Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) *2024 25(12):1108-1114*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Correspondence

https://doi.org/10.1631/jzus.B2300790

Check for updates

Olig2$^+$ single-colony-derived cranial bone-marrow mesenchymal stem cells achieve improved regeneration in a cuprizone-induced demyelination mouse model

Deqing PENG[1*], Ruijie LU[2*], Leyao LÜ[2], Qing YAO[3], Kaichuang YANG[1], Yunfeng XU[4], Xiaoming FENG[4✉], Ruolang PAN[5], Yuyuan MA[1✉]

[1]*Center for Rehabilitation Medicine, Department of Neurosurgery, Zhejiang Provincial People's Hospital (Affiliated People's Hospital, Hangzhou Medical College), Hangzhou 310014, China*
[2]*Second Clinical Medical College, Wenzhou Medical University, Wenzhou 325035, China*
[3]*School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou 325035, China*
[4]*Department of Neurosurgery, the First Hospital of Jiaxing (Affiliated Hospital of Jiaxing University), Jiaxing 314001, China*
[5]*Key Laboratory of Cell-Based Drug and Applied Technology Development in Zhejiang Province, Hangzhou 311122, China*

Oligodendrocytes are the myelinating cells of the central nervous system. Brain injury and neurodegenerative disease often lead to oligodendrocyte death and subsequent demyelination-related pathological changes, resulting in neurological defects and cognitive impairment (Spaas et al., 2021; Zhang J et al., 2022). Multiple sclerosis (MS) is a major demyelinating disease of the central nervous system. The pathology of MS is characterized by the loss of myelin, oligodendrocytes, and axons in the brain, brain stem, and spinal cord, as well as by white matter lesions (Lassmann et al., 2007). Unfortunately, no definitive cure for MS has been developed. Immunomodulatory and anti-inflammatory drugs are effective in the relapsing-remitting phase of MS because they reduce the frequency of relapses and the formation of inflammatory lesions; however, they do not alter the course of progressive MS and are insufficient to cure chronic neurological dysfunction (Xiao et al., 2015; Zhang et al., 2021). The treatment outcome is even worse for MS patients with primary and secondary progressions. Mesenchymal stem cells (MSCs) are stromal cells that can self-renew and exhibit multilineage differentiation. MSCs are easy to expand in vitro and exhibit low immunogenicity, no tumorigenic risks, and ethical controversies, making them a promising candidate for regenerative medicine (Zhang L et al., 2022; Xu et al., 2023). Many studies have confirmed the neural differentiation potential of MSCs under certain conditions, making them a prime candidate for treating neurodegenerative diseases (Jang et al., 2010; Yan et al., 2013). The present study investigated the effects of cranial bone-marrow mesenchymal stem cells (cBMMSCs) and oligodendrocyte-specific protein 2-positive (Olig2$^+$) single-colony-derived cBMMSC (sc-cBMMSC), isolated in our previous work (Yang et al., 2022), in a central nervous system demyelination mouse model.

First, we established the mouse model of central nervous system demyelination by feeding mice with 0.2% (2 g/L) cuprizone (CPZ) for six weeks (see supplementary "Materials and methods" for the specific methods) (Hedayatpour et al., 2013), and the animals were randomly divided into a model group, vehicle group, cBMMSC group, and Olig2$^+$ sc-cBMMSC group (n=7). The cBMMSCs and sc-cBMMSCs were isolated from the mononuclear cell content of cranial bones by adherent culture and passaging. To establish an sc-cBMMSC isolate, individual colonies were identified under a phase-contrast microscope and harvested using sterile cloning cylinders (see supplementary "Materials and methods" for the specific methods). Cells at the 5th passage were used in the following treatments.

✉ Xiaoming FENG, fengxiaoming2022@163.com
Yuyuan MA, yuyuan_ma98246@163.com
* The two authors contributed equally to this work
Xiaoming FENG, https://orcid.org/0009-0004-3663-2787
Yuyuan MA, https://orcid.org/0000-0001-7193-6023

Received Oct. 30, 2023; Revision accepted Apr. 24, 2024;
Crosschecked Sept. 18, 2024; Published online Sept. 25, 2024

The mice were administered cell isolates via tail vein at 1×10^6 cells per 100 μL phosphate-buffered saline (PBS) once a week for two weeks. One week after the completion of treatment, mice in each group were evaluated for the relevant indicators. In the rod-rotating experiment, the rod-rotating time of CPZ model mice was significantly shorter than that of normal (P<0.01). Both cBMMSC and sc-cBMMSC transplantation substantially improved rod-rotating time (P<0.05 and P< 0.01, respectively), and the effect was more significant in the sc-cBMMSC group (P<0.05) (Fig. 1a). The same change trend was observed in the suspension experiment; the hanging time of animals in the CPZ model group was significantly shorter than that of those in the normal group (P<0.01). Administration of cBMMSC slightly expanded mouse hanging time, but the difference was not significant (P>0.05). The animals in the sc-cBMMSC transplantation group exhibited significantly improved hanging time compared to the model group (P<0.05; Fig. 1b). We also conducted a water-maze experiment. During Days 2−5 of positioning and navigation training, the time it took for mice in each group to find the platform gradually shortened with the increasing number of training days. The results on Day 5 showed that compared with the model group, both the cBMMSC and sc-cBMMSC transplantation groups achieved significantly reduced time (P<0.01), and the effect was more significant in the sc-cBMMSC group (P<0.05) (Fig. 1c). The number of crossings for the CPZ model group was significantly lower than that for the normal group (P<0.01), and those for the cBMMSC and Olig2$^+$ sc-cBMMSC transplantation groups were significantly higher than those for the model group (P<0.05 and P< 0.01, respectively) (Fig. 1d). These findings suggest that cranial-derived MSC transplantation improves the movement coordination, muscle strength, and learning and memory abilities of CPZ mice, and that the effect of sc-cBMMSC transplantation is more pronounced.

Next, we assessed the levels of peripheral and central inflammatory factors in each group of mice (Avşar et al., 2021). The enzyme-linked immunosorbent assay (ELISA) results showed that the levels of

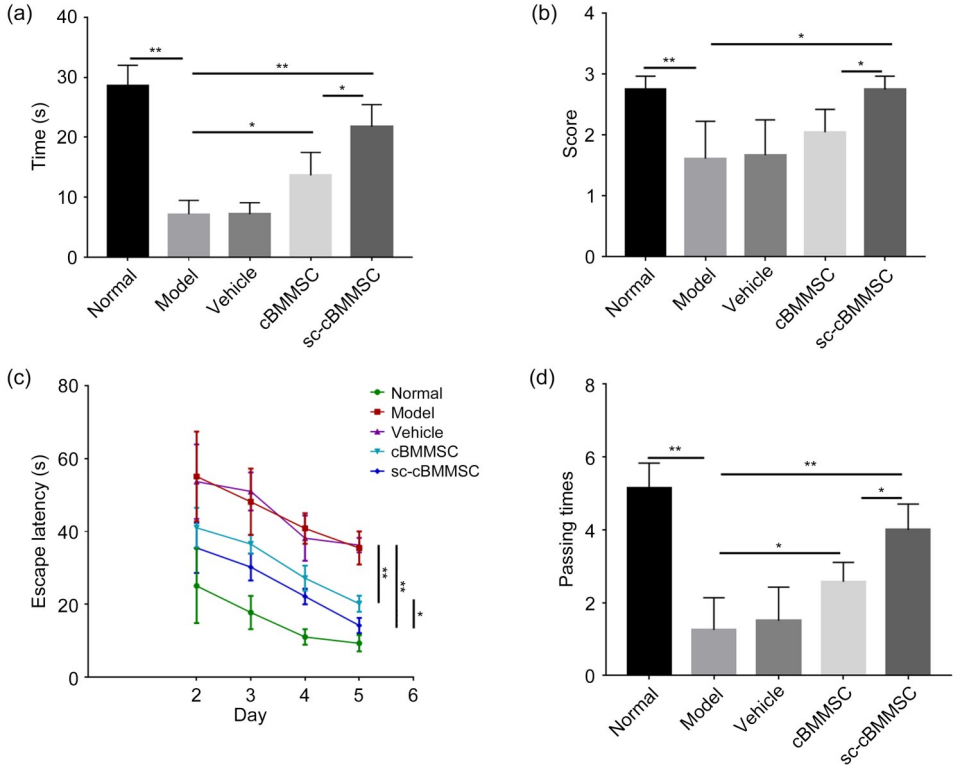

Fig. 1 Assessment of mouse behavior. (a) Rotation time for each group of mice in the rotarod experiment; (b) Hanging time for each group of mice in the suspension experiment. (c) Positioning cruise training for the water-maze experiment, with the time it took for each group of animals to find the platform at different time points. (d) Number of times the animals in each group crossed the water maze on Day 6. Data are presented as mean±standard deviation (SD), n=5. * P< 0.05; ** P<0.01. cBMMSC: cranial bone-marrow mesenchymal stem cell; sc-cBMMSC: single-colony-derived cBMMSC.

serum tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and other inflammatory factors in the peripheral blood of animals in the CPZ model group were significantly increased. MSC transplantation significantly suppressed the levels of the above inflammatory factors (P<0.05; Fig. 2a). There was no significant difference between the cBMMSC and sc-cBMMSC groups (P>0.05). The detection results for brain tissue protein were similar to those for peripheral blood (Fig. 2b). T-cell subpopulations that were sensitive to CPZ were further analyzed (Solti et al., 2015). Compared with the normal group, the proportions of cluster of differentiation 3-positive ($CD3^+$) and $CD4^+$ T cells in the peripheral blood of the CPZ model group were significantly higher, as determined by flow assays (P<0.05). In the MSC transplantation groups, the proportions of $CD3^+$ and $CD4^+$ T cells were significantly suppressed (P<0.05; Fig. 3), suggesting that these cells have excellent immune-regulatory capabilities.

Myelin basic protein (MBP), a marker of mature oligodendrocytes, is widely present in myelin sheaths (Kashani et al., 2015). MBP is essential for maintaining the stability of central nervous system (CNS) myelin-sheath structure and function (Vassall et al., 2015). Western blotting showed that MBP expression in CPZ model mice was significantly reduced (P<0.01), while MSC transplantation significantly increased MBP expression (P<0.05), especially in the sc-cBMMSC group (Figs. 4a and 4b). Hematoxylin and eosin (H&E) staining and transmission electron microscopy were employed to observe the myelin-like structure in the corpus callosum of the brain tissue of experimental animals according to previous report (el Sharouny et al., 2022). The results showed that the myelin-like structure was largely diminished in CPZ model mice. We observed myelin-like structure in the MSC transplantation groups, among which recovery was more obvious in the sc-cBMMSC group (Figs. 4c and S1a). Immunohistochemistry staining of Olig2 and Synapsin (Syn) was performed in the corpus callosum, showing that Olig2 and Syn signals were significantly decreased in CPZ model mice. The number of positive signals was increased in both MSC transplantation groups, with more obvious recovery in the sc-cBMMSC group (Figs. 4d and S1b). These results suggest that $Olig2^+$ cBMMSC transplantation can promote the regeneration of oligodendrocyte progenitor cells and nerve cells, thereby promoting the repair of myelin structure, with a more significant effect of sc-cBMMSC.

The observed improvements in motor, strength, and cognitive functions following cBMMSC and sc-cBMMSC transplantation align with previous research demonstrating the potential of stem-cell therapy in various

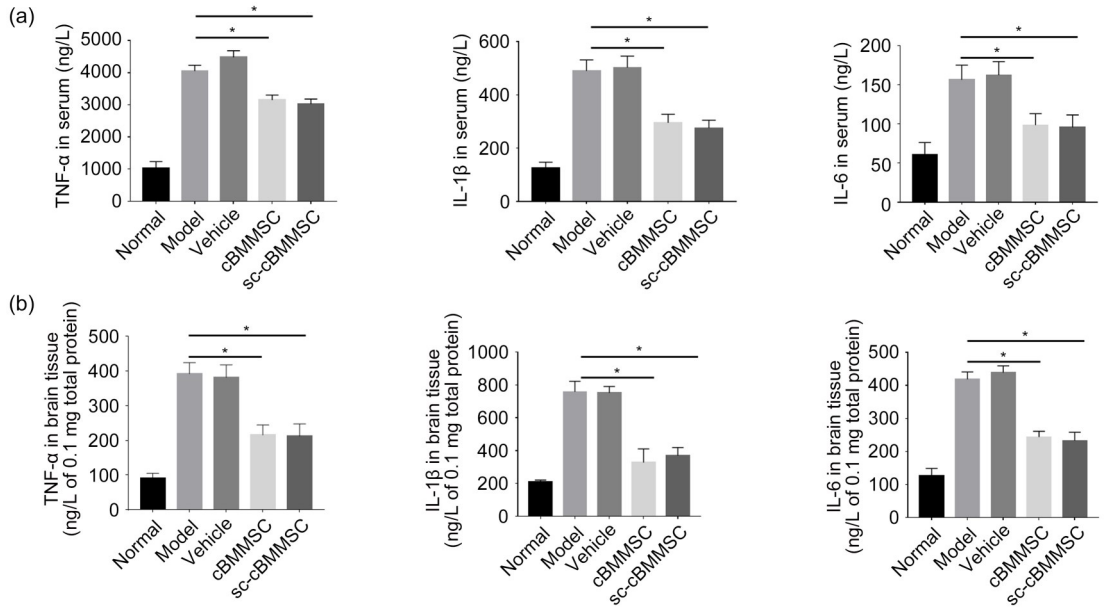

Fig. 2 Expression levels of inflammatory factors. (a) Enzyme-linked immunosorbent assay (ELISA) detection results for serum inflammatory factors. (b) ELISA detection results for brain tissue inflammatory factors. Data are presented as mean± standard deviation (SD), n=5. * P<0.05. TNF-α: tumor necrosis factor-α; IL: interleukin; cBMMSC: cranial bone-marrow mesenchymal stem cell; sc-cBMMSC: single-colony-derived cBMMSC.

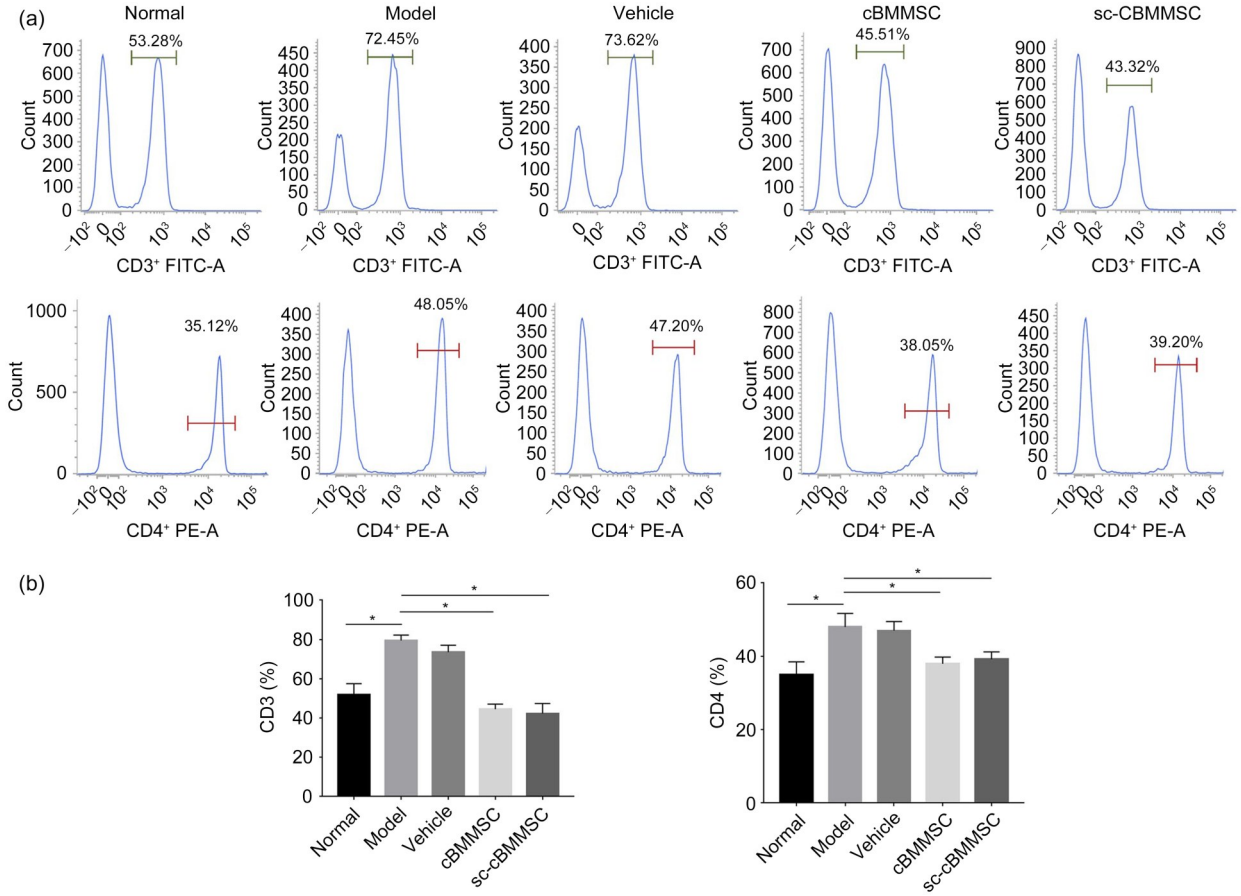

Fig. 3 Detection of T-cell ratio in peripheral blood. (a) Flow cytometry showing the proportion of $CD3^+$ and $CD4^+$ cells. (b) Statistical results for the proportion of $CD3^+$ and $CD4^+$ T cells. Data are presented as mean±standard deviation (SD), n=5. * P<0.05. $CD3^+$: cluster of differentiation 3-positive; FITC-A: fluorescein isothiocyanate-A; PE-A: phycoerythrin-A; cBMMSC: cranial bone-marrow mesenchymal stem cell; sc-cBMMSC: single-colony-derived cBMMSC.

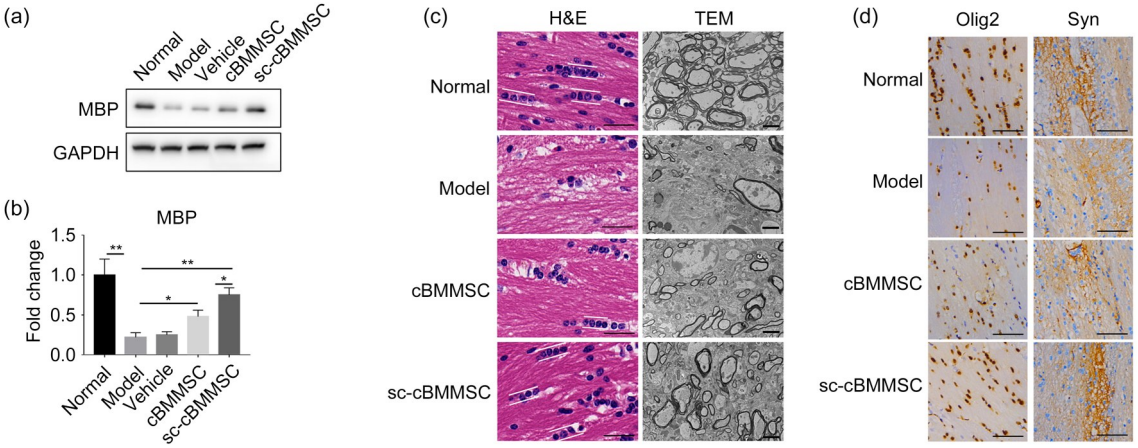

Fig. 4 Analyses of demyelination and remyelination. (a, b) Western blotting and quantitative results of myelin basic protein (MBP) expression in brain tissue. Data are presented as mean±standard deviation (SD), n=5. * P<0.05; ** P<0.01. (c) Pathological examination of brain-tissue corpus callosum. The nuclei of oligodendrocytes appear darkly stained and arranged in parallel longitudinal rows, as bracketed by lines. H&E: hematoxylin and eosin (scale bar=50 μm); TEM: transmission electron microscopy (scale bar=2 μm). (d) Immunohistochemical detection of Olig2 and Syn. Scale bar=100 μm. GAPDH: glyceraldehyde-3-phosphate dehydrogenase; cBMMSC: cranial bone-marrow mesenchymal stem cell; sc-cBMMSC: single-colony-derived cBMMSC; Olig2: odendrocyte-specific protein 2; Syn: synapsin.

neurodegenerative diseases. For instance, studies exploring stem-cell applications in pre-clinical models of neurodegenerative disorders, such as Parkinson's disease and Huntington's disease, have reported positive effects on motor control, cognitive abilities, and neurofunctional outcomes (Biglari et al., 2023; Conner et al., 2023; Kandeel et al., 2023). These findings highlight the remarkable potential of stem-cell transplantation to promote functional recovery in the context of neurodegeneration.

The immunomodulatory effects of MSCs are increasingly recognized and have been studied extensively. MSCs have been shown to secrete a wide range of bioactive molecules that can exert potent immunosuppressive effects, such as reducing pro-inflammatory cytokines while promoting the release of anti-inflammatory factors (Eldaly et al., 2022; Lyamina et al., 2023). The reduction observed in TNF-α, IL-1β, and IL-6 levels in the peripheral and central nervous systems of the model mice in this study is in agreement with the known immunomodulatory capabilities of MSCs (Mo et al., 2022). By suppressing inflammatory response, cBMMSC and sc-cBMMSC transplantation may attenuate immune-mediated damage and promote a suitable environment for tissue repair.

The potential of MSCs to facilitate myelin repair has been a significant area of interest. Studies have also underscored the critical role of MSCs in disease modification and myelin restoration beyond symptomatic treatment in various demyelinating disorders, including MS (Allegretta et al., 2022; Islam et al., 2023; Zhang et al., 2023). The increase in MBP expression and the formation of myelin-like structures observed here further support the regenerative potential of cBMMSC and sc-cBMMSC transplantation. Our findings highlight the ability of these stem cells to promote the repair and formation of myelin sheaths, which are crucial for restoring normal neuronal communication and function.

Furthermore, the added value of using Olig2$^+$ sc-cBMMSCs that became apparent in this study corresponds with emerging research emphasizing the importance of enhancing the purity and specificity of stem-cell populations; recent studies have demonstrated that the enrichment or selection of specific cell subpopulations, such as Olig2$^+$ cells, can enhance the therapeutic efficacy of stem-cell-based therapies (Yang et al., 2022). By focusing on specific stem-cell subtypes, researchers can potentiate the desired therapeutic effects for improved outcomes (Kashani et al., 2023).

Overall, the findings of this study contribute to the growing body of evidence supporting the therapeutic and immunomodulatory potential of stem cells, particularly cBMMSC and Olig2$^+$ sc-cBMMSC, in the context of demyelinating diseases like MS. Our results highlight the potential of this avenue for future clinical applications and underline the need for further research. This should include well-designed clinical trials to validate these findings and translate them into effective therapies for patients in need. The expanding knowledge in this field fosters optimism about harnessing the regenerative capabilities of stem cells to address the complex challenges posed by neurodegenerative diseases.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Acknowledgments

This work was supported by the Zhejiang Provincial Natural Science Foundation of China (Nos. LQ21H090016 and LTGY23H090016).

Author contributions

All authors contributed to the conception and design of this study. Deqing PENG and Ruijie LU designed the experiment and drafted the manuscript. Leyao LÜ, Qing YAO, and Kaichuang YANG conducted the study. Yunfeng XU analyzed the data. Ruolang PAN revised the manuscript. Xiaoming FENG and Yuyuan MA designed the experiment and monitored the project progression. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of such data.

Compliance with ethics guidelines

Deqing PENG, Ruijie LU, Leyao LÜ, Qing YAO, Kaichuang YANG, Yunfeng XU, Xiaoming FENG, Ruolang PAN, and Yuyuan MA declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed. This study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital (October 10, 2019; No. 2019KY15).

References

Allegretta C, D'Amico E, Manuti V, et al., 2022. Mesenchymal stem cell-derived extracellular vesicles and their therapeutic use in central nervous system demyelinating disorders. *Int J Mol Sci*, 23(7):3829. https://doi.org/10.3390/ijms23073829

Avşar T, Erdem GÇ, Terzioğlu G, et al., 2021. Investigation of neuro-inflammatory parameters in a cuprizone induced mouse model of multiple sclerosis. *Turk J Biol*, 45(5):644-655. https://doi.org/10.3906/biy-2104-88

Biglari N, Mehdizadeh A, Vafaei Mastanabad M, et al., 2023. Application of mesenchymal stem cells (MSCs) in neurodegenerative disorders: history, findings, and prospective challenges. *Pathol Res Pract*, 247:154541. https://doi.org/10.1016/j.prp.2023.154541

Conner LT, Srinageshwar B, Bakke JL, et al., 2023. Advances in stem cell and other therapies for Huntington's disease: an update. *Brain Res Bull*, 199:110673. https://doi.org/10.1016/j.brainresbull.2023.110673

Eldaly AS, Mashaly SM, Fouda E, et al., 2022. Systemic anti-inflammatory effects of mesenchymal stem cells in burn: a systematic review of animal studies. *J Clin Transl Res*, 8(4):276-291. https://doi.org/10.18053/JCTRES.08.202204.003

el Sharouny SH, Shaaban MH, Elsayed RM, et al., 2022. N-acetylcysteine protects against cuprizone-induced demyelination: histological and immunohistochemical study. *Folia Morphol (Warsz)*, 81(2):280-293. https://doi.org/10.5603/FM.a2021.0044

Hedayatpour A, Ragerdi I, Pasbakhsh P, et al., 2013. Promotion of remyelination by adipose mesenchymal stem cell transplantation in a cuprizone model of multiple sclerosis. *Cell J*, 15(2):142-151.

Islam MA, Alam SS, Kundu S, et al., 2023. Mesenchymal stem cell therapy in multiple sclerosis: a systematic review and meta-analysis. *J Clin Med*, 12(19):6311. https://doi.org/10.3390/jcm12196311

Jang S, Cho HH, Cho YB, et al., 2010. Functional neural differentiation of human adipose tissue-derived stem cells using bFGF and forskolin. *BMC Cell Biol*, 11:25. https://doi.org/10.1186/1471-2121-11-25

Kandeel M, Morsy MA, Alkhodair KM, et al., 2023. Mesenchymal stem cell-derived extracellular vesicles: an emerging diagnostic and therapeutic biomolecules for neurodegenerative disabilities. *Biomolecules*, 13(8):1250. https://doi.org/10.3390/biom13081250

Kashani IR, Hedayatpour A, Pasbakhsh P, et al., 2015. Progesterone enhanced remyelination in the mouse corpus callosum after cuprizone induced demyelination. *Iran J Med Sci*, 40(6):507-514.

Kashani SA, Navabi R, Amini A, et al., 2023. Immunomodulatory potential of human clonal mesenchymal stem cells and their extracellular vesicle subpopulations in an inflammatory-mediated diabetic rhesus monkey model. *Life Sci*, 329:121950. https://doi.org/10.1016/j.lfs.2023.121950

Lassmann H, Brück W, Lucchinetti CF, 2007. The immunopathology of multiple sclerosis: an overview. *Brain Pathol*, 17(2):210-218. https://doi.org/10.1111/j.1750-3639.2007.00064.x

Lyamina S, Baranovskii D, Kozhevnikova E, et al., 2023. Mesenchymal stromal cells as a driver of inflammaging. *Int J Mol Sci*, 24(7):6372. https://doi.org/10.3390/ijms24076372

Mo QY, Zhang W, Zhu AJ, et al., 2022. Regulation of osteogenic differentiation by the pro-inflammatory cytokines IL-1β and TNF-α: current conclusions and controversies. *Hum Cell*, 35(4):957-971. https://doi.org/10.1007/s13577-022-00711-7

Solti I, Kvell K, Talaber G, et al., 2015. Thymic atrophy and apoptosis of $CD4^+CD8^+$ thymocytes in the cuprizone model of multiple sclerosis. *PLoS ONE*, 10(6):e0129217. https://doi.org/10.1371/journal.pone.0129217

Spaas J, van Veggel L, Schepers M, et al., 2021. Oxidative stress and impaired oligodendrocyte precursor cell differentiation in neurological disorders. *Cell Mol Life Sci*, 78(10):4615-4637. https://doi.org/10.1007/s00018-021-03802-0

Vassall KA, Bamm VV, Harauz G, 2015. Myelstones: the executive roles of myelin basic protein in myelin assembly and destabilization in multiple sclerosis. *Biochem J*, 472(1):17-32. https://doi.org/10.1042/BJ20150710

Xiao J, Yang RB, Biswas S, et al., 2015. Mesenchymal stem cells and induced pluripotent stem cells as therapies for multiple sclerosis. *Int J Mol Sci*, 16(5):9283-9302. https://doi.org/10.3390/ijms16059283

Xu YZ, Fan P, Liu L, et al., 2023. Novel perspective in transplantation therapy of mesenchymal stem cells: targeting the ferroptosis pathway. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 24(2):115-129. https://doi.org/10.1631/jzus.B2200410

Yan ZJ, Hu YQ, Zhang HT, et al., 2013. Comparison of the neural differentiation potential of human mesenchymal stem cells from amniotic fluid and adult bone marrow. *Cell Mol Neurobiol*, 33(4):465-475. https://doi.org/10.1007/s10571-013-9922-y

Yang KC, Lu RJ, Lu JN, et al., 2022. Phenotypic and functional characterizations of mesenchymal stem/stromal cells isolated from human cranial bone marrow. *Front Neurosci*, 16:909256. https://doi.org/10.3389/fnins.2022.909256

Zhang J, Buller BA, Zhang ZG, et al., 2022. Exosomes derived from bone marrow mesenchymal stromal cells promote remyelination and reduce neuroinflammation in the demyelinating central nervous system. *Exp Neurol*, 347:113895. https://doi.org/10.1016/j.expneurol.2021.113895

Zhang L, Li Y, Dong YC, et al., 2022. Transplantation of um-

bilical cord-derived mesenchymal stem cells promotes the recovery of thin endometrium in rats. *Sci Rep*, 12:412. https://doi.org/10.1038/s41598-021-04454-7

Zhang RB, Liu J, Xu B, et al., 2021. Cornuside alleviates experimental autoimmune encephalomyelitis by inhibiting Th17 cell infiltration into the central nervous system. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 22(5):421-430. https://doi.org/10.1631/jzus.B2000771

Zhang YY, Gu JB, Wang XS, et al., 2023. Opportunities and challenges: mesenchymal stem cells in the treatment of multiple sclerosis. *Int J Neurosci*, 133(9):1031-1044. https://doi.org/10.1080/00207454.2022.2042690

Supplementary information

Materials and methods; Fig. S1