ejphar.2017.02.012.
5. Brancewicz J, Wójcik N, Sarnowska Z, Robak J, Król M. The multifaceted role of macrophages in biology and diseases. *Int J Mol Sci*. 2025;26(5):2107. DOI: 10.3390/ijms26052107.
6. Yurtseven B, Aydemir E, Ayaz F. The role of intestinal microbiota and immune system interactions in autoimmune diseases. *Immunotargets Ther*. 2025;14:1347–72. DOI: 10.2147/ITT.S569016.
7. Cui H, Wang M, Jiao S, Tian S, Liu H, Luo B. Macrophages in chronic infections: regulation and remodeling. *Front Immunol*. 2025;16:1594988. DOI: 10.3389/fimmu.2025.1594988.
8. García-Domínguez M. Targeting GM-CSF in rheumatoid arthritis: advances in cytokine-directed immunotherapy and clinical implications. *Life (Basel)*. 2025;15(11):1737. DOI:10.3390/life15111737.
9. Qianyi Wu, Jiyuan Shi, Luojin Wu, et al. HCX3 mitigates LPS-induced inflammatory responses in macrophages by suppressing the activation of the NF- κ B signaling pathway. *Curr Issues Mol Biol*. 2025;47(10):100809. DOI: 10.3390/cimb47100809.
10. Ayaz F, Demir D, Bölgen N. Injectable chitosan cryogel microspheres with biocompatible properties on mammalian macrophages in vitro. *J Mater Sci*. 2021;56(30):17268–77. DOI: 10.1007/s10853-021-06399-8.

11. Yurtseven B, Başkan ÖM, Aydemir E, Ayaz F. Investigation of the immune system effects of desloratadine on lipopolysaccharide-stimulated mammalian macrophage cells. *Fundam Clin Pharmacol.* 2025;39(6):e70056. DOI: 10.1111/fcp.70056.
12. Mayoral V, Galvez R, Ferrándiz M, et al. Pregabalin vs. gabapentin in the treatment of neuropathic pain: a comprehensive systematic review and meta-analysis of effectiveness and safety. *Front Pain Res (Lausanne).* 2024;5:1513597. DOI: 10.3389/fpain.2024.1513597.
13. Rusciano D. Molecular mechanisms and therapeutic potential of gabapentin with a focus on topical formulations to treat ocular surface diseases. *Pharmaceuticals (Basel).* 2024;17(5):623. DOI: 10.3390/ph17050623.
14. Rossi A, Murta V, Auzmendi J, Ramos AJ. Early gabapentin treatment during the latency period increases convulsive threshold, reduces microglial activation and macrophage infiltration in the lithium-pilocarpine model of epilepsy. *Pharmaceuticals (Basel).* 2017;10(4):93. DOI: 10.3390/ph10040093.
15. Jang D-i, Lee A-H, Shin H-Y, et al. The role of tumor necrosis factor alpha (TNF- α) in autoimmune disease and current TNF- α inhibitors in therapeutics. *Int J Mol Sci.* 2021;22(5):2719. DOI: 10.3390/ijms22052719.
16. Goleij P, Amini A, Sanaye PM, et al. The IL-12 family cytokines in neurodegenerative diseases: dual roles in neurotoxicity and neuroprotection. *Inflammopharmacology.* 2025;33(9):5235–56. DOI: 10.1007/s10787-025-01901-z.

Надійшла до редакції 24.10.2025.

Прийнята до друку 26.02.2026.

Електронна адреса для листування furkan1988ayaz@gmail.com