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LETTERS

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# Folic acid ameliorated sevoflurane exposure-induced decrease in differentiation capacity of oligodendrocyte precursor cells

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Dear Editor,

During early childhood, the human nervous system's growth is particularly essential, and myelination is a crucial element of this. Myelination is linked to the development of children's perception, motor skills, learning, and memory [1]. Research has shown that sevoflurane anesthesia can lead to detriments in myelination and cognitive performance in mice [2]. Folate, a B vitamin of utmost importance, is necessary for a range of cellular activities and healthy growth. Our previous studies have indicated that young patients undergoing surgery with sevoflurane anesthesia tend to have lower blood folate levels, and preoperative folate supplementation can help reduce the neurotoxicity caused by sevoflurane [2]. To explore sevoflurane's effect on the nervous system and its toxicity on the development of myelination, we used a primary oligodendrocyte cell line as our research subject and looked into folic acid's potential protective effects on oligodendrocytes.

To explore the effect of folate on oligodendrocyte precursor cells (OPCs) in the differentiation of newborn rats, we conducted a series of experiments. To examine the effect of OPC differentiation, OPCs initially taken from the brain tissue of newborn rats were exposed to two varying concentrations of folate, 10 μM and 50 μM.

We were delighted to observe a marked increase in OPC differentiation when folate was added, as evidenced by immunofluorescence (Fig. 1A, B). Folate appears to be essential for advancing the transformation of OPCs into fully developed oligodendrocytes. We did not observe any noteworthy distinctions in the capability to differentiate OPCs when different doses of folate were used. The results of this experiment indicate that folate may have a promotion effect that is independent of dose within the tested dosage ranges, which has implications for improving folate applications.

As a point of comparison, we also included thyroid hormone 3,3',5-triiodothyronine (T3) as a positive control group. T3, a thyroid hormone, plays an essential role in the development of neural cells [3, 4]. By contrasting the folate-treated group with the T3 positive control group (Fig. 1A, B), we can better understand the effects of folate on OPC differentiation and assess its potency compared to the neural cell differentiation (Fig. 1A, B).

The purpose of our study was to investigate the regulatory processes responsible for sevoflurane anesthesia-induced myelination impairment and the influence of sevoflurane anesthesia on folate metabolism. Our research revealed that sevoflurane anesthesia has an impact on folate metabolism, resulting in myelination defects which are mediated by ERMN [2]. Previous research has largely been conducted in a living organism. To better understand the mechanisms, we set up an experimental model in a laboratory setting. OPCs

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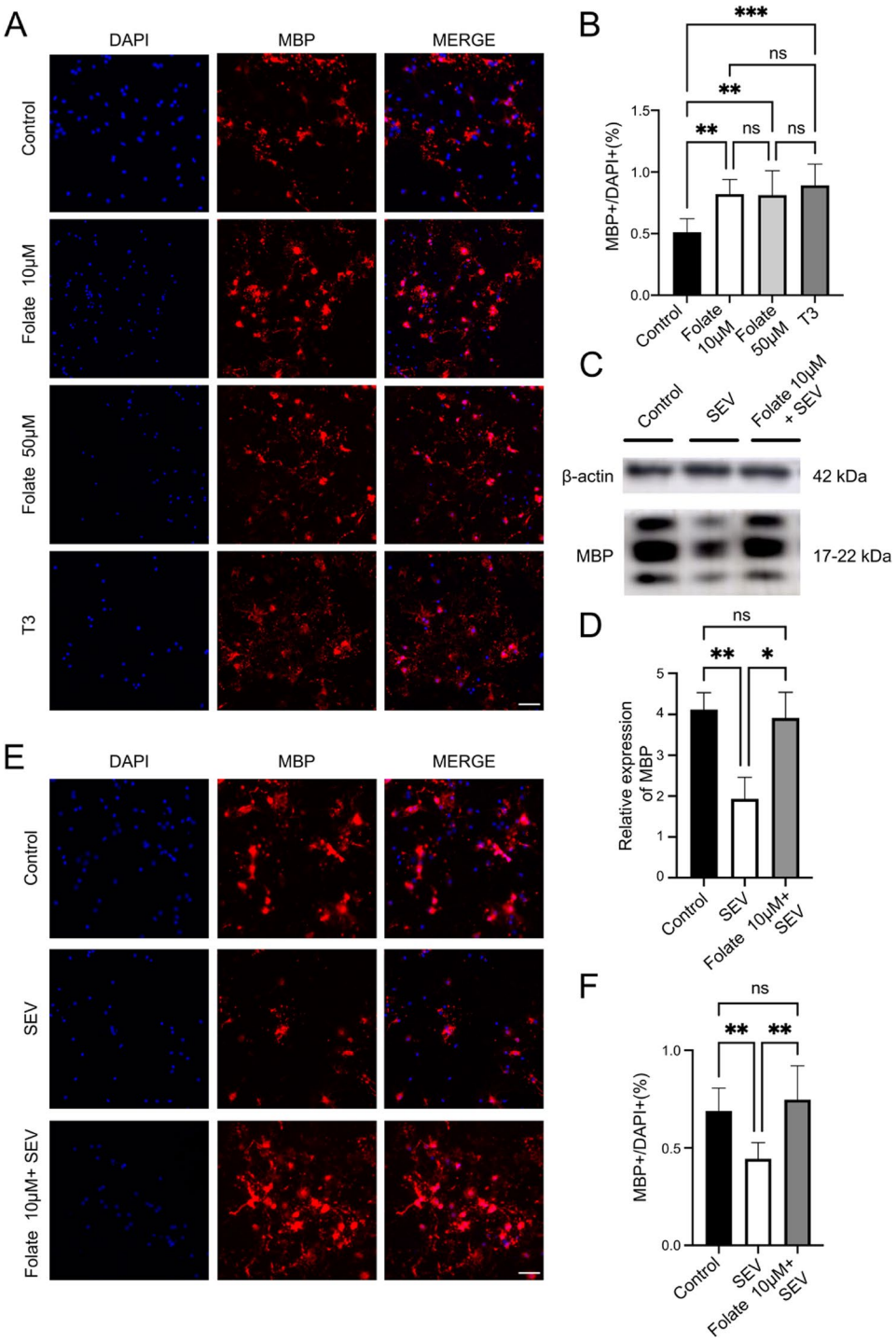


**Fig. 1** The use of Sevoflurane was found to decrease the differentiation capacity of OPC, however, this decrease was rescued by the administration of folic acid. **A** Immunofluorescent staining revealed enhanced levels of myelin basic protein (MBP) protein in OPCs (quantified by the number of MBP + cells) following the addition of folic acid. **B** The number of MBP + cells was normalized to the number of DAPI + cells ($n = 3$, control vs Folate 10 μM, $p = 0.004$; control vs Folate 50 μM, $p = 0.0083$; control vs T3, $p = 0.0008$; Folate 10 μM vs Folate 50 μM, $p = 0.9996$; Folate 10 μM vs T3, $p = 0.7944$; Folate 50 μM vs T3, $p = 0.7711$; one-way analysis of variance (ANOVA)). **C**, **D** Western blot showed the expression of MBP ($n = 3$, control vs SEV, $p = 0.0076$; control vs Folate 10 μM + SEV, $p = 0.8994$; SEV vs Folate 10 μM + SEV, $p = 0.0181$; one-way ANOVA) in the protein levels. **E** Immunofluorescent staining showed that folic acid pretreatment rescued sevoflurane-induced impairment of myelin differentiation (quantified by the number of MBP + cells). **F** The number of MBP + cells was normalized to the number of DAPI + cells ($n = 3$, control vs SEV, $p = 0.0042$; control vs Folate 10 μM + SEV, $p = 0.6747$; SEV vs Folate 10 μM + SEV, $p = 0.0012$; one-way ANOVA). The scale bar indicates 50 μm

were chosen as the research subjects due to their essential part in the formation of myelination. For three consecutive days, the primary OPCs were subjected to 2.5% sevoflurane for two hours on a daily basis. Results from multiple sevoflurane exposures indicated a significant decrease in the capacity of OPCs to differentiate (Fig. 1E, F). The findings of this study indicate that sevoflurane might have a detrimental effect on OPCs, resulting in myelination defects and consequent disruption to the normal functioning of the nervous system. With the aim of comprehending better and amplifying this effect, we opted to study the role of folate.

Following this, we pre-treated the initial OPCs with a folate solution before subjecting them to sevoflurane anesthesia. The immunofluorescence experiment was encouraging, as it showed that the differentiation capability of OPCs in the Folate 10 μM + SEV group was successfully restored (Fig. 1E, F). The Western blot results mirrored this trend, as illustrated in Fig. 1C and D. Folate pre-treatment was shown to positively affect the reduction in differentiation caused by sevoflurane. Our discovery further emphasizes the importance of folate in neural glial cell differentiation and myelination formation.

In this research, we investigated the influence of sevoflurane on an oligodendrocyte cell line and discovered that sevoflurane has a detrimental effect on the differentiation capacity of OPCs. OPCs are essential to the central nervous system, since they can transform into the cells responsible for myelination. Myelination is a process that is essential for the proper transmission and functioning of neural signals [5–8].

We conducted additional experiments to determine the effects of supplementing folate while under sevoflurane exposure. We were pleased to find that folate could reverse the negative effect of sevoflurane on the differentiation of OPCs. Research has suggested that folate administration can reduce neuroinflammation and demyelination, thus stimulating the regeneration and repair of impaired nerve fibers [9]. It is possible that folate may be able to lessen the neurotoxic effects of sevoflurane, thus preserving neural cells and their associated growth. These discoveries have far-reaching implications for research into neurological disorders and advancing treatments for neurodegenerative diseases.

The recent research findings offer a greater insight into sevoflurane's neurotoxicity mechanisms and provide useful hints for developing preventive measures against anesthesia-induced neural damage. Our findings provide valuable information on the neurotoxicity of sevoflurane, however, further in vivo experiments and clinical trials are necessary to validate the efficacy and safety of folate in clinical applications. It is essential to be mindful when utilizing the findings of this research as a reference or source of motivation for further investigations into anesthetics and neuroprotection.

**Abbreviations**

| | |
|---|---|
| ANOVA | Analysis of variance |
| MBP | Myelin basic protein |
| OPCs | Oligodendrocyte precursor cells |

## Supplementary Information

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**Supplementary Material 1.**

**Authors' contributions**

Conceptualization, Y.C and L.Z; Methodology, L.Z., L.S, and Z.M; Writing, Original Draft Preparation, Y.C and L.Z; Writing, Review & Editing, Y.C and L.Z.

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**Availability of data and materials**

Further inquiries can be directed to the corresponding authors.

## Declarations

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

All authors gave their content for publication.

**Competing interests**

The authors declare no competing interests.

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