Hyperbaric Oxygen Therapy Alleviates the Autoimmune Encephalomyelitis via the Reduction of IL-17a and GM-Csf Production of Autoreactive T Cells as Well as Boosting the Immunosuppressive IL-10 in the Central Nervous System Tissue Lesions

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Abstract

Multiple sclerosis (MS) is a chronic autoimmune disease mainly caused by autoreactive T cells, followed by neuronal demyelination and disabling paralysis. Hyperbaric oxygen therapy (HBOT) is usually an adjunct to therapy for the treatment of neurological disorders. However, it remains still controversial whether HBOT is an effective option for the treatment of MS. Experimental autoimmune encephalomyelitis (EAE) is a well-studied mouse model investigated for the MS pathogenesis and the efficacy of the therapeutic intervention. Both encephalitogenic Th1 and Th17 are pivotal T cell subsets immunopathogenically producing several disease-initiating/modifying cytokines in the central nervous system (CNS) lesions to further exacerbate/ameliorate the progression of EAE or MS. However, it remains unclear whether HBOT modulates the context of T helper cell subsets in CNS lesions. We employed EAE in the presence of HBOT to assess whether disease amelioration is attributed to alterations of CNS-infiltrating T cell subsets. Our results demonstrated that semi-therapeutic HBOT significantly alleviated the progression of EAE, at least, via the suppression of Th17 response, the downregulation of CD4 T helper cells expressing GM-CSF or TNF-α, and the boosting

of immunomodulatory IL-4 or IL-10-expressed CD4 T cells in the CNS lesions. Conclusively, HBOT attenuated EAE through the modulation of T cell responses in an earlier stage.

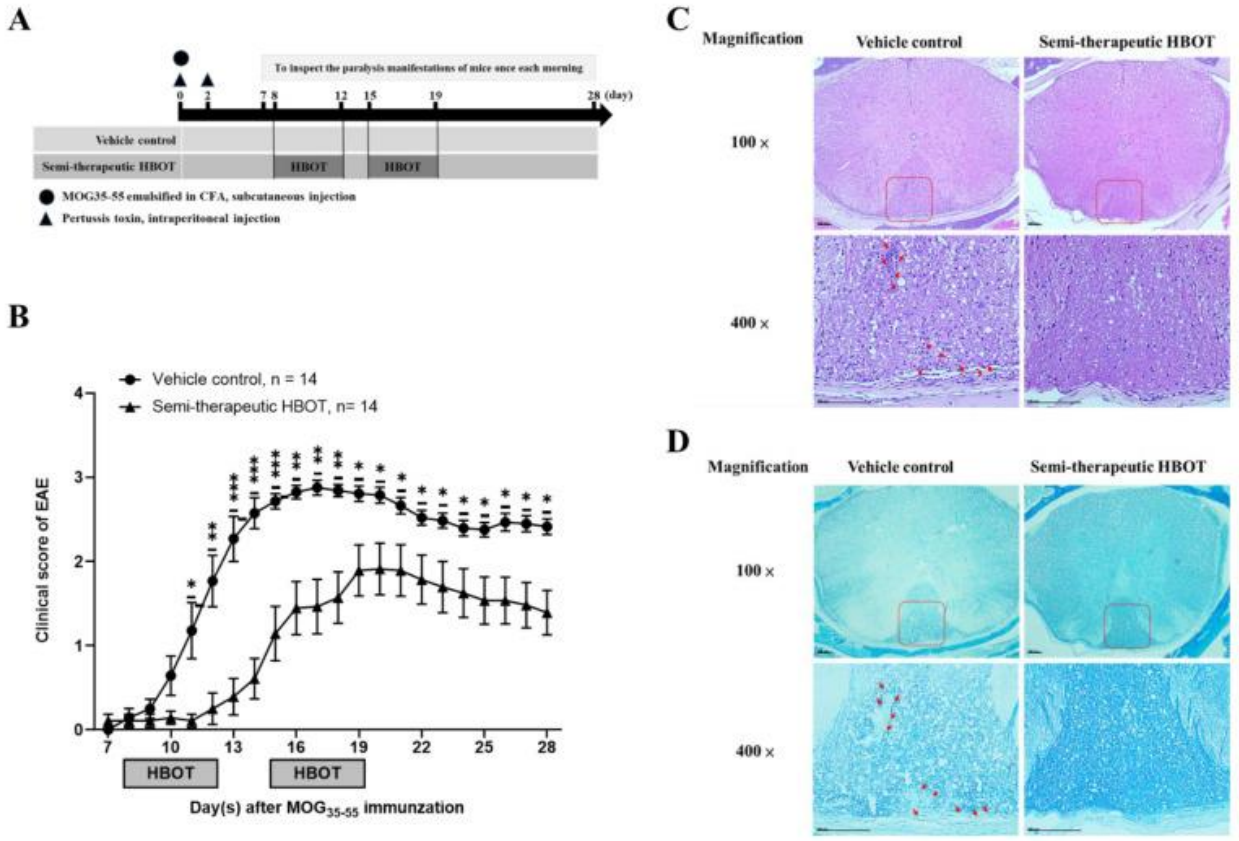

Figure 1 HBOT effectively ameliorated the disease progressions of MOG35–55-immunized EAE and improved pathological outcomes of leukocyte infiltration and demyelination. (A) Schematic diagram of the procedures of MOG-induced EAE in mice treated with semi-therapeutic HBOT or vehicle control. (B) Clinical scores of EAE in mice receiving HBOT (n = 14, filled circle) or vehicle control (n = 14, filled triangle). Clinical manifestations and disease progression of EAE were monitored once in the morning per day from day 7 to 28 after MOG immunization. All data are representative of three independent experiments and were presented as mean ± SEM from fourteen mice in each group. * < 0.05, ** <0.001, or *** < 0.0001 was analyzed by the two-way ANOVA test. (C,D) Pathological analysis of (C) H&E and (D) LFB stains of the thoracic spinal cord tissue cross-sections from EAE mice in the absence or presence of HBOT. The thoracic spinal cords were dissected from EAE mice on day 14 after MOG immunization and subsequently were subjected to the procedures of histochemical staining. Representative images are representative of two independent experiments, with at least six mice in each group. The parenchymal mononuclear leukocyte infiltration and vacuolization were displayed as red arrows in the H&E-stained tissue sections (C). The signs of neuronal demyelination were indicated as red arrows in the LFB-stained tissue sections (D). The original magnifications of images taken by the inverted light microscopy were shown as ×100 and ×400. The scale bars were shown as 100 μm in pictures.

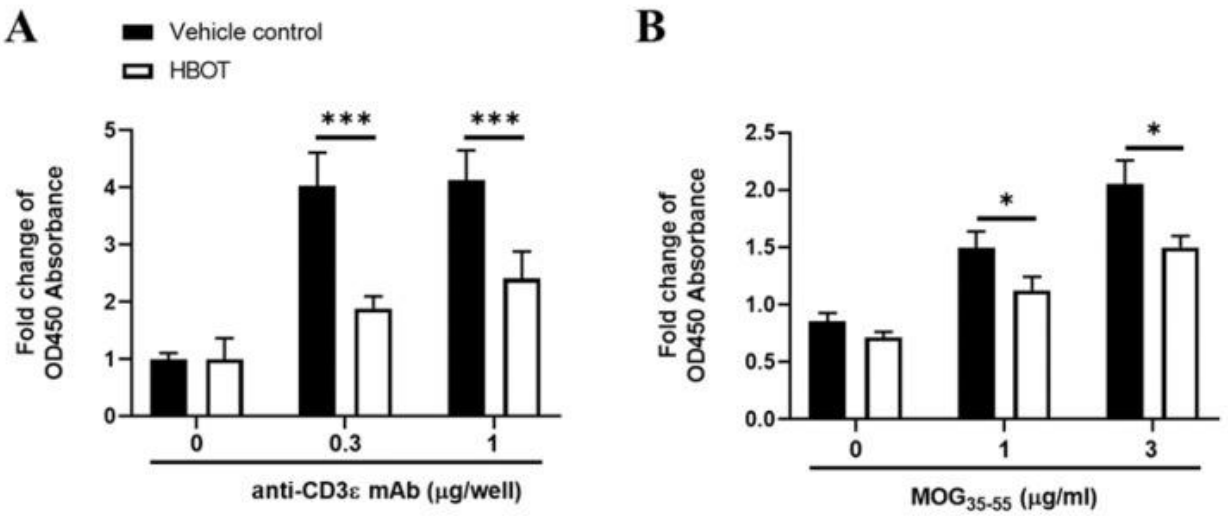

Figure 2 HBOT significantly downregulated the cell expansions of splenic T cells in vitro in the presence of TCR signaling or MOG autoantigen. (A,B) The proliferation of splenic T cells was measured in the presence of stimulation by TCR signaling (A) or autoantigen (B), respectively. The single-cell suspensions were isolated from the spleen of EAE mice with (open bar) or without (closed bar) HBOT on day 14 after MOG immunization. Cells were counted then seeded as 5 × 105 per well in a 96-well plate in the presence of the concentrations of 0, 0.3, or 1 μg anti-CD3ε mAb per well for a total culture time of 48 h, respectively. Besides, those isolated single-cell suspensions, pooled with lymphocytes from inguinal lymph nodes, were stimulated with MOG autoantigen by the concentration of 0, 1, or 3 μg/mL MOG35–55 peptides for a total stimulation of 72 h, respectively. The cell numbers of cultured splenic T cells were measured by the CCK-8 colorimetric assay kit. The absorbance of OD at 450 nm was measured by a microplate reader and the fold change of absorbance was further calculated by the normalization to the negative control. All data are representative of three independent experiments and were presented as mean ± SEM. The two-way ANOVA test was employed for the statistical analysis. * < 0.05, *** < 0.0001.

Keywords: CNS; EAE; GM-CSF; IL-10; Th17; hyperbaric oxygen; multiple sclerosis.

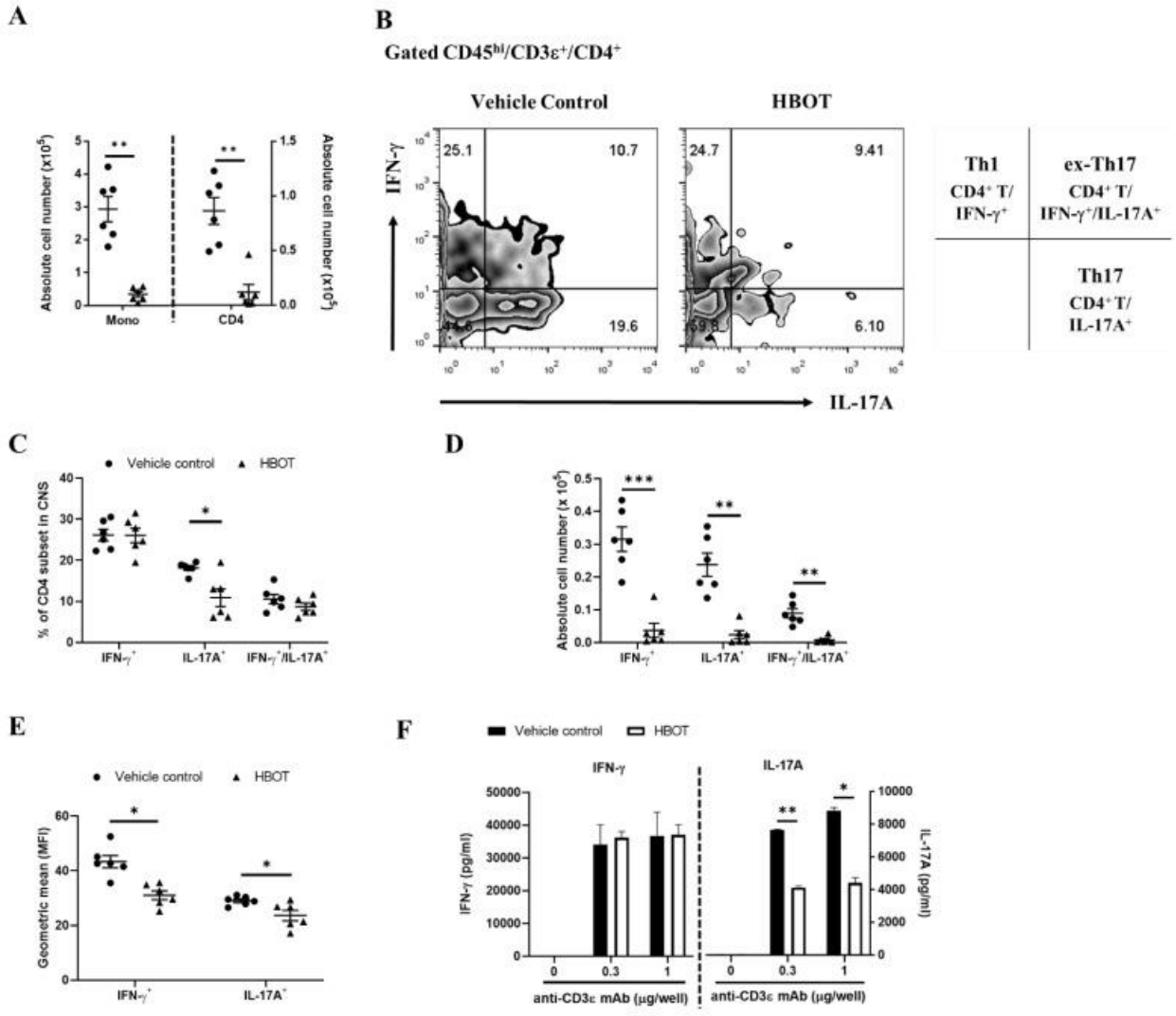

Figure 3 HBOT notably dampened the frequency of the Th17 subset and the signature cytokine expressions of both Th1 and Th17 in the CNS lesions. On day 14 after MOG immunization, the total numbers of parenchymal mononuclear cells and CD4 T cells (A) and the representative flow cytometric plots (B), the percentages (C), cell numbers (D), or geometric mean of fluorescence index (E) of cytokine-producing CD4 subsets were measured in the CNS lesions of EAE mice in the absence (filled circle) or presence (filled triangle) of HBOT, respectively. Those data of IFN-γ+ (Th1), IL-17A+ (Th17), and IFN-γ+/IL-17A+ (ex-Th17) CD4 subsets were shown in panel A, B, and C, respectively. All data are representative of two independent experiments and were presented as mean ± SEM from six mice in each group. The two-way ANOVA test was used for the statistical analysis. * < 0.05, **< 0.001, *** < 0.0001. (F) The IFN-γ and IL-17A productions of splenic T cells in culture supernatants were measured in the presence of TCR stimulations. The single-cell suspensions were isolated from the spleen of EAE mice with (open bar) or without (closed bar) HBOT on day 14 after MOG immunization. The splenic single-cell suspensions were counted then seeded as 2 × 106 per well of 12-well plate. Subsequently, those cell suspensions were stimulated by TCR signaling in the presence of coating anti-CD3ε mAb of 0, 0.3, or 1 μg per well for a total culture of 48 h, respectively. Accordingly, culture supernatants were harvested and immediately stored at −80 °C refrigerators temporarily. Those cytokine concentrations of IFN-γ and IL-17A in culture supernatants were measured by the ELISA assay kits, respectively. All data are representative of three independent experiments and were presented as mean ± SEM. The two-way ANOVA test was used for the statistical analysis. * < 0.05, **< 0.001.

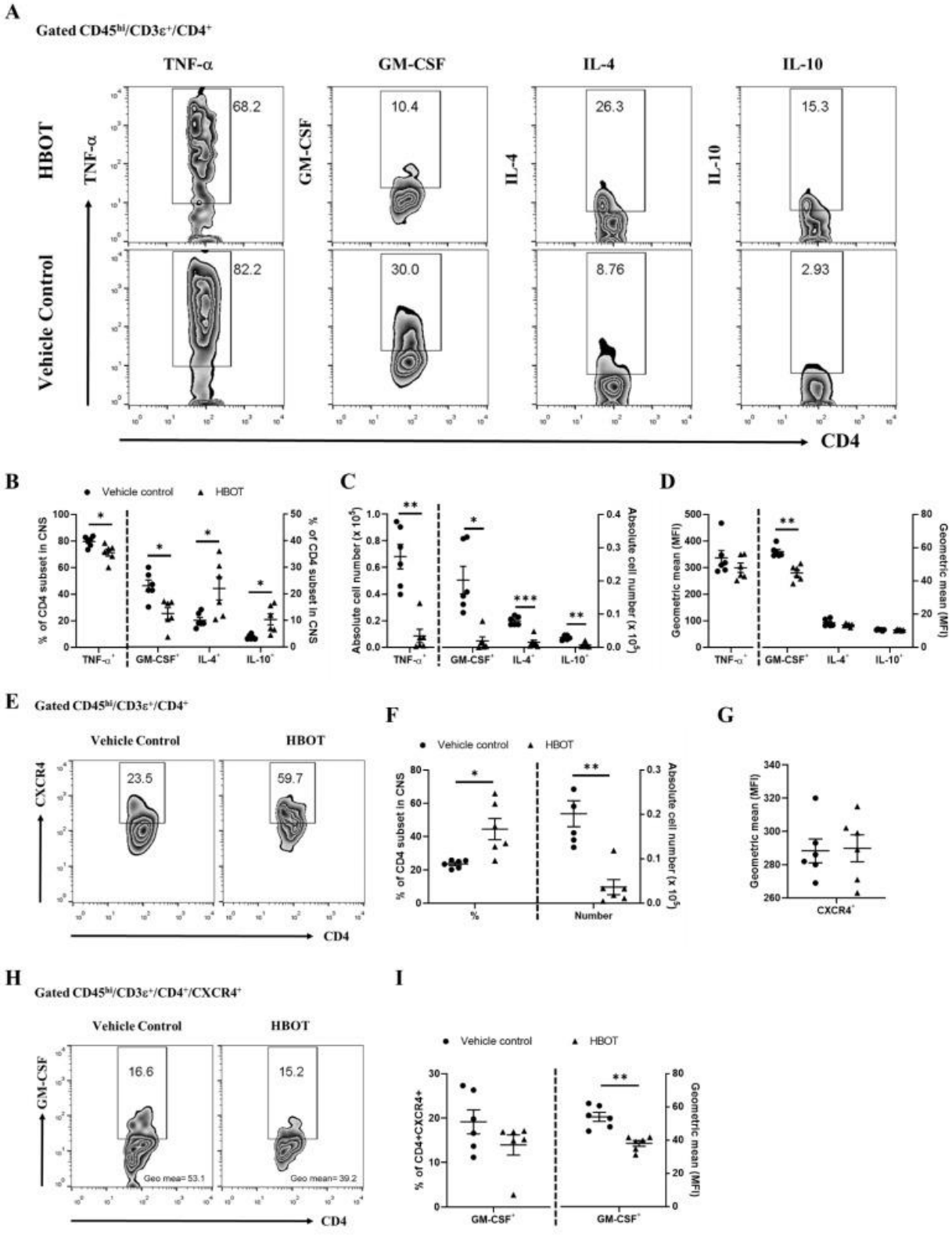
A
Gated CD45hi/CD3ε+/CD4+
TNF-α
GM-CSF
IL-4
IL-10
HBOT
Vehicle Control
68.2
10.4
26.3
15.3
82.2
30.0
8.76
2.93
CD4
B
Vehicle control
HBOT
% of CD4 subset in CNS
% of CD4 subset in CNS
TNF-α+
GM-CSF+
IL-4+
IL-10+
C
Absolute cell number (x 10⁵)
Absolute cell number (x 10⁵)
D
Geometric mean (MFI)
Geometric mean (MFI)
E
Gated CD45hi/CD3ε+/CD4+
Vehicle Control
HBOT
CXCR4
23.5
59.7
CD4
F
Vehicle control
HBOT
% of CD4 subset in CNS
Absolute cell number (x 10⁵)
%
Number
G
Geometric mean (MFI)
CXCR4+
H
Gated CD45hi/CD3ε+/CD4+/CXCR4+
Vehicle Control
HBOT
GM-CSF
16.6
15.2
Geo mean= 53.1
Geo mean= 39.2
CD4
I
Vehicle control
HBOT
% of CD4+CXCR4+
Geometric mean (MFI)
GM-CSF+
GM-CSF+

Figure 4 HBOT markedly attenuated the contexts of TNF-α and GM-CSF pro-inflammatory cytokines, as well as augmented the frequencies of IL-4 and IL-10 anti-inflammatory cytokines, in the CNS lesions-infiltrating CD4 T cell subsets. On day 14 after MOG35–55 immunization, the representative flow cytometric plots (A), the percentages (B), cell numbers (C), or geometric mean of fluorescence index (D) of cytokine-producing CD4 subsets were measured in the CNS lesions of EAE mice with (filled triangle) or without (filled circle) HBOT, respectively. The pro-inflammatory cytokines, TNF-α and GM-CSF, as well as anti-inflammatory cytokines, IL-4 and IL-10, were measured in the CD4 T cell subsets of the CNS lesions. All data are representative of two independent experiments and were presented as mean ± SEM from six mice in each group. The two-way ANOVA test was used for the statistical analysis. * < 0.05, **< 0.001. (E–G) The context of the CXCR4-expressing CD4 T cell subset was explored in the CNS lesions of EAE mice. On the day 14 after MOG immunization, the representative flow cytometric plots (E), the percentages ((F), left panel), absolute cell numbers ((F), right panel), and geometric mean fluorescence index (G) of CXCR4 in the CNS-infiltrating CD4 T cell subsets were measured in HBOT- or vehicle control-treated EAE mice. (H,I) The expression of GM-CSF in CXCR4-positive CD4 T cell subset. On the day 14 after MOG immunization, the representative flow cytometric plots (H), the frequencies ((I), left panel), or geometric mean of fluorescence index ((I), right panel) of GM-CSF in CD4 T cell subset were measured in the CNS lesions of HBOT-treated and vehicle control-treated EAE mice, respectively. All data are representative of two independent experiments were presented as mean ± SEM from six mice in each group. The two-way ANOVA test was used for the statistical analysis. * < 0.05, **< 0.001.

Conflict of interest statement

The authors declare no conflict of interest.