Original Article

Dimethyl Fumarate Preconditioning can Reinforce the Therapeutic Potential of Bone Marrow Mesenchymal Stem Cells through Trophic Factor Profile Enhancement

Sareh Pandamooz[1], Anahid Safari[1], Nasrin Ghorbani[2], Iman Jamhiri[3], Shahrokh Zare[1], Ivaldo Jesus Almeida Belém-Filho[4], Parisa Dolati[1], Mohammad Saied Salehi[5]

[1]Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [2]Department of Nursing, College of Nursing, Lebanese French University, Erbil, Kurdistan, Iraq, [3]Molecular Dermatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [4]Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil, [5]Clinical Neurology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Numerous studies have confirmed the therapeutic efficacy of bone marrow-derived mesenchymal stem cells (BM-MSCs) in addressing neurologic disorders. To date, several preconditioning strategies have been designed to improve the therapeutic potential of these stem cells. This study was designed to evaluate the preconditioning effect of dimethyl fumarate (DMF) on the expression of main trophic factors in human BM-MSCs.

Materials and Methods: Initially, the identity of stem cells was confirmed through the evaluation of surface markers and their capacity for osteogenic and adipogenic differentiation using flow cytometry and differentiation assay, respectively. Subsequently, stem cells were subjected to different concentrations of DMF for 72 hours and their viability was defined by MTT assay. Following 72-hour preconditioning period with 10 μM DMF, gene expression was assessed by quantitative RT-PCR.

Results: Our findings demonstrated that the isolated stem cells expressed cardinal MSC surface markers and exhibited osteogenic and adipogenic differentiation potential. MTT results confirmed that 10 μM DMF was an optimal dose for maintaining cell viability. Preconditioning of stem cells with DMF significantly upregulated the expression of *BDNF*, *NGF*, and *NT-3*. Despite a slight increase in transcript level of *GDNF* and *VEGF* after DMF preconditioning, this difference was not statistically significant.

Conclusions: Our findings suggest that DMF preconditioning can enhance the expression of major neurotrophic factors in human BM-MSCs. Given the curative potential of both BM-MSCs and DMF in various neurological disease models and preconditioning outcomes, their combined use may synergistically enhance their neuroprotective properties.

Keywords: Bone marrow mesenchymal stem cells, dimethyl fumarate, neurological diseases, preconditioning, trophic factors

Address for correspondence: Dr. Sareh Pandamooz, Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail: sareh_pandamooz@sums.ac.ir
Submitted: 14-Aug-2023; **Revised:** 18-Oct-2023; **Accepted:** 21-Oct-2023; **Published:** 29-Jul-2024

Introduction

Stem cell therapy is an emerging strategy to treat neurological impairments. According to the regenerative capacity of stem cell-based therapies, their application has been widely explored in basic research and preclinical studies. Up until now, the majority of investigations report the trophic and immunomodulatory effects of transplanted stem cells that are linked to their paracrine activity rather than their ability to replace neural cells. In this context, mesenchymal stem cells (MSCs), known for their

Access this article online

Quick Response Code:

Website: www.advbiores.net

DOI: 10.4103/abr.abr_298_23

How to cite this article: Pandamooz S, Safari A, Ghorbani N, Jamhiri I, Zare S, Almeida Belem-Filho IJ, *et al*. Dimethyl fumarate preconditioning can reinforce the therapeutic potential of bone marrow mesenchymal stem cells through trophic factor profile enhancement. Adv Biomed Res 2024;13:37.

potential paracrine effects, stand out as a highly sought-after cell source.[1] MSCs can be obtained from various sources including adipose tissue, bone marrow, teeth, and birth-associated tissues like the umbilical cord and placenta. Currently, bone marrow-derived mesenchymal stem cells (BM-MSCs) are the most widely used source of MSCs that have been considered the gold standard in cell therapy due to their well-characterized properties.[2] The therapeutic potential of BM-MSCs is attributed to their secretome, which is a complex mixture of soluble fraction constituted by cytokines and growth factors and vesicular fraction composed of microvesicles and exosomes.[3] This secretome plays a pivotal role in mediating antiapoptotic, angiogenic, and immunomodulatory effects, making BM-MSCs a promising candidate for various neurological diseases in animal models and clinical trials, notably ischemic stroke. Since 2005, several clinical trials assessed the safety, feasibility, and efficacy of BM-MSCs transplantation as an ideal candidate for destructive conditions of stroke.[4] Despite promising outcomes, BM-MSCs may lose their biological functions during isolation and *in vitro* expansion and face a hostile environment post-transplantation, limiting their therapeutic efficacy. Hence, the development of strategies to preserve their characteristics, prolong their survival, and enhance their neuroprotective properties has become imperative.[5] Among these strategies, preconditioning approaches, including exposure to hypoxic conditions and pretreatment with various biological, chemical, and pharmacological agents have been developed. Preconditioning with pharmacological agents has been used to optimize the secretory profile of BM-MSCs and improve their restorative potential.[6] Dimethyl fumarate (DMF), marketed as Tecfidera® (formerly known as BG12; Biogen, USA), serves as a first-line treatment of relapsing-remitting multiple sclerosis[7] and has a history of application in psoriasis dating back to the 1990s.[8] The therapeutic benefits of DMF mainly stem from its immunomodulatory and antioxidative mechanisms.[9] Accumulating data suggests that DMF can strongly reduce neuronal cell death upon ischemic insult through pleiotropic cytoprotective and anti-inflammatory effects.[10] Preclinical studies have demonstrated its ability to reduce brain edema and improve neurological outcomes in animal models of ischemic stroke.[11,12] Furthermore, previous research has confirmed DMF's preconditioning effect in enhancing the secretory profile of hair follicle stem cells.[13] These collective findings raise the possibility that DMF may induce the upregulation of trophic factors in BM-MSCs, potentially enhancing their performance post-transplantation. Combining DMF with BM-MSCs, each with its own therapeutic potential and preconditioning benefits, may synergistically boost their protective and neurorestorative capabilities in the context of neural damage, such as ischemic stroke. Therefore, this study was designed to investigate the impact of DMF preconditioning on the trophic factor profile of human-derived BM-MSCs. In this study, we characterized isolated stem cells, determined the optimal DMF dosage, and subjected the cells to 24, 48, and 72 hours of preconditioning with DMF. We then assessed the expression of key trophic factors, including brain-derived neurotrophic factor (*BDNF*), glial cell line-derived neurotrophic factor (*GDNF*), nerve growth factor (*NGF*), neurotrophin-3 (*NT-3*), and vascular endothelial growth factor (*VEGF*) in treated stem cells. The objectives of this study are rooted in the evolving landscape of stem cell therapy for neurological impairments. This research holds the promise of not only improving our understanding of the interplay between DMF and BM-MSCs but also paving the way for more effective treatments in the realm of neurological disorders, offering hope to patients suffering from conditions like ischemic stroke. Ultimately, this study contributes to the ongoing efforts to optimize stem cell therapies for neurological impairments, taking us one step closer to realizing their full potential in clinical applications.

Materials and Methods

In vitro expansion of human BM-MSCs

Human BM-MSCs were purchased from the Royan Institute for Stem Cell Biology and Technology (Royan Stem Cell Bank, #RSCB0178, Tehran, Iran). These stem cells were fed with alpha-modified minimum essential medium (α-MEM, Bio Idea, #BI-1010-05) supplemented with 10% fetal bovine serum (FBS, Bio Idea, # BI-1201), 1% penicillin/streptomycin (Bio Idea, # BI-1203), and 1% L-glutamine (Bio Idea, # BI-1202), and standard incubator condition (37°C, saturated humidity and 50 mL/L CO_2) were used to expand the cells. The third passage of BM-MSCs, characterized by a desirable confluence (70%), was used for DMF treatment.

Identification of human BM-MSCs

To assess cell morphology, BM-MSCs in the third passage were stained with crystal violet and fluorescein diacetate (FDA #F7378 Sigma, 25 μg/ml) staining was carried out to reveal both morphology and viability of stem cells as described previously.[14,15] Finally, cells were visualized using a ZOE fluorescent microscope (Bio-Rad, USA).

For the identification of BM-MSCs, flow cytometry was employed to evaluate the expression of cardinal cell surface markers. The cells were incubated with primary conjugated antibodies targeting CD73, CD90, CD105, CD29, CD45, and CD34 at 4°C for 30 min. Here, a minimum of 10^5 cells per sample was analyzed. Antibodies details are listed in Table 1.

Flow cytometric analysis was conducted using a BD FACSCalibur (BD, Biosciences), and the final histograms of target markers were generated with FlowJo v10 software (FlowJo LLC).

Table 1: The list of conjugated primary antibodies used in immunophenotyping experiment

Primary antibody	Company	Cat. No.
CD73-PE/Cyanine7	BioLegend	344009
CD90-APC	BioLegend	328113
CD105-PE	BioLegend	323205
CD29-PerCP	ImmunoSteps	080210
CD45-FITC/CD34-PE	BD Biosciences	341071

To confirm the multipotency of isolated stem cells, their osteogenic and adipogenic potentials were evaluated by culturing them in OsteoPlus medium (Bioidea, #BI-1102) and AdipoPlus medium (Bioidea, #BI-1101), respectively, for 21 days. Next, cells were fixed with 4% paraformaldehyde for 20 min and stained with Alizarin Red (Bioidea, #BI-1801) to detect calcified extracellular matrix deposits or Oil Red O (Bioidea, #BI-1802) to stain neutral lipids followed by several washing steps. Images were captured using an Olympus IX71 inverted microscope (Olympus, Japan).

Study design and group assignment

To compare the inducing effects of DMF treatment on the trophic factor profile of BM-MSCs in this *in vitro* study, three groups were assigned as follows: control (CTRL), vehicle, and DMF-treated. The control group comprised stem cells incubated with α-MEM supplemented with 10% FBS. The vehicle group was composed of cells exposed to α-MEM with 10% FBS and 1% dimethyl sulfoxide as the drug solvent. The DMF-treated group involved stem cells treated with α-MEM, 10% FBS, and 10 μM DMF.

Cell viability assay for DMF dose-response

To determine the optimal DMF dose for BM-MSCs treatment, cell viability was assessed following exposure to different DMF concentrations (1, 5, 10, 25, 50, and 100 μM). In this regard, the mitochondrial activity of treated stem cells was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The selection of DMF concentrations is informed by existing literature, clinical relevance, and our preliminary study.

Here, stem cells were seeded at a density of 10^4 cells per well in a 96-well plate, followed by replacement of the growth medium with a medium containing 10% FBS and various DMF concentrations. After a 72-hour treatment period of stem cells with DMF in normal culture conditions, the medium was discarded, and 200 μl MTT solution (0.5 mg/ml, # M5655, Sigma-Aldrich) was added to each well. After approximately 3 hours of incubation, the MTT solution was gently aspirated, and acidic isopropanol (0.01 N HCl in absolute isopropanol, 100 μl/well) was added to dissolve formazan crystals. Finally, the developed color was measured at 570 nm using a microplate absorbance reader (BioTek, USA).

Preconditioning of human BM-MSCs with DMF

Once human BM-MSCs in the third passage reached 70%–80% confluence, cells were preconditioned with 10 μM DMF (selected dose based on the MTT assay) for 24, 48, and 72 hours under a 37ºC and 5% CO_2 environment. Corresponding control and vehicle groups were considered for each time point (24, 48, and 72-hour culture) with the same culture conditions. Notably, the culture medium for each experimental group remained unchanged during the treatment period.

RNA extraction, cDNA synthesis, and qRT-PCR

Total RNA was extracted from all experimental groups at the end of each time point using YTzol (#YT9063, Yekta Tajhiz Azma, Iran). cDNA was synthesized from 1 μg total RNA using a reverse transcriptase kit (Cat No: YT4500; Yekta Tajhiz Azma, Iran). Next, quantitative real time-PCR (qRT-PCR) was carried out using primers [listed in Table 2] and RealQ Plus 2x MasterMix Green (Cat. No: A325402; Ampliqon, Denmark) on an ABI StepOne Real-Time PCR system (Applied Biosystems, USA). β-actin served as an internal housekeeping gene, and each sample was examined in triplicate to calculate the mean gene expression. Finally, the arithmetic formula of $2^{-}\Delta\Delta^{CT}$ was applied to calculate the fold changes of relative gene expressions.

Statistical analysis

GraphPad Prism (Version 7.03, GraphPad Software, Inc., San Diego, CA) was applied to analyze the collected data. After normality assessments by the Shapiro–Wilk test, a one-way analysis of variance (ANOVA) with Tukey *post hoc* correction was performed to detect the statistical differences among the experimental groups. A $P < 0.05$ was considered statistically significant, and the data are presented as means ± SEM.

Results

Identification of human BM-MSCs

Microscopic evaluation of cultured human BM-MSCs in the third passage (P3) coupled with crystal violet and FDA staining revealed their characteristic spindle-shaped morphology [Figure 1a-c]. Also, flow cytometry analysis confirmed the identity of these isolated stem cells, which exhibited positivity for cardinal MSC markers, including CD29 (99.8%), CD105 (99.7%), CD73 (99.9%), and CD90 (98.7%), while displaying negligible expression of CD34 (0.095%) and CD45 (0.021%), markers associated with hematopoietic stem cells [Figure 1d-i]. In addition, the Alizarin red staining of cultured cells in an osteogenic medium demonstrated the presence of abundant calcified nodules [Figure 1j] and Oil Red O staining of cells cultured

Table 2: Primer sequences (5′–3′) used in quantitative polymerase chain reaction (qPCR)

Gene	Sequence	Amplicon length (bp)
BDNF F	TGTGCCGGGTGTGTAATC	102
BDNF R	CTCACCTGGTGGAACTGG	
GDNF F	GTGACTCAAATATGCCAGAGGA	115
GDNF R	GGAAGCACTGCCATTTGTTTAT	
NGF F	GTCCGGACCCAATAACAGTTT	76
NGF R	GGACATTACGCTATGCACCTC	
NT-3 F	CGTCCACCTTTCTCTTCATGTC	82
NT-3 R	CACCTGTAAGATCGTGGCAAA	
GFAP F	GAGAACCGGATCACCATTCC	144
GFAP R	CTGGTGAGCCTGTATTGGTATAA	
VEGF F	CTCCACCATGCCAAGTGGTC	105
VEGF R	GCAGTAGCTGCGCTGATAGA	
ACTB F	ATCAAGATCATTGCTCCTCCTG	111
ACTB R	GTCATACTCCTGCTTGCTGAT	

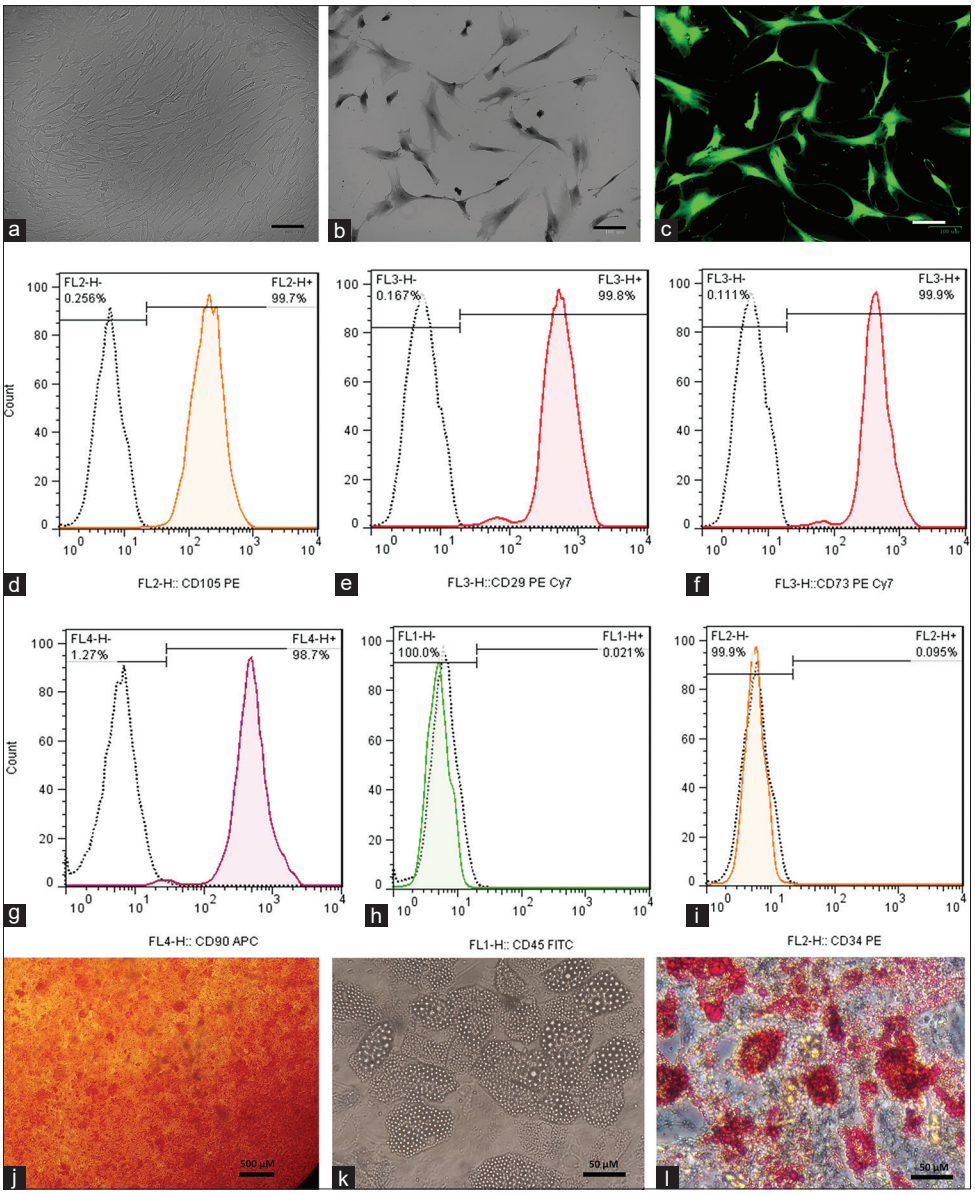

Figure 1: Characterization of human BM-MSCs. The microscopic evaluation of cultured human BM-MSCs (a) in third passage and crystal violet (b) and FDA (c) staining showed typical spindle-shaped cell morphology of isolated stem cells, scale bar: 100 µm. Flow cytometric analysis of key mesenchymal stem cell surface markers revealed that cultured cells were positive for CD105, CD29, CD73, and CD90, while they were negative for CD45 and CD34 which are hematopoietic markers (d-i). The Alizarin red staining of cultured cells in osteogenic medium demonstrated the presence of abundant calcified nodules (j), sale bar: 500 µm. The Oil Red O staining of cells cultured under adipogenic condition revealed the accumulation of lipid-filled cells (k and l), scale bar: 50 µm. These results confirm the osteogenic and adipogenic differentiation potential of isolated stem cells

under adipogenic condition revealed the accumulation of lipid-filled cells [Figure 1k and l]. These morphological characteristics, immunophenotypic properties, and differentiation potential collectively authenticate the identity of the cultured stem cells.

Viability of BM-MSCs treated with DMF

The MTT assay was employed to ascertain the optimal DMF dose for BM-MSC treatment. According to the obtained data, there was no significant difference between the viability of stem cells in CTRL, vehicle, and cells treated with 1, 5, 10, and 25 µM DMF. However, treatment with higher concentrations of DMF (50 and 100 µM) for 72 hours resulted in significant decrease in cell viability ($P < 0.001$) [Figure 2a]. In addition, the morphological evaluation of the treated stem cells prior to MTT assay [Figure 2b] and following crystal violet staining [Figure 2c] corroborated these findings. Cells treated with 1, 5, 10, and 25 µM DMF maintained normal morphology, whereas higher drug concentrations not only induced cell death but also caused morphological alterations. Based on these results and previous studies,[13] a concentration of 10 µM DMF was selected as the suitable dose to investigate its preconditioning impact on trophic factor expression in human BM-MSCs.

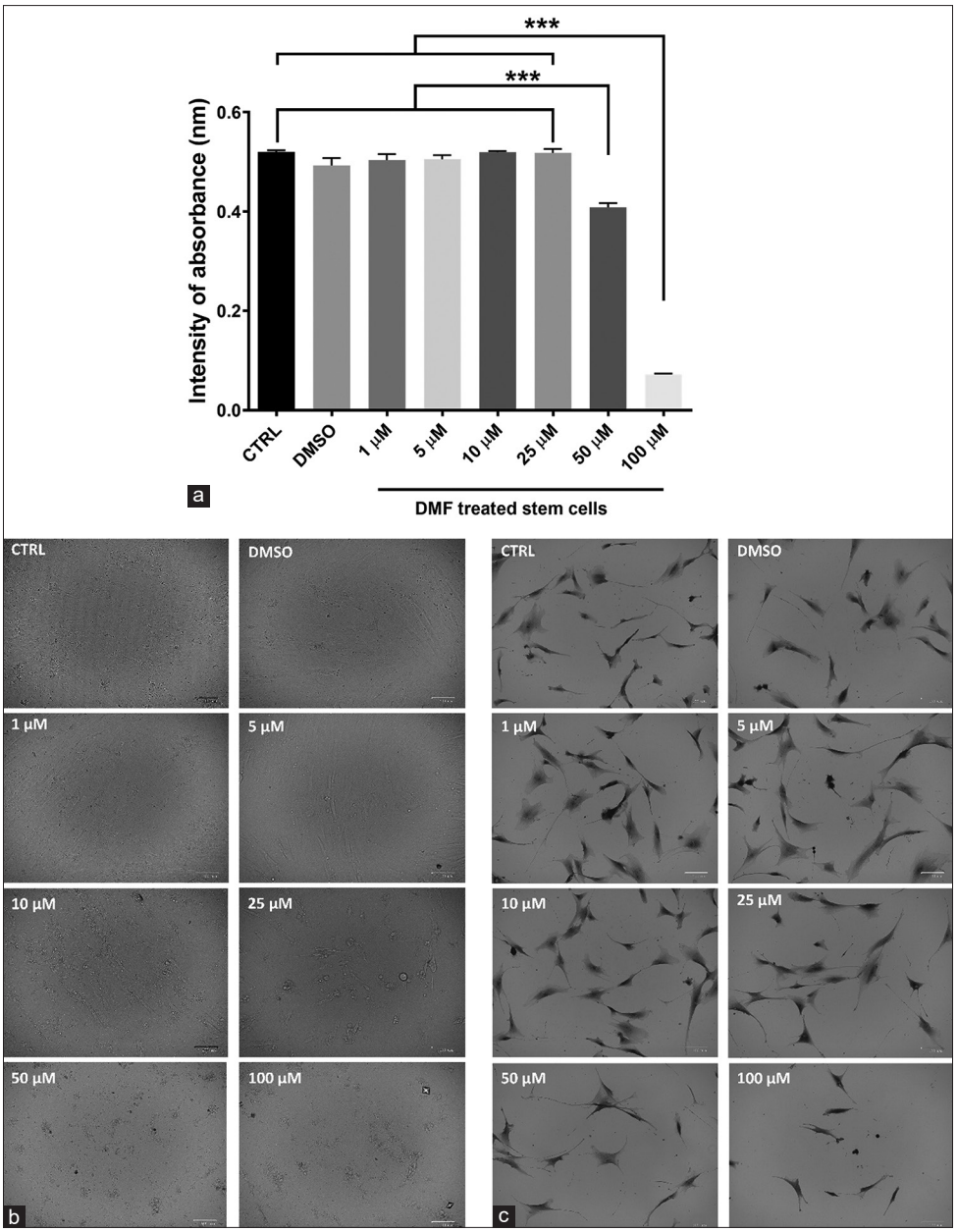

Figure 2: The effect of DMF treatment on survival and morphology of human BM-MSCs. The MTT assay was performed 72 hours after human BM-MSCs treatment with various concentration of DMF. (a) The result indicated that 50 and 100 μM DMF can significantly reduce the survival rate of stem cells, while no significant difference was detected between rests of experimental groups. The data are expressed as mean ± SEM and their analysis was performed using one-way ANOVA ($n = 3$). Also, morphological evaluation of treated stem cells prior MTT assay (b) and following crystal violet staining (c) revealed that cells treated with 1, 5, 10, and 25 μM DMF present normal morphology, while higher drug concentration leads to cell death and morphological alteration

DMF preconditioning enhances the BDNF expression

Treatment of BM-MSCs with DMF resulted in the upregulation of several trophic factors. BDNF is one of the trophic factors that its expression affected by DMF preconditioning. On day one, the relative expression of *BDNF* was significantly higher in DMF-treated group than in other experimental groups (CTRL vs. DMF, $P = 0.041$; Vehicle vs DMF, $P = 0.0377$). On day two, a meaningful difference was detected only between DMF-treated cells and vehicle group ($P = 0.0987$). However, on the third day of treatment, *BDNF* expression was significantly upregulated in preconditioned stem cells compared to CTRL ($P = 0.0028$) and vehicle groups ($P = 0.0026$) [Figure 3].

DMF preconditioning increases NGF transcript level

Another trophic factor that is expression upregulated following DMF treatment is NGF. Here, an increasing pattern of expression was observed in DMF preconditioned stem cells. While no significant difference was detected in NGF mRNA levels among experimental groups after 24 hours of treatment, on day two, transcript levels of *NGF* significantly increased in DMF-treated cells compared to the CTRL group ($P = 0.0275$).

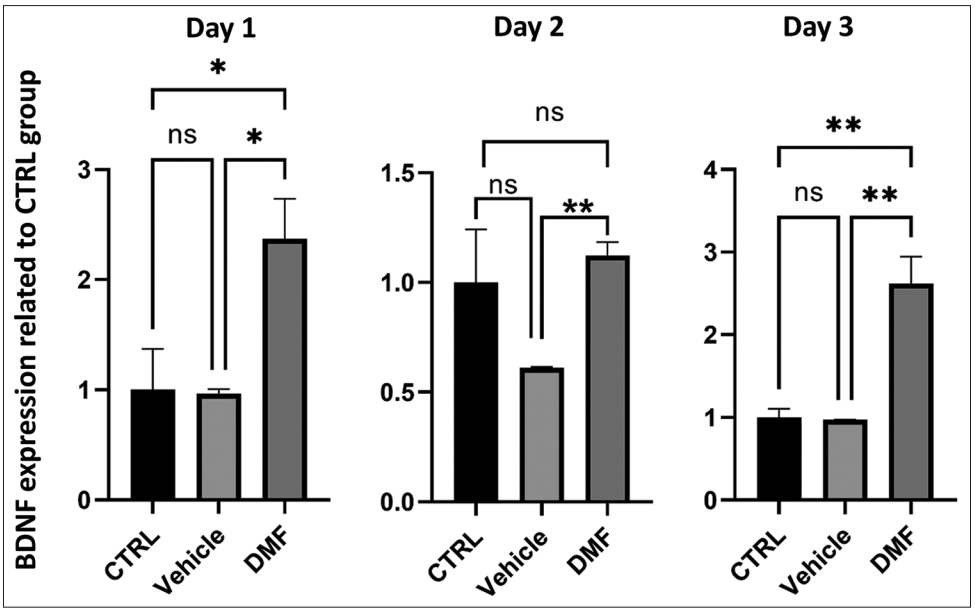

Figure 3: The effect of DMF treatment on *BDNF* expression. The expression of *BDNF* was assessed following 24, 48 and 72 hours of preconditioning with 10 μM DMF. The qRT-PCR results revealed that the transcript level of *BDNF* in DMF-treated group significantly increased after 24 hours of treatment compared to other groups. Also, expression level of *BDNF* was significantly higher in DMF preconditioned stem cells after 72 hours of treatment. The data are expressed as mean ± SEM and their analysis was performed using one-way ANOVA and Tukey *post hoc* test ($n = 3$). *$P < 0.05$; **$P < 0.01$

A more pronounced difference was observed following 72 hours of treatment in the preconditioned groups (CTRL vs. DMF, $P = 0.0019$; Vehicle vs DMF, $P = 0.0101$) [Figure 4].

DMF preconditioning upregulates NT-3 expression level

NT-3 is another member of NTs family. In this study, the transcript level of NT-3 altered in BM-MSCs after undergoingDMF treatment. The quantification of the obtained data revealed that expression of *NT-3* significantly increased following 72 hours of preconditioning with 10 μM DMF (CTRL vs. DMF, $P < 0.001$; Vehicle vs DMF, $P < 0.001$) [Figure 5].

DMF preconditioning has no significant effect on GDNF and VEGF expression level

Following preconditioning of BM-MSCs with 10 μM DMF, the expression levels of *GDNF* and *VEGF* were detected by qRT-PCR. Here, no significant differences in transcript levels of *GDNF* and *VEGF* were observed among experimental groups. Although, on day three, an upregulation was observed in the DMF-preconditioned group, this alteration was not significantly higher than CTRL group (*GDNF*: CTRL vs. DMF, $P = 0.1069$; Vehicle vs DMF, $P = 0.0359$; *VEGF*: CTRL vs. DMF, $P = 0.0908$; Vehicle vs DMF, $P = 0.0391$) [Figure 6].

Discussion

In the current study, we evaluated the effect of DMF preconditioning on the trophic factors profile of human BM-MSCs, highlighting the potential implications for neuroprotection and regenerative medicine. Our collected results revealed that DMF preconditioning significantly increased the transcript levels of *BDNF*, *NGF*, and *NT-3* in these stem cells. Although DMF pretreatment increased the expression of *GDNF* and *VEGF*, these upregulations were not statistically significant. The regenerative potential of BM-MSCs has garnered substantial attention in the field of regenerative medicine. Their therapeutic efficacy primarily stems from their multilineage differentiation capabilities and, notably, their paracrine effects. Rather than replacing damaged cells, BM-MSCs contribute to tissue repair predominantly by paracrine action of their secretome.[16] This secretome contains a wide range of angiogenic, antiapoptotic, immunomodulatory, and growth factors, which support cell survival and tissue regeneration in neurological diseases. In this context, preconditioning strategies using pharmacological agents have gained attention for their ability to optimize the secretory profile of MSCs, enhancing their therapeutic potential for various conditions.[17] Previous studies have explored the preconditioning effects of pharmacological agents such as lithium chloride and valproic acid, which significantly increase the expression of genes associated with neuroprotection including *BDNF*, *GDNF*, *NGF*, and *NT-3*.[18-21] In our study, we focused on DMF preconditioning as a potential strategy for enhancing the therapeutic potential of BM-MSCs in the context of ischemic stroke. We found that a 10 μM DMF treatment led to a significant increase in *BDNF* expression in BM-MSCs, with the maximum effect observed on the third day. This finding is consistent with previous investigations that reported *BDNF* upregulation in hair follicle stem cells, following a 72 hour treatment.[13] BDNF is the most prevalent growth factor in the brain, promoting neuronal survival and differentiation through interaction with tyrosine kinase receptors.[22] BDNF is one of the important mediators in the MSC secretome that prevents glutamate-induced neuronal death.[23] Animal

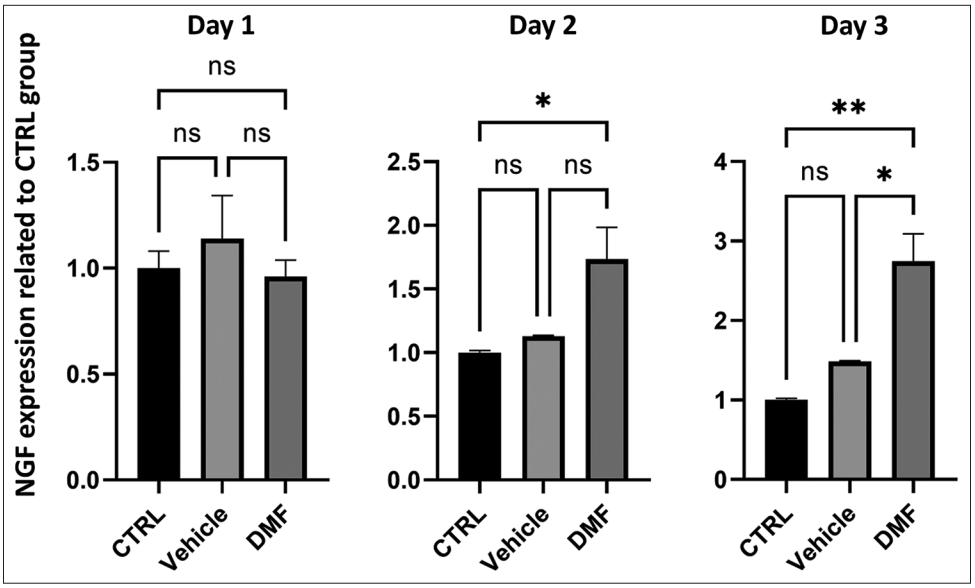

Figure 4: The effect of DMF treatment on *NGF* expression. The evaluation of *NGF* transcript level by qRT-PCR revealed no significant difference between groups after one day treatment with 10 μM DMF. However an increasing pattern of *NGF* transcript was detected on day two and three. Following three days culture, the *NGF* expression level significantly upregulated in DMF preconditioned group compared to vehicle and CTRl groups. The data are expressed as mean ± SEM and their analysis was performed using one-way ANOVA and Tukey *post hoc* test ($n = 3$). *$P < 0.05$; **$P < 0.01$

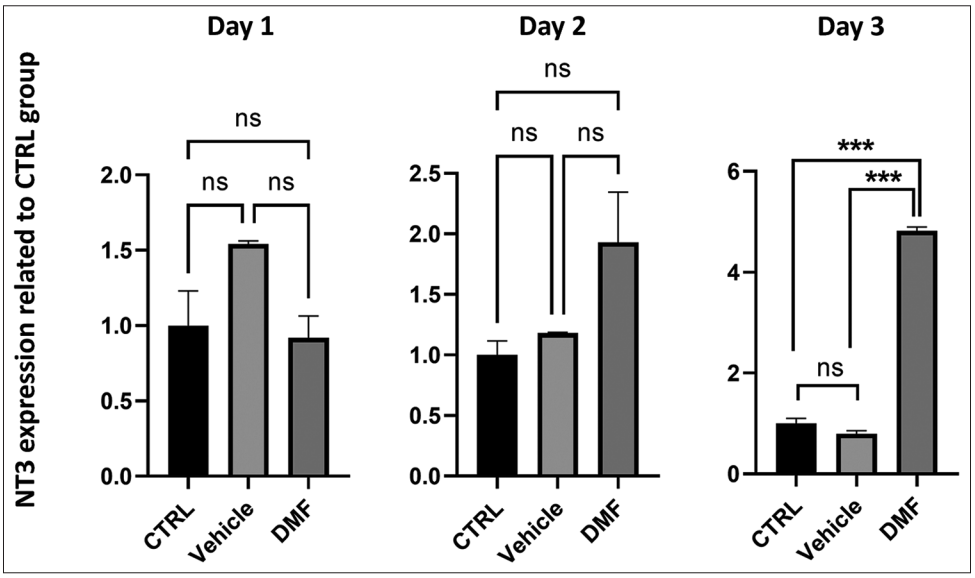

Figure 5: The influence of DMF preconditioning on *NT-3* expression. The analysis of data obtained from qRT-PCR demonstrated that preconditioning with 10 μM DMF significantly increase transcript level of *NT-3* on day three. However no significant was detected between groups on day one and two. The data are expressed as mean ± SEM and their analysis was performed using one-way ANOVA and Tukey *post hoc* test ($n = 3$). ***$P < 0.001$

models of stroke have shown promising outcomes with direct BDNF administration or grafting of engineered stem cells that stably express this trophic factor, resulting in reduced infarct volume, enhanced neurogenesis, and improved functional recovery.[24,25] Therefore, DMF preconditioning may serve as a priming approach to elevate baseline *BDNF* expression in BM-MSCs, enhancing their therapeutic potential for conditions like ischemic stroke.

NGF is another trophic factor whose expression affected by DMF exhibited increased expression after 48 and 72 hours of preconditioning. NGF is the first discovered member of NT family, which plays a crucial role in the functional integrity of cholinergic neurons in the central nervous system (CNS) and the development and maintenance of peripheral nervous system neurons.[26] In CNS, the cortex is the primary site for NGF production. However, in the context of cerebral ischemia, there is a significant decrease in the endogenous NGF expression within the infarcted cortex. To date, several strategies have been developed to deliver NGF to the ischemic brain.[27,28] Thus, transplantation of BM-MSCs following DMF preconditioning could serve as an excellent strategy

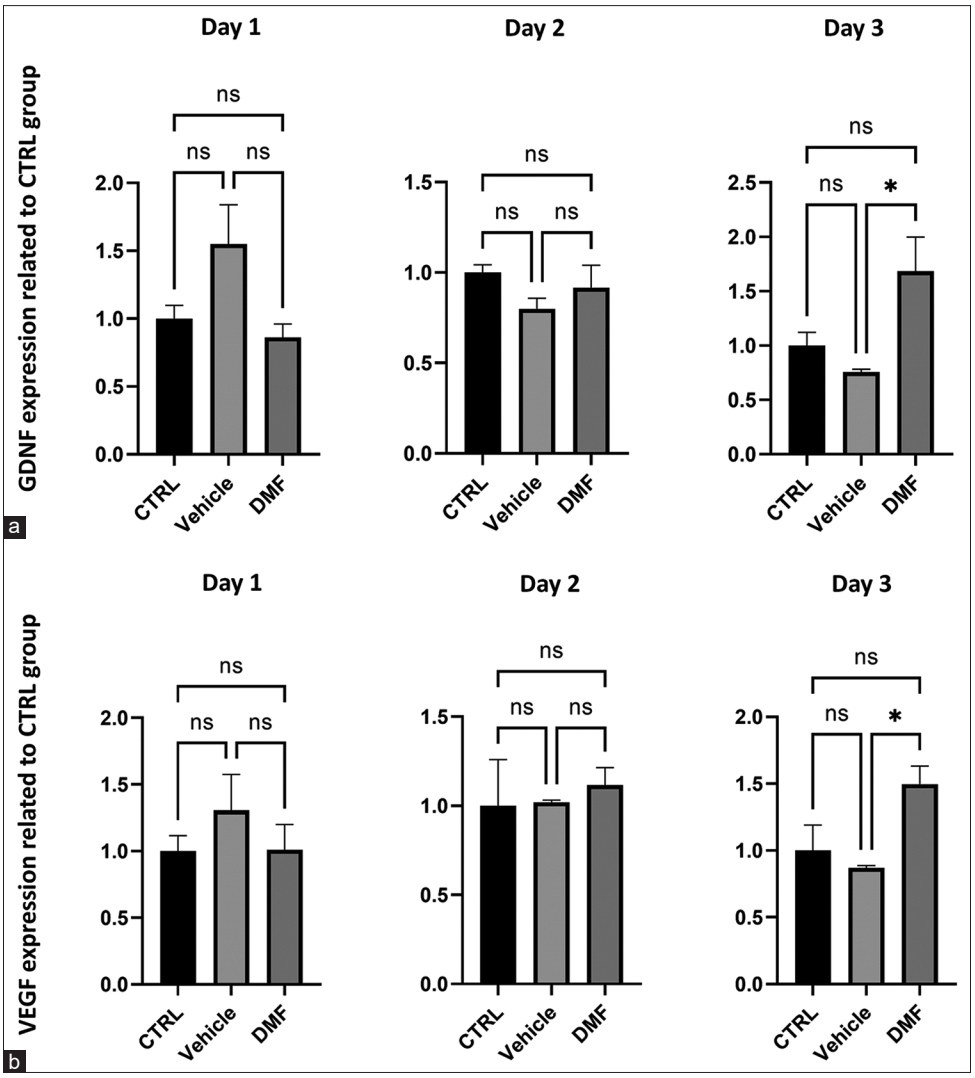

Figure 6: The effect of DMF preconditioning on *GDNF* and *VEGF* expression. The assessment of *GDNF* (a) and *VEGF* (b) expression following DMF preconditioning demonstrated no significant difference between experimental groups. The data are expressed as mean ± SEM and their analysis was performed using one-way ANOVA and Tukey *post hoc* test ($n = 3$)

for locally delivering NGF to damaged tissue, promoting the survival of host neurons. Additionally, the upregulation of *NGF* may directly influence BM-MSCs, enhancing their immunomodulatory potential and paracrine activity through NGF receptors. Recently, Babaie and colleagues have reported overexpression of *BDNF* and *NGF* in the hippocampus of a rat model of Alzheimer's disease following the transplantation of DMF-preconditioned rat adipose-derived MSCs.[29] *NT-3*, a member of NT family, exhibited significant upregulation following 72 hours of preconditioning with 10 μM DMF. NT-3 is the third neurotrophic factor in NGF family, which has been characterized after NGF and BDNF. NT-3 has been shown to have a neuroprotective effect in rat models of middle cerebral artery occlusion, lessening ischemic brain injury.[30] NT-3 contributes to the function and development of locomotor circuits, including corticospinal tract (CST) and descending serotonergic axons and afferents from skin and muscle that mediate tactile and proprioception sensation. It has been well established that delivery of NT-3 to CNS can promote the recovery of animal models of CNS injury.[31] Duricki and colleagues showed that injection of viral vector expressing NT-3 into forelimb muscles, 24 hours after ischemic stroke, can improve CST sprouting and sensorimotor recovery.[32] The recent finding of this group demonstrated that 24-hour-delayed intramuscular infusion of NT-3 protein can improve sensorimotor function after ischemic stroke.[33] Moreover, several studies indicated the functional recovery in animal models of brain and spinal cord injury following transplantation of engineered MSC to overexpress NT-3 or administration of their enriched supernatants.[34-36] Hence, transplantation of the DMF-preconditioned BM-MSCs or their secretome, owing to overexpression of *NT-3*, can be considered as a neuroprotective strategy in various neurological conditions. Notably, this *NT-3* upregulation in human BM-MSCs is consistent with a previous study by Salehi and colleagues that reported an eight-fold

increase in *NT-3* expression level in hair follicle stem cells following three days of DMF treatment.[13] We also evaluated the expression of *VEGF* and *GDNF* in our study. Despite a slight increase in their transcript levels after DMF preconditioning, these differences were not statistically significant. This observation is in line with an earlier report that demonstrated DMF preconditioning did not significantly alter *VEGF* expression at various time points.[13] However, due to high baseline expression of *VEGF* in BM-MSCSs, as assessed by qRT-PCR, these stem cells can still contribute to angiogenesis in target injured tissue upon transplantation. This higher expression of *VEGF* in BM-MSCs primarily depends on their source of isolation.[37] GDNF, a member of the transforming growth factor-β superfamily, is a potent neurotrophic factor promoting the survival of dopaminergic neurons and motoneurons.[38] Our data revealed that *GDNF* expression level in human BM-MSCs remained unchanged following DMF preconditioning. In contrast, a previous study showed that treatment of hair follicle stem cells with DMF significantly decreased the *GDNF* transcripts at various time points.[13] While the precise mechanisms by which DMF induces the expression of trophic factors in human BM-MSCs require further investigation, several potential pathways can be considered. One potential mechanism is the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway by DMF. DMF has been shown to activate Nrf2, a transcription factor that plays a key role in cellular antioxidant responses. Activation of Nrf2 can lead to the transcriptional upregulation of various cytoprotective genes, including those encoding neurotrophic factors like BDNF.[39] Therefore, it is possible that DMF-induced Nrf2 activation in BM-MSCs may contribute to the increased expression of BDNF and other trophic factors observed in our study. Furthermore, DMF has been reported to modulate various signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway.[40] The MAPK pathway is involved in the regulation of gene expression, and its activation can lead to the upregulation of neurotrophic factors. DMF's ability to modulate MAPK signaling in BM-MSCs may play a role in the observed increase in trophic factor expression. Another potential mechanism is the modulation of epigenetic factors by DMF.[41] Epigenetic modifications, such as DNA methylation and histone acetylation, can regulate gene expression. DMF has been shown to exert epigenetic effects, including histone deacetylase (HDAC) inhibition. HDAC inhibition can result in a more permissive chromatin state, allowing for increased gene transcription. It is possible that DMF-induced epigenetic changes in BM-MSCs contribute to the upregulation of trophic factors like NGF and NT-3.

To contextualize our study within the broader landscape of cell-based therapies, it is essential to highlight recent FDA and EMA-approved cell therapy products. These approvals exemplify the practical application of innovative medical treatments in clinical settings and underscore the diversity of cell-based approaches. Notable examples include Kymriah and Yescarta, both CAR-T cell therapies designed for leukemia and lymphoma treatment, Provenge, an autologous dendritic cell-based immunotherapy for prostate cancer, and Alofisel, an allogeneic stem cell therapy indicated for Crohn's disease. These therapies serve as striking illustrations of how cell-based approaches are offering tailored and personalized solutions for a wide array of diseases. They also emphasize the ongoing challenges and opportunities within the dynamic field of cell therapy, underlining the importance of continued research and innovation to expand the horizons of these groundbreaking treatments.

Conclusion

In conclusion, given the growing interest in the application of human MSCs for treating various neurological conditions, including stroke[42], our study proposed DMF preconditioning as a simple yet effective method to enhance the therapeutic potential of human BM-MSCs. The combination of DMF and BM-MSCs, leveraging the therapeutic potential of each, along with the preconditioning outcome, may present a synergistic effect that enhances their neuroprotective and regenerative potential in the context of ischemic stroke. Nevertheless, further *in vitro* investigations are needed to explore the impact of DMF preconditioning on BM-MSCs at various passage numbers, with the aim of maximizing the therapeutic efficacy of this preconditioning strategy. Moreover, future *in vivo* studies can further elucidate the therapeutic potential of this pharmacological preconditioning in animal models of ischemic stroke and other neurological conditions.

Study approval statement

This study protocol was reviewed and approved by the Research Ethics Committees of Shiraz University of Medical Sciences, approval number [IR.SUMS.REC.1400.414]. According to this approval, working with purchased human stem cells from cell banks does not require written informed consent from donors.

Author contributions

Conceptualization: Sareh Pandamooz, Anahid Safari, Mohammad Saied Salehi; Methodology: Sareh Pandamooz, Iman Jamhiri, Shahrokh Zare; Writing-original draft: Sareh Pandamooz, Anahid Safari, Nasrin Ghorbani; Analysis: Sareh Pandamooz, Parisa Dolati; Writing- review and editing: Sareh Pandamooz, Mohammad Saied Salehi; Supervision: Sareh Pandamooz, Anahid Safari; Funding acquisition: Sareh Pandamooz

Data Availability Statement

The authors declare that all data supporting the findings of this study are available within the article or are available from the corresponding author upon request.

Financial support and sponsorship

This study was supported by Shiraz University of Medical Sciences (Grant No: 19546).

Conflicts of interest

The authors declare that there is no financial or other potential conflict of interest regarding the publication of this paper.

References

1. Andrzejewska A, Dabrowska S, Lukomska B, Janowski M. Mesenchymal stem cells for neurological disorders. Adv Sci (Weinh) 2021;8:2002944.
2. Soares MBP, Gonçalves RGJ, Vasques JF, da Silva-Junior AJ, Gubert F, Santos GC, *et al*. Current status of mesenchymal stem/stromal cells for treatment of neurological diseases. Front Mol Neurosci 2022;15:883378.
3. Teixeira FG, Salgado AJ. Mesenchymal stem cells secretome: Current trends and future challenges. Neural Regen Res 2020;15:75-77.
4. Zhou L, Zhu H, Bai X, Huang J, Chen Y, Wen J, *et al*. Potential mechanisms and therapeutic targets of mesenchymal stem cell transplantation for ischemic stroke. Stem Cell Res Ther 2022;13:195.
5. Schepici G, Gugliandolo A, Mazzon E. Mesenchymal stromal cells preconditioning: A new strategy to improve neuroprotective properties. Int J Mol Sci 2022;23:2088.
6. Linares GR, Chiu CT, Scheuing L, Leng Y, Liao HM, Maric D, *et al*. Preconditioning mesenchymal stem cells with the mood stabilizers lithium and valproic acid enhances therapeutic efficacy in a mouse model of Huntington's disease. Exp Neurol 2016;281:81-92.
7. Bomprezzi R. Dimethyl fumarate in the treatment of relapsing-remitting multiple sclerosis: An overview. Ther Adv Neurol Disord 2015;8:20-30.
8. Blair HA. Dimethyl fumarate: A review in moderate to severe plaque psoriasis. Drugs 2018;78:123-30.
9. Narapureddy B, Dubey D. Clinical evaluation of dimethyl fumarate for the treatment of relapsing-remitting multiple sclerosis: Efficacy, safety, patient experience and adherence. Patient Prefer Adherence 2019;13:1655-66.
10. Kunze R. Dimethyl fumarate for ischemic stroke. Oncotarget 2017;8:14281-2.
11. Kunze R, Urrutia A, Hoffmann A, Liu H, Helluy X, Pham M, *et al*. Dimethyl fumarate attenuates cerebral edema formation by protecting the blood-brain barrier integrity. Exp Neurol 2015;266:99-111.
12. Owjfard M, Bigdeli MR, Safari A, Namavar MR. Therapeutic effects of dimethyl fumarate on the rat model of brain ischemia. Braz J Pharm Sci 2022;58:e19677.
13. Salehi MS, Borhani-Haghighi A, Pandamooz S, Safari A, Dargahi L, Dianatpour M, *et al*. Dimethyl fumarate up-regulates expression of major neurotrophic factors in the epidermal neural crest stem cells. Tissue Cell 2019;56:114-20.
14. Pandamooz S, Jurek B, Dianatpour M, Haerteis S, Limm K, Oefner PJ, *et al*. The beneficial effects of chick embryo extract preconditioning on hair follicle stem cells: A promising strategy to generate Schwann cells. Cell Prolif 2023;56:e13397.
15. Karimi-Haghighi S, Pandamooz S, Jurek B, Fattahi S, Safari A, Azarpira N, *et al*. From hair to the brain: The short-term therapeutic potential of human hair follicle-derived stem cells and their conditioned medium in a rat model of stroke. Mol Neurobiol 2023;60:2587-601.
16. Soares MBP, Gonçalves RGJ, Vasques JF, da Silva-Junior AJ, Gubert F, Santos GC, *et al*. Current status of mesenchymal stem/stromal cells for treatment of neurological diseases. Front Mol Neurosci 2022;15:883378.
17. Li M, Jiang Y, Hou Q, Zhao Y, Zhong L, Fu X. Potential pre-activation strategies for improving therapeutic efficacy of mesenchymal stem cells: Current status and future prospects. Stem Cell Res Ther 2022;13:146.
18. Linares GR, Chiu CT, Scheuing L, Leng Y, Liao HM, Maric D, *et al*. Preconditioning mesenchymal stem cells with the mood stabilizers lithium and valproic acid enhances therapeutic efficacy in a mouse model of Huntington's disease. Exp Neurol 2016;281:81-92.
19. Pandamooz S, Salehi MS, Safari A, Azarpira N, Heravi M, Ahmadiani A, *et al*. Enhancing the expression of neurotrophic factors in epidermal neural crest stem cells by valproic acid: A potential candidate for combinatorial treatment. Neurosci Lett 2019;704:8-14.
20. Baharvand Z, Nabiuni M, Tahmaseb M, Amini E, Pandamooz S. Investigating the synergic effects of valproic acid and crocin on BDNF and GDNF expression in epidermal neural crest stem cells. Acta Neurobiol Exp (Wars) 2020;80:38-46.
21. Ahmadi S, Nabiuni M, Tahmaseb M, Amini E. Enhanced neural differentiation of epidermal neural crest stem cell by synergistic effect of lithium carbonate and crocin on BDNF and GDNF expression as neurotrophic factors. Iran J Pharm Res 2021;20:95-106.
22. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. Arch Med Sci 2015;11:1164-78.
23. Tejeda GS, Esteban-Ortega GM, San Antonio E, Vidaurre ÓG, Díaz-Guerra M. Prevention of excitotoxicity-induced processing of BDNF receptor TrkB-FL leads to stroke neuroprotection. EMBO Mol Med 2019;11:e9950.
24. Cunningham CJ, Redondo-Castro E, Allan SM. The therapeutic potential of the mesenchymal stem cell secretome in ischaemic stroke. J Cereb Blood Flow Metab 2018;38:1276-92.
25. Salehi MS, Safari A, Pandamooz S, Jurek B, Hooshmandi E, Owjfard M, *et al*. The beneficial potential of genetically modified stem cells in the treatment of stroke: A review. Stem Cell Rev Rep 2022;18:412-40.
26. Aloe L, Rocco ML, Bianchi P, Manni L. Nerve growth factor: From the early discoveries to the potential clinical use. J Transl Med 2012;10:239.
27. Li X, Li F, Ling L, Li C, Zhong Y. Intranasal administration of nerve growth factor promotes angiogenesis via activation of PI3K/Akt signaling following cerebral infarction in rats. Am J Transl Res 2018;10:3481-92.
28. Yang J, Wu S, Hou L, Zhu D, Yin S, Yang G, *et al*. Therapeutic effects of simultaneous delivery of nerve growth factor mRNA and protein via exosomes on cerebral ischemia. Mol Ther Nucleic Acids 2020;21:512-22.
29. Babaei H, Kheirollah A, Ranjbaran M, Cheraghzadeh M, Sarkaki A, Adelipour M. Preconditioning adipose-derived mesenchymal stem cells with dimethyl fumarate promotes their therapeutic efficacy in the brain tissues of rats with Alzheimer's disease. Biochem Biophys Res Commun 2023;672:120-7.
30. Zhang WR, Kitagawa H, Hayashi T, Sasaki C, Sakai K, Warita H, *et al*. Topical application of neurotrophin-3 attenuates ischemic brain injury after transient middle cerebral artery occlusion in rats. Brain Res 1999;842:211-4.
31. Kathe C, Hutson TH, McMahon SB, Moon LD. Intramuscular Neurotrophin-3 normalizes low threshold spinal reflexes, reduces spasms and improves mobility after bilateral corticospinal tract injury in rats. Elife 2016;5:e18146.
32. Duricki DA, Hutson TH, Kathe C, Soleman S, Gonzalez-Carter D, Petruska JC, *et al*. Delayed intramuscular human neurotrophin-3 improves recovery in adult and elderly rats after stroke. Brain 2016;139:259-75.
33. Duricki DA, Drndarski S, Bernanos M, Wood T, Bosch K, Chen Q, *et al*. Stroke recovery in rats after 24-hour-delayed intramuscular neurotrophin-3 infusion. Ann Neurol 2019;85:32-46.
34. Wu K, Huang D, Zhu C, Kasanga EA, Zhang Y, Yu E, *et al*. NT3(P75-2) gene-modified bone mesenchymal stem cells improve neurological function recovery in mouse TBI model. Stem Cell Res Ther 2019;10:311.
35. Stewart AN, Kendziorski G, Deak ZM, Bartosek NC, Rezmer BE, Jenrow K, *et al*. Transplantation of mesenchymal stem cells that overexpress NT-3 produce motor improvements without axonal regeneration following complete spinal cord transections in rats. Brain Res 2018;1699:19-33.
36. Huang D, Bao H, Wu J, Zhuge Q, Yang J, Ye S. Overexpression of NT3(P75-2) gene modified bone marrow mesenchymal stem cells supernatant promotes neurological function recovery in ICH rats. Neurosci Lett 2023;796:137067.
37. Petrenko Y, Vackova I, Kekulova K, Chudickova M, Koci Z, Turnovcova K, *et al*. A comparative analysis of multipotent mesenchymal stromal cells derived from different sources, with a focus on neuroregenerative potential. Sci Rep 2020;10:4290.
38. Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Ishii K, Kobune M, *et al*. Mesenchymal stem cells that produce neurotrophic factors reduce

ischemic damage in the rat middle cerebral artery occlusion model. Mol Ther 2005;11:96-104.
39. Cuadrado A. Brain-protective mechanisms of transcription factor NRF2: Toward a common strategy for neurodegenerative diseases. Annu Rev Pharmacol Toxicol 2022;62:255-77.
40. Wang Q, Chuikov S, Taitano S, Wu Q, Rastogi A, Tuck SJ, *et al*. Dimethyl fumarate protects neural stem/progenitor cells and neurons from oxidative damage through Nrf2-ERK1/2 MAPK pathway. Int J Mol Sci 2015;16:13885-907.
41. Kalinin S, Polak PE, Lin SX, Braun D, Guizzetti M, Zhang X, *et al*. Dimethyl fumarate regulates histone deacetylase expression in astrocytes. J Neuroimmunol 2013;263:13-9.
42. Zhang Y, Dong N, Hong H, Qi J, Zhang S, Wang J. Mesenchymal stem cells: Therapeutic mechanisms for stroke. Int J Mol Sci 2022;23:2550.