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Modifications in gamma-aminobutyric acid type A receptor subunit gene expression after macrophage differentiation and propofol administration in THP-1 cells

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Background: Gamma-aminobutyric acid type A ($GABA_A$) receptors are thought to play a role in the functioning of the immune system. They have 19 types of subunits that determine their physiological functions. However, the subunits expressed in immune cells during inflammation have not been fully investigated. Recent reports have shown that anesthetic agents may affect the gene expression of their subunits in immune cells. Therefore, we aimed to examine the changes in $GABA_A$ receptor subunit gene expression after macrophage differentiation and propofol administration to clarify the relationship between the expression of these receptors and the immunosuppressive effect of propofol.

Methods: Human acute monocytic leukemia cells were differentiated into macrophage-like cells, which were subsequently differentiated into inflammatory macrophage-like cells. Propofol was administered during the differentiation into inflammatory macrophage-like cells. Using reverse transcription polymerase chain reaction (RT-PCR), we examined which $GABA_A$ receptor subunit genes were expressed and whether there were changes in gene expression after macrophage differentiation and propofol administration in human acute monocytic leukemia cells.

Results: The expression of *α1*, *α4*, *β1*, *β2*, *γ1*, and *γ2* subunits increased after differentiation into macrophage-like cells. The expression of *α1*, *α4*, *β1*, *β2*, *γ2*, and *δ* subunits decreased, and that of the *γ1* subunit increased after differentiation into inflammatory macrophage-like cells. The gene expression of *α1*, *α4*, and *β2* subunits increased upon administering propofol after differentiation into inflammatory macrophage-like cells.

Conclusions: The gene expression of $GABA_A$ receptor subunits changed after macrophage differentiation in human acute monocytic leukemia cells. The expressions of *α1* and *α4* increased following propofol administration during the differentiation into inflammatory macrophage-like cells, indicating that $GABA_A$ receptors are involved in the immunosuppressive effects of propofol. This study can help choose anesthetic agents for proinflammatory conditions like highly invasive surgery.

Keywords: Propofol; THP-1; macrophage; gamma-aminobutyric acid type A subunit ($GABA_A$ subunit)

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Introduction

Background

Gamma-aminobutyric acid type A ($GABA_A$) receptors are ligand-gated anion channels activated when gamma-aminobutyric acid (GABA) binds to them. GABA is a major inhibitory neurotransmitter. The function of GABA has been well studied in the central nervous system; however, few studies are available on $GABA_A$ receptor subunit expression in relation to physiological functions in other tissues. In particular, the expression of $GABA_A$ receptors and their functions in immune cells have not been fully investigated. $GABA_A$ receptor expression has been confirmed in monocytes (1), human acute monocytic leukemia cell lines (THP-1 cells), macrophages (2,3), and T-cells (4,5). Monocytes and macrophages produce GABA, inhibiting inflammatory cytokine production (6-8). Hence, it has been hypothesized that the GABAergic signaling system is also present in immune cells, regulating cellular functions such as cell proliferation, cytokine production, phagocytosis, and chemotaxis (2,6,8,9).

The $GABA_A$ receptor is a pentamer consisting of three different subunits. There are 19 different types of $GABA_A$ receptor subunits ($\alpha 1–6$, $\beta 1–3$, $\gamma 1–3$, δ, ε, π, θ, and $\rho 1–3$). $GABA_A$ receptors are mainly composed of 2α, 2β, and 1γ or 1δ subunits (10). Since the difference in composition determines the channel's specific function and pharmacological properties, identifying subunit expression in cells is crucial. The hypnotic effect of anesthetics is produced by the $GABA_A$ receptors (11). Propofol, a predominant hypnotic agent in the anesthetic field, is known to act on immune cells, resulting in immunosuppressive effects (12,13).

Highlight box

Key findings

- Gene expressions of *α1* and *α4* (which have anti-inflammatory effect) gamma-aminobutyric acid type A ($GABA_A$) receptor subunits increased following propofol administration after differentiation into inflammatory macrophage-like THP-1 cells. $GABA_A$ receptors can be involved in the immunosuppressive effects of propofol.

What is known and what is new?

- Propofol has an immunosuppressive effect. However, the precise mechanism of immunosuppressive effects is unknown. Propofol has hypnotic activity through the activation of $GABA_A$ receptors. The GABAergic signaling system is present in immune cells.
- The gene expression of $GABA_A$ receptor subunits was modified after macrophage differentiation in THP-1 cells. The gene expression of *α1* and *α4* subunits was increased by propofol administration after macrophage differentiation.

What is the implication, and what should change now?

- Propofol can exert an anti-inflammatory effect through the GABAA receptor. The findings of this study can aid clinicians in the choice of anesthetic agents for proinflammatory conditions.

Rationale and knowledge gap

Macrophages are immune cells that are present in various tissues throughout the body. Monocytes differentiate into macrophages when they migrate to tissues throughout the body. During inflammatory processes, macrophages differentiate into M1 macrophages, which act as inflammatory cells, and M2 macrophages, responsible for tissue repair. These macrophages play an important role in the immune response (14,15). THP-1, a human acute monocytic leukemia cell line, has been widely used to investigate the function of human macrophages. THP-1 cells can differentiate into macrophage-like and M1/M2 macrophage-like cells (16,17). In a previous study using THP-1-derived macrophages, we found that propofol suppresses interleukin (IL)-6 and IL-1β production without affecting M1/M2 differentiation and that $GABA_A$ receptors could be involved in the suppression of cytokine production (18).

The expression of $GABA_A$ receptor subunits on T cells and monocytes can be altered by influenza infection. The administration of diazepam affects immune function and increases susceptibility to infection (19). This finding indicates that changes in $GABA_A$ receptor subunit expression are involved in immune function and exacerbate the immunosuppressive effect of diazepam.

Objective

Since the $GABA_A$ subunits expressed in immune cells during inflammation have not been fully investigated, we aimed to analyze the expression of $GABA_A$ receptor subunit genes in THP-1 cells after macrophage differentiation. Furthermore, we aimed to investigate the changes induced by propofol administration to clarify the precise mechanism of the immunosuppressive effect of propofol. We present this article in accordance with the MDAR reporting checklist (available at https://amj.amegroups.com/article/view/10.21037/amj-23-58/rc).

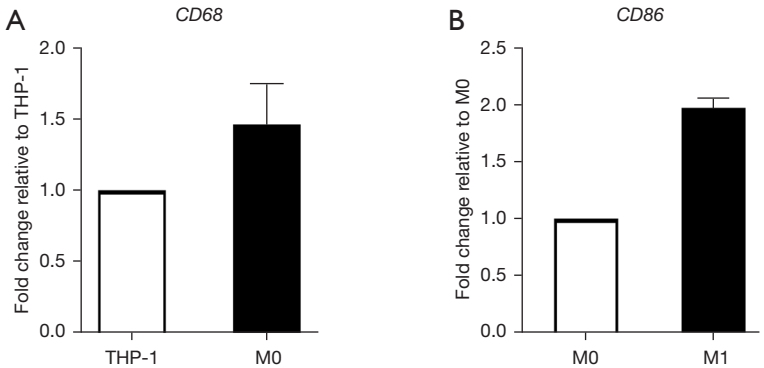

Figure 1 Changes in differentiation marker gene expression after differentiation of THP-1 cells into M0-THP-1 cells and M0-THP-1 cells into M1-THP-1 cells. THP-1 cells were differentiated into M0-THP-1 cells, and M0-THP-1 cells were differentiated into M1-THP-1 cells (A,B). RT-PCR assays of *CD68* and *CD86* mRNA levels in THP-1 cells. The gene expression of *CD68* was upregulated after differentiation into M0 THP-1 cells. The gene expression of *CD86* was upregulated after differentiation into M1 THP-1 cells. Data were normalized relative to *β-actin* mRNA (internal control) and presented as mean ± SD (n=6 per group). *CD*, cluster of differentiation; RT-PCR, reverse transcription polymerase chain reaction; mRNA, messenger RNA; SD, standard deviation.

Methods

Materials

Roswell Park Memorial Institute (RPMI) 1640 medium, dimethyl sulfoxide (DMSO), lipopolysaccharide (LPS) from *Escherichia coli* strain O111:B4, and phorbol-12-myristate-13-acetate (PMA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Interferon (IFN)-γ was obtained from R&D Systems (Minneapolis, MN, USA). Propofol was obtained from Wako Pure Chemical Industries (Osaka, Japan).

Cell culture and differentiation

We used the following cell line: (RRID:CVCL 0006). THP-1 cells (ATCC; Manassas, VA, USA) resemble primary monocytes and macrophages in morphology and differentiation properties. When exposed to PMA, THP-1 cells adhere to culture plates and start differentiating into a macrophage-like phenotype; these cells are generally used to study human macrophage functions (16). THP-1 cells were differentiated into macrophage-like cells (M0 THP-1) through incubation for 3 days with 200 nM PMA in RPMI 1640 supplemented with 5% fetal bovine serum (FBS), penicillin (100 IU/mL), and streptomycin (100 μg/mL) (16). M0 THP-1 cells were polarized into inflammatory macrophage-like cells (M1 THP-1) via incubation with 100 ng/mL of LPS and 20 ng/mL of IFN-γ for 18 h (17). We confirmed the appropriate differentiation into M0 and M1 macrophages by analyzing the differentiation marker gene expressions under our experimental conditions. Cluster of differentiation (*CD*)*68* (macrophage marker) gene expression was upregulated approximately 1.5-fold when THP-1 cells were differentiated into M0 THP-1 cells. *CD86* (M1 marker) gene expression was upregulated approximately 2.0-fold when M0 THP-1 cells were differentiated into M1 THP-1 cells (*Figure 1*).

M0 THP-1 cells were differentiated into M1 THP-1 cells in the presence of propofol (25–100 μM) or in that of the solvent alone (0.05% DMSO) to evaluate the effects of propofol on $GABA_A$ subunit gene expression after M1 differentiation. Under these experimental conditions, propofol had little effect on the viability of polarized THP-1 cells. Cell viability measured by trypan blue staining was 97.9%±3.6%, 96.6%±2.8%, and 96.6%±1.2% for propofol concentrations of 25, 50, and 100 μM, respectively when control was 100% (*Figure 2*).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays

Total cellular RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Subsequently, complementary DNA (cDNA) was synthesized from the total RNA preparations using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) by following the guidelines. Furthermore, qRT-PCR

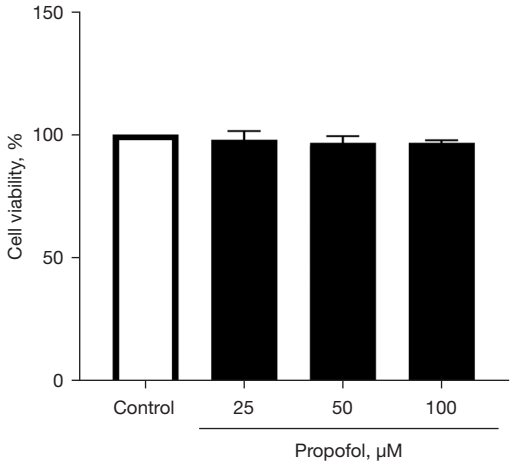

Figure 2 Influence of propofol administration on cell viability in M1-THP-1 cells. M0 THP-1 cells were differentiated into M1 THP-1 cells in the presence of propofol (25–100 μM) or solvent alone (0.05% DMSO). Cell viability measured by trypan blue staining was 97.9%±3.6%, 96.6%±2.8%, and 96.6%±1.2% for propofol concentrations of 25, 50, and 100 μM, respectively, when control was 100%. Under our experimental conditions, propofol had little effect on the viability of polarized THP-1 cells. DMSO, dimethyl sulfoxide.

was performed using PowerUp SYBR Green Master Mix (Applied Biosystems) and specific primers (Takara, Kusatsu, Japan; *Table 1*) on a 7500 Fast Real-Time PCR System (Applied Biosystems). Target messenger RNA (mRNA) levels were normalized against housekeeping *β-actin* mRNA levels, and the expression level relative to that of the control was calculated using the ΔΔCt method. The relative mRNA expression was expressed as fold expression over the control gene expression. The expression level with the control treatment was assumed to be 1.

Statistical analysis

Values are expressed as the mean ± standard deviation (SD), and the results were obtained from six separate experiments. While differences between two groups were analyzed using an unpaired two-tailed *t*-test, differences among multiple groups were analyzed using a one-way analysis of variance (ANOVA), followed by Bonferroni's post-hoc test. All statistical analyses were performed using GraphPad Prism software program V. 6.00 (GraphPad Software; La Jolla, CA, USA), with $P<0.05$ defined as statistically significant.

Table 1 Primer sequences for quantitative real-time RT-PCR

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
β-actin	TGGCACCCAGCACAATGAA	CTAAGTCATAGTCCGCCTAGAAGCA
α1	CAGCAAGTATGAACCACATGGAAC	ATGTGTGGAATGACTTGAACAGAGA
α2	TCCCAAGTTTCATTCTGGCTTAACA	ACCAGTCCATGGCAGTTGCATA
α3	GTCACAAGTGTCGTTCTGGCTCA	AGTCCATGGCCGTCGCATA
α4	GTGCAGCATGTTGGCTTGTC	TCGCATAGATACACCTTTGCATGA
α5	ATCTTGGATGGGCTCTTGGATG	CCGAAGCTGGTGACGTAGATG
α6	AGGAGTCCGTCCCAGCAAGA	GTTGACAGCTGCGAACTCGATAAG
β1	TCTGCAGCCAGAGTCGCACTA	ATACTCCAGCAGAGCCAGGAACA
β2	CTTTGAGTTCCCAAACCAAATGTC	TGGAACTGTCAACTTGCTTCAAATG
β3	GCAGAACTGCACTCTGGAAATTGA	TCCACTCCGGTAACAGCCTTG
γ1	GCAGCCTTGATGGAATATGGAAC	TGGATCCAGGATGGAGACCAG
γ2	TCTGGCAAATCTCTGTGCTG	TCACTTGACAACACCTATGTGAGAA
γ3	TGGATCACCACACCCAATCAG	ATCAGCGGGCAGGAGTGTTC
δ	GTGCATGCTGGACCTGGAGA	CGGTAGCTGGTGATGGTGAAC
CD68	TTGCAGCAACTCGAGCATCA	CAGCAAGATGGACCGGTCAC
CD86	CTGTAACTCCAGCTCTGCTCCGTA	GCCCATAAGTGTGCTCTGAAGTGA

RT-PCR, reverse transcription polymerase chain reaction.

Ethical consideration

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study used THP-1 cells of the cell line which were commercially available in cell culture experiments, not with human primary cells. The ethics registration and informed consent for this study were waived.

Results

Increase in the gene expression of α1, α4, β1, β2, and γ2 $GABA_A$ receptor subunits after differentiation into M0 THP-1 cells

$GABA_A$ receptor subunit gene expression was investigated after macrophage differentiation. The gene expression of *α1*, *α4*, *β1*, *β2*, and *γ2* $GABA_A$ receptor subunits in THP-1 cells significantly increased after the differentiation into M0 THP-1 cells. The expression of *α3*, *α5*, *α6*, *β3*, and *γ3* was not detected in THP-1 cells. The expression of *α2* and *δ* did not change after macrophage differentiation (*Figure 3*).

Decrease in the expression of α1, α4, β1, β2, γ2, and δ subunits and increase in the expression of γ1 subunit after differentiation into M1 THP-1 cells

We examined $GABA_A$ receptor subunit gene expression in THP-1 cells after M1 differentiation. The gene expression of *α1*, *α4*, *β1*, *β2*, *γ2*, and *δ* decreased significantly after the differentiation into M1 THP-1 cells, while that of *γ1* showed a significant increase (*Figure 3*).

Increase in the expression of α1, α4, and β2 GABAA receptor subunits following propofol administration after differentiation into M1 THP-1 cells

We evaluated the effect of propofol on the expression of $GABA_A$ receptor subunits after M1 differentiation in THP-1 cells. Propofol significantly increased the gene expression of *α1*, *α4*, and *β2*, but did not affect the expression of *β1*, *γ1*, *γ2*, or *δ* (*Figure 4*).

Discussion

Key findings

In this study, we detected the gene expression of *α1*, *α2*, *α4*, *β1*, *β2*, *γ1*, *γ2*, and *δ* $GABA_A$ receptor subunits in THP-1 cells. The gene expression of *α1*, *α4*, *β1*, *β2*, *γ1*, and *γ2* increased after macrophage differentiation. The gene expression of *α1*, *α4*, *β1*, *β2*, *γ2*, and *δ* decreased after M1 differentiation; additionally, propofol administration increased the expression of *α1*, *α4*, and *β2* after M1 differentiation.

Strengths and limitations

We suggested that THP-1 cells actually express several $GABA_A$ receptor subunits gene expression, which is altered after macrophage differentiation and propofol administration. These changes can be the mechanism of propofol's immunosuppressive effects.

This study has several limitations. First, an artificial THP-1 cell line was used; therefore, macrophages from human peripheral blood should be examined in a clinical setting in a future study. This is because the results from peripheral blood more likely resemble those acquired in actual situations and may easily be applied in clinical therapy. Second, only gene expression was analyzed in this study; therefore, changes should be examined at the protein level to clarify the precise mechanism of the immunosuppressive effect via the $GABA_A$ receptor. Third, we analyzed only 13 major subunits; however, all 19 $GABA_A$ receptor subunits should be investigated in order to acquire more accurate results. Further studies are required to address these limitations.

Comparison with similar research

GABA is a major inhibitory neurotransmitter in the mammalian central nervous system. $GABA_A$ receptors, the primary target of GABA, are pentameric complexes consisting of three different subunits. Various combinations of $GABA_A$ receptor subunits determine receptor function. Mammals express 20–30 different $GABA_A$ receptor isoforms. The most common combination of $GABA_A$ receptor subunits consists of two *α* subunits, two *β* subunits, and one *γ* or *δ* subunit (20). In this study, we investigated 13 $GABA_A$ receptor subunits: *α1–6*, *β1–3*, *γ1–3*, and *δ*. Most of the hypnotic effects of anesthetic agents are produced by activating $GABA_A$ receptors. Classical benzodiazepines, such as diazepam, bind to two distinct binding sites on this receptor. They may bind to the *α*-*γ* subunit interface in the receptor's extracellular domain. Benzodiazepines may also bind to the *β*-*α* and *γ*-*β* interfaces, which are present in the

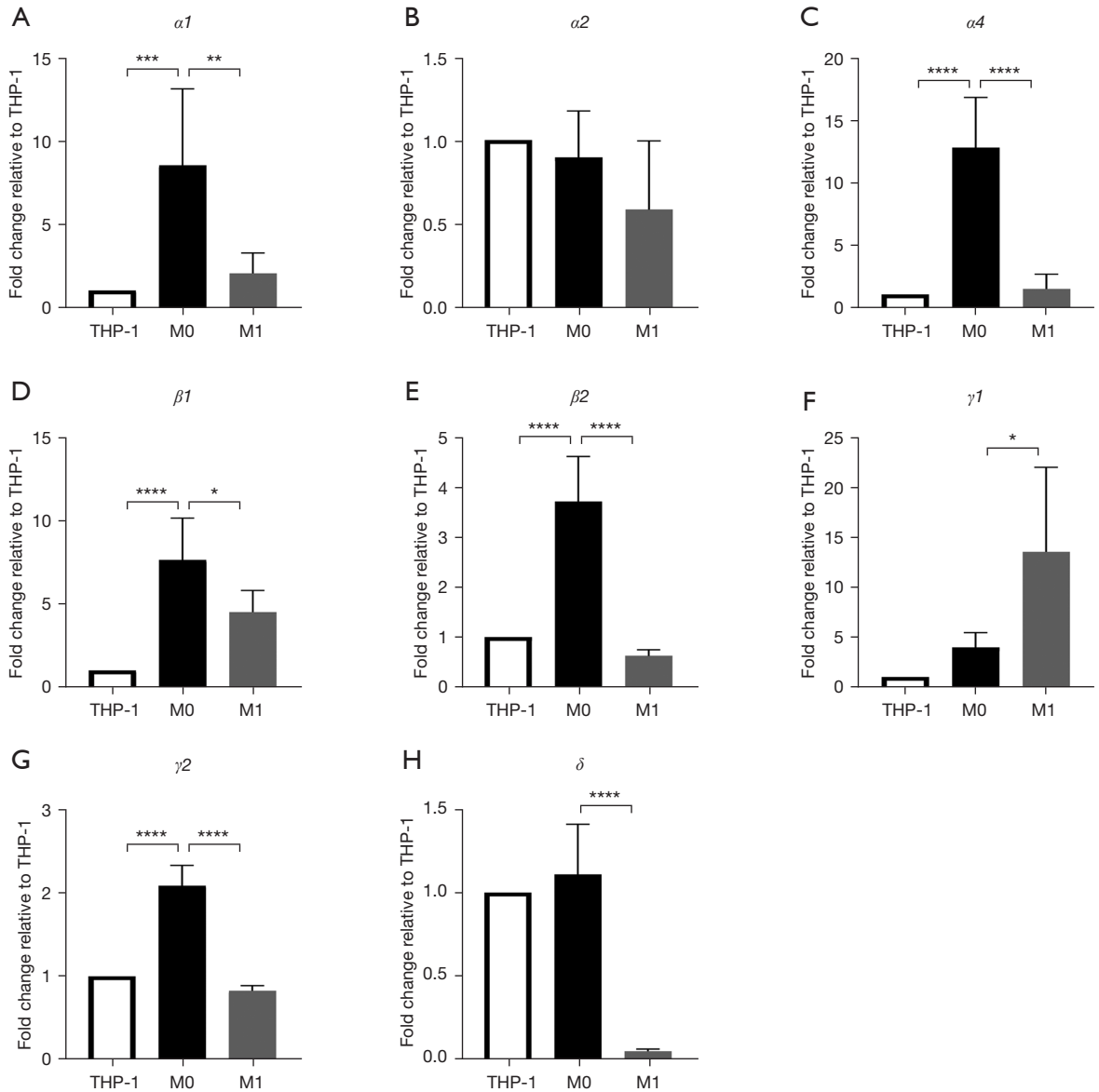

Figure 3 Changes in $GABA_A$ receptor subunit gene expression after differentiation of THP-1 cells into M0-THP-1 cells and M0-THP-1 cells into M1-THP-1 cells. THP-1 cells were differentiated into M0-THP-1 cells, and M0-THP-1 cells were differentiated into M1-THP-1 cells (A-H). RT-PCR assays of $GABA_A$ subunit mRNA levels in THP-1 cells. The gene expression of *α1*, *α4*, *β1*, *β2*, *γ1*, and *γ2* $GABA_A$ receptor subunits was increased after differentiation into M0 THP-1 cells. The gene expression of *α1*, *α4*, *β1*, *β2*, *γ2*, and *δ* $GABA_A$ receptor subunits was decreased after differentiation into M1-THP-1 cells. The gene expression of *γ1* $GABA_A$ receptor subunit was increased after differentiation into M1-THP-1 cells. Data were normalized relative to *β-actin* mRNA (internal control) and presented as mean ± SD (n=6 per group). *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001 compared with control cells by a one-way ANOVA and Bonferroni's post-hoc test. $GABA_A$, gamma-aminobutyric acid type A; RT-PCR, reverse transcription polymerase chain reaction; SD, standard deviation; ANOVA, analysis of variance.

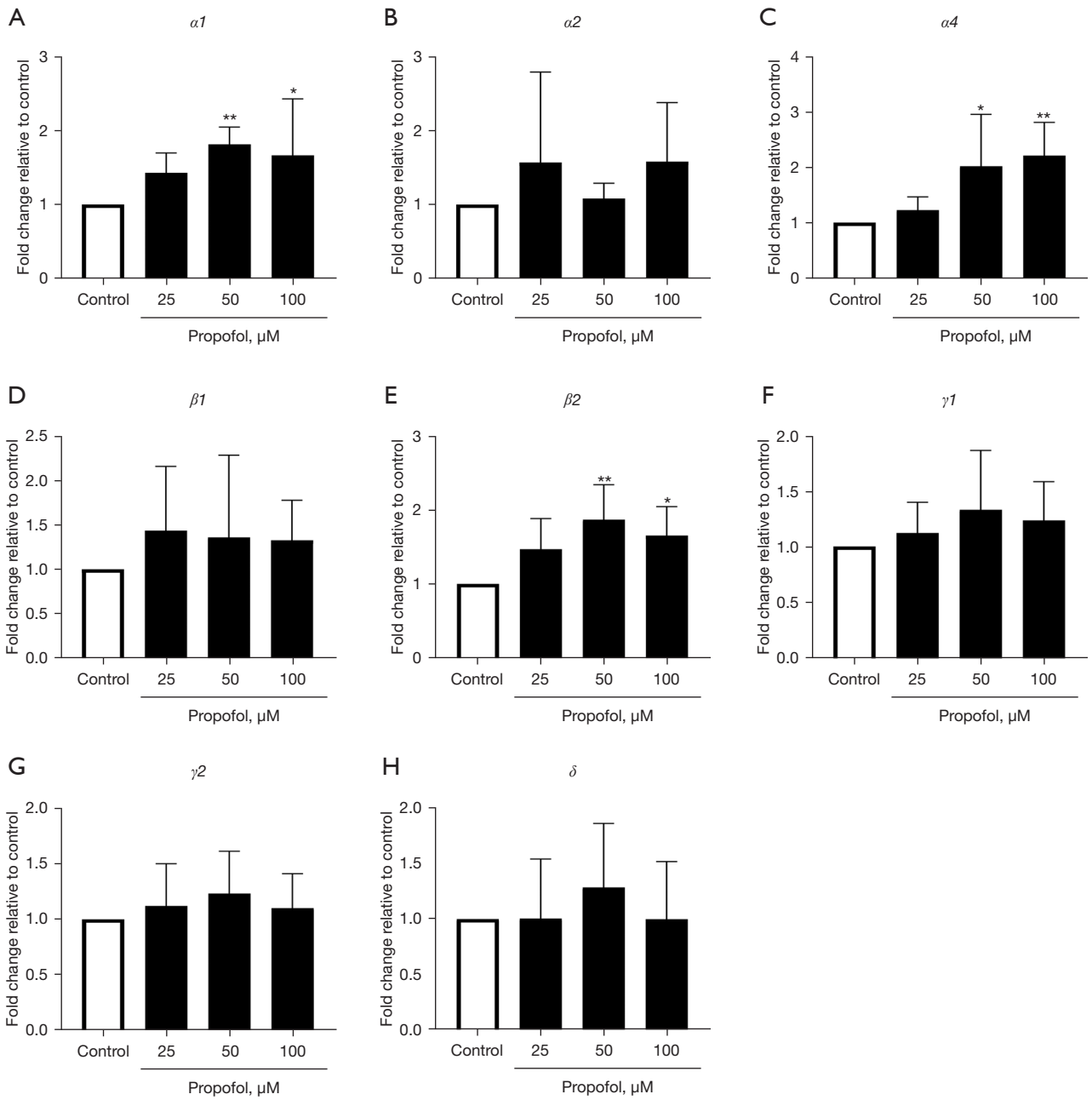

Figure 4 Changes in $GABA_A$ receptor subunit gene expression following propofol administration after M1 differentiation. M0-THP-1 cells were differentiated into M1-THP-1 cells in the presence of 0.05% DMSO as the control solvent or in the presence of propofol (25–100 μM). (A-H) RT-PCR assays of $GABA_A$ subunit mRNA levels in THP-1 cells. The administration of propofol (50 and 100 μM) after differentiation into M1 THP-1 cells increased the gene expression of *α1*, *α4*, and *β2* $GABA_A$ receptor subunits. Data were normalized relative to *β-actin* mRNA (internal control) and presented as mean ± SD (n=6 per group). *, P<0.05; **, P<0.01 compared with control cells by a one-way ANOVA and Bonferroni's post-hoc test. $GABA_A$, gamma-aminobutyric acid type A; DMSO, dimethyl sulfoxide; RT-PCR, reverse transcription polymerase chain reaction; mRNA, messenger RNA; SD, standard deviation; ANOVA, analysis of variance.

transmembrane domain. Propofol binds to the *β-α* interface in the transmembrane domain of the receptor (21).

$GABA_A$ receptors are present in immune cells, and $GABA_A$ signaling modifies immune function. $GABA_A$ receptors have been found on $CD4^+$ and $CD8^+$ T cells, macrophages, monocytes, and THP-1 cells (1-5). The administration of diazepam in a mouse pneumonia model leads to immunosuppression, resulting in higher mortality. In contrast, administering of the $GABA_A$ receptor antagonist, bicuculline counteracts the immunosuppressive effect of diazepam and decreases mortality (22). Another study reported that benzodiazepines are involved in immunosuppressive effects, resulting in increased mortality and the incidence of pneumonia (23). The expression of $GABA_A$ receptor subunits on T cells and monocytes is modified by influenza infection, and diazepam administration affects immune function and increases susceptibility to infection (19). These results indicate that the expression of $GABA_A$ receptor subunits can be modified by external stimuli, such as inflammation, differentiation, and drug administration, including the administration of intravenous anesthetics.

Explanations of findings

Our previous study showed that via the effects on $GABA_A$ receptors in THP-1 cells, propofol suppresses the production of inflammatory cytokines, IL-6 and IL-1β, after the differentiation into inflammatory M1 macrophage-like cells without affecting M1 differentiation (18). The present study shows that the gene expression of *α1*, *α4*, *β2*, and other subunits of $GABA_A$ receptors decreased after M1 differentiation. Conversely, the addition of propofol increased the gene expression of *α1*, *α4*, and *β2* subunits after M1 differentiation. Combined, our previous and present data suggest that the suppression of inflammatory cytokines production after M1 differentiation in THP-1 cells by propofol administration may be associated with increasing the gene expression of *α1*, *α4*, and *β2* subunits.

$GABA_A$ receptors are reportedly involved in inflammatory diseases, such as asthma, intestinal inflammation, and pulmonary fibrosis. The $GABA_A$ receptor, *α1*, is observed in human alveolar macrophages and monocytes and is responsible for diazepam-induced immunosuppression (19,22). When the $GABA_A$ receptor, *α4*, is knocked out in mice suffering from asthma, lung inflammation and airway reactivity deteriorate further (24). In mice with stress-induced intestinal inflammation, *α1*, *α4*, and *α5* $GABA_A$ receptor agonists exert anti-inflammatory effects, while *α3* receptor agonists exacerbate inflammation (25). Diazepam administration activates $GABA_A$ *α4* receptors, suppressing LPS-induced lung injury and the development of pulmonary fibrosis (26). These results suggest that *α1* and *α4* $GABA_A$ receptors are involved in suppressing inflammatory responses. In addition to such previous data, our data suggest that propofol suppresses the production of inflammatory cytokines in THP-1 cells after the M1 differentiation and increases gene expressions of *α1*, *α4*, and *β2* subunits of $GABA_A$ receptors after M1 differentiation. Considering these findings together, it is quite possible that propofol exerts its immunosuppressive effect by increasing the expressions of the *α1* and *α4* subunits of $GABA_A$ receptors.

Implications and actions needed

In this study, we suggested the gene expression of $GABA_A$ receptor subunits was changed after macrophage differentiation and propofol administration in THP-1 cells. The gene expression of *α1* and *α4* subunits which are involved in anti-inflammatory effects was increased by propofol administration. Therefore, the immunosuppressive effect of propofol may be related to changes in gene expressions. Propofol can be beneficial in highly inflammatory situations.

Conclusions

In this study, the gene expression of $GABA_A$ receptor subunits was modified after macrophage differentiation in THP-1 cells. In particular, propofol administration increased the gene expression of *α1* and *α4* subunits after M1 differentiation. These results suggest that the immunosuppressive effect of propofol may be related to changes in the gene expression of $GABA_A$ receptor subunits. These results can help clinicians choose appropriate anesthetic agents for proinflammatory treatments, such as invasive surgery.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://amj.amegroups.com/article/view/10.21037/amj-23-58/rc

Data Sharing Statement: Available at https://amj.amegroups.com/article/view/10.21037/amj-23-58/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://amj.amegroups.com/article/view/10.21037/amj-23-58/coif). I.K. serves as an unpaid editorial board member of *AME Medical Journal* from February 2023 to January 2025. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study used THP-1 cells of the cell line which were commercially available in cell culture experiments, not with human primary cells. The ethics registration and informed consent for this study were waived.

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