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# Effect of Combination Electroacupuncture and Tenuigenin on the Migration and Differentiation of Mesenchymal Stem Cells following Ischemic Stroke

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**Objectives:** Since stroke is a serious health issue, novel therapeutic strategies are required. In a mouse model of ischemic stroke, this study analyzed the potential of electroacupuncture (EA) and tenuigenin (TE) to improve the efficacy of human mesenchymal stem cell (hMSC) transplantation.
**Methods:** Middle cerebral artery occlusion (MCAO) with reperfusion was used to generate ischemic stroke. Forty-eight male C57BL/6 mice were randomly divided into five groups: control, MCAO-operated, MCAO-EA, MCAO-TE, or MCAO + EA + TE. Subsequently, hMSCs were transplanted into the ischemic region and EA, TE, or the combination was administered. Behavior assessments and immunohistochemistry were conducted to evaluate motor and cognitive recovery and hMSCs survival, migration, and differentiation.
**Results:** The combined treatment of EA and TE exhibited enhanced hMSCs survival, migration and differentiation into neural cell lineages while suppressing astrocyte formation. Immunohistochemistry demonstrated increased neurogenesis through hMSCs transplantation in the ischemic brain. Immediate behavioral improvements were not significantly different between groups, but there was a gradual recovery in motor and cognitive function over time.
**Conclusion:** These findings highlight the potential of EA and TE co-treatment as a therapeutic strategy for ischemic stroke, opening avenues for further research to optimize treatment protocols and elucidate underlying mechanisms.

**Keywords:** electroacupuncture, mesenchymal stem cell, middle cerebral artery occlusion, stroke, tenuigenin

## INTRODUCTION

The World Health Organization ranks stroke as the second leading cause of death globally and a significant contributor to long-term disability [1]. Ischemic stroke, characterized by an abrupt cessation of cerebral blood flow (CBF), triggers ischemic brain damage, resulting in a spectrum of neurological deficits. Although thrombolysis and endovascular clot retrieval are crucial existing treatment options, their effectiveness is time-dependent and not universally applicable [2]. Therefore, there is a pressing need for novel strategies that can enhance neuronal regeneration and functional recovery in patients with stroke.

A promising avenue of research in the field of stroke therapy revolves around mesenchymal stem cells (MSCs). These multipotent stromal cells are capable of self-renewal and differentiation into various cell types and hold great promise in regenerative medicine [3]. Their potential as a therapeutic option for neurological disorders, including stroke, has drawn significant attention. However, MSC transplantation presents challenges, including issues related to cell survival, targeted migration, and differentiation into neural cell lineages for functional integration into damaged brain tissue [4]. Additionally, achieving suc-

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cessful integration within the ischemic brain is a complex and multifaceted endeavor.

Electroacupuncture (EA) has emerged as a promising therapeutic modality in the field of brain disorder research. EA, an evolution of traditional acupuncture, involves the electrical stimulation of acupuncture points and has been studied for its neuroprotective and neuroregenerative effects [5]. The integration of ancient acupuncture practices with modern electrostimulation techniques augments its potential to promote neural repair and functional recovery after brain injuries. EA is believed to exert therapeutic effects by modulating neuroinflammation, enhancing neuroprotection, and promoting neuroplasticity [6]. Notably, EA can stimulate the release of neurotrophic factors crucial for neuronal survival, growth, and differentiation, including brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) [7, 8]. Furthermore, EA may influence the activation of endogenous neural stem cells, amplifying the restoration processes within the injured brain [9].

Emerging reports indicate natural compounds sourced from traditional oriental herbal remedies can enhance brain function and stimulate the proliferation of stem cells [10]. Tenuigenin (TE), a bioactive compound derived from the Chinese herb *Polygala tenuifolia*, is renowned for its neuroprotective properties and potential to stimulate neurogenesis [11, 12].

In this study, we hypothesized that the combination of EA and TE treatment would increase the survival, migration, and differentiation of human MSC (hMSC) into neural cells within the ischemic brain by creating a supportive microenvironment. We aimed to evaluate the therapeutic efficacy of this approach using a mouse model of middle cerebral artery occlusion (MCAO), a widely accepted model that mirrors ischemic stroke in humans.

## MATERIALS AND METHODS

### 1. Animals and groups

A total of 48 male C57BL/6 mice (Dooyeol Biotech, Seoul, Korea) were housed under controlled temperature and humidity, maintaining a 12-hour light-dark cycle. The mice were allowed free access to food and water. All animal procedures in this study were approved by the Institutional Animal Care and Use Committee at Pusan National University (Approval Number PNU-2017-1617). The mice were randomly assigned to five groups: control, MCAO-operated, MCAO + EA, MCAO + TE, and MCAO + EA + TE.

### 2. MCAO model

Male C57BL/6 mice (8 weeks old) were anesthetized using 2% isoflurane. The anesthetic was supplied with a calibrated vaporizer (Model VIP 3000, Midmark, Orchard Park, OH, USA) in a carrier gas composed of 20% $O_2$ and 80% $N_2O$. The progression of anesthesia was monitored by assessing CBF using the PeriFlux Laser Doppler System 5000 (Perimed, Stockholm, Sweden), which was attached to the skull. MCAO was performed by inserting a 7-0 silicone rubber-coated monofilament (701956PK5Re, Doccol Corporation, Sharon, MA, USA) through the common and internal carotid arteries. After 40 minutes, the monofilament was removed to facilitate the reperfusion of the vessel.

### 3. hMSC culture and cell labeling

Briefly, hMSCs (Lonza; PT-2501, Basel, Basel-Stadt, Switzerland) were incubated at 37℃ in 5 % $CO_2$ in a flask for one day, and nonadherent cells were removed by replacing the medium. To monitor the distribution of hMSCs in brain tissue, the highly lipophilic carbocyanine dye CM-DiI (V-22888, Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to label the hMSCs. The hMSCs were then detached using Accutase (AT-104, Innovative Cell Technologies, Inc., San Diego, CA, USA). Passage 3 cells were washed three times and suspended at a density of $1 \times 10^6$/mL in hMSC growth medium. The cell-labeling solution was then added to the cell suspension at a ratio of 5 μL per mL of cell suspension. The resulting suspension was then incubated for 20 minutes at 37℃. Following the incubation period, the labeled cells were centrifuged at 600 g for 5 minutes. The cells were then gently resuspended in a warm medium following two gentle rinses and the removal of the supernatant. This procedure was repeated three times.

### 4. TE treatment

TE was purchased from Chemfaces (CFN99109, Wuhan, China). Oral treatment with TE (0.16 mg/kg) began on the $5^{th}$ day after the MCAO surgery and continued once a day for 22 days (Fig. 1). The same amount of double-distilled water was given to the animals in the control, MCAO-operated, and MCAO + EA groups.

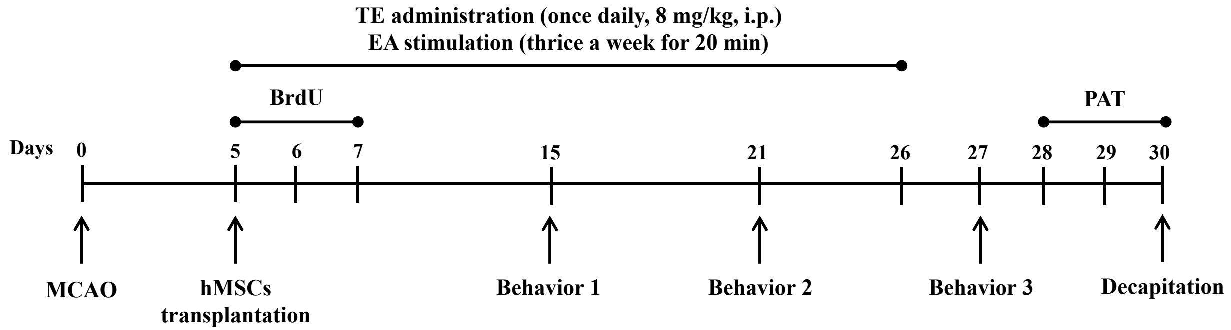


**Figure 1.** Experimental schedule. BrdU, bromodeoxyuridine; EA, electroacupuncture; hMSC, human mesenchymal stem cells; MCAO, middle cerebral artery occlusion; TE, tenuigenin.

## 5. hMSC transplantation

The prepared passage 3 hMSCs, at a concentration of $2 \times 10^5$/5 μL, were stereotaxically implanted into the left striatum of the mice (ML: 2.5 mm, DV: 4.0 mm). A 26-gauge Hamilton syringe was used for the transplantation at a rate of 0.5 μL/minute. After 5 minutes, the syringe was removed from the brain.

## 6. EA treatment

Mice were anesthetized using 2% isoflurane in a carrier gas composed of 80% $N_2O$ and 20% $O_2$ and were placed on a heating pad maintained at 37℃. The acupoints used for the procedure were the Baihui (GV20, the midpoint of the line connecting the apexes of the two ears on the parietal bone) and Dazhui (GV14, the posterior midline in the depression below the spinous process of the 7th cervical vertebrae). Stainless steel needles (Cat. DB106, Dongbang Healthcare Co., Ltd., Seoul, Korea) were employed for the acupuncture. A Grass S88 electrostimulator (Astro-Med Inc., West Warwick, RI, USA) was connected to the needles. Consistent with previous research [13], the output voltage was set at 2 V, and EA stimulation was applied at a frequency of 2 Hz for 20 minutes. EA was performed once every day for 22 days, starting from day 5 to day 26 following MCAO (Fig. 1). However, the MCAO, MCAO + hMSC, and MCAO + hMSC + TE groups were anesthetized but did not receive EA stimulation.

## 7. Bromodeoxyuridine (BrdU) injection

Cell proliferation was observed using BrdU (B5002–5G, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), a synthetic nucleoside for labeling. During the period of peak cell proliferation following MCAO, specifically from day 5 to 7, BrdU (10 mg/mL; 50 mg/kg) was administered daily.

## 8. Behavioral tests

Corner, cylinder, rotarod, and wire grip tests were conducted on days 15, 21, and 27 after MCAO. Additionally, the passive avoidance test was carried out on days 28-30 following MCAO (Fig. 1).

### 1) Corner test

The corner test assesses sensorimotor dysfunction on the ipsilateral side after ischemic stroke. Two boards (size: 30 cm × 20 cm) were joined at a 30° angle, with one side left open. When a mouse approached the left corner, the direction in which it raised its body was observed. The test was conducted 10 times and the average percentage of rotations that were toward the ipsilateral side was recorded.

### 2) Cylinder test

The cylinder test assesses locomotor imbalances and sensorimotor function by analyzing forelimb usage. A mouse contacts the front surface as it enters a 9 cm × 9 cm × 15 cm clear acrylic cylinder. After 20 attempts, we tallied the touches made by the right, left, and both paws, presenting each paw's usage in percentage.

### 3) Rotarod test

A rotarod device (Panlab S.I.U., Barcelona, Spain) was used to evaluate balance and motor coordination. Each mouse was tested on the spinning rod three times a day for 3 minutes at a speed of 18 rpm following acclimatization trials. The duration each mouse remained on the rod was recorded.

#### 4) Wire grip test

The wire grip test evaluates grip strength, balance, and endurance. A five-point scale was used to determine the wire grip score: 0, unable to stay attached to the wire for 30 seconds; 1, failure to grab the wire with both fore and hind paws at the same time; 2, grasping the wire with the fore paws but not the tail; 3, grasping the wire with the tail and both fore and hind paws; 4, traveling along the wire on all four paws and the tail. Each mouse received an average of three trials.

#### 5) Passive avoidance test

The passive avoidance test was used to evaluate memory and short-term learning. Mice were placed in a bright area and, when they attempted to escape, they were shocked on the foot. The following day, their recall of the foot shock was evaluated using a passive avoidance device (MED-APA-D1, Med Associates Inc., St. Albans, VT, USA), consisting of bright and dark compartments. The mice were placed in the bright compartment, and after 180 seconds, when they entered the dark compartment with the closed guillotine door, they received a small electric shock (0.5 mA, 3 seconds). The following day, with a maximum testing time of 600 seconds, the latency time for switching from the bright compartment to the dark compartment was measured.

### 9. Immunohistochemistry

Coronal serial cryosections of both hemispheres, each 25-μm-thick, were obtained. The cryosections were post-fixed with a 4% paraformaldehyde solution for 15 minutes before being washed with phosphate-buffered saline (PBS). Following that, the samples were processed using a standard immunofluorescence methodology for cell signaling studies, which involved blocking the samples with antibody dilution buffer (1× PBS with 5% normal goat serum and 0.3% Triton X-100). The following primary antibodies were used to stain the samples at 4℃ overnight: BrdU (OBT0030G, AbD Serotec, Oxford, UK), Dcx (Ab18723, Abcam, Cambridge, UK), NeuN (MAB377 and ABN78, Millipore Corporation, Billerica, MA, USA), GFAP (Z0334, DAKO, Santa Clara, CA, USA), and Sox2 (ab79351, Abcam). The following day, after three washes with PBS with Tween 20 (PBST), the samples were incubated with secondary antibodies for 2 hours. The stained brain tissues were mounted using a solution of 4',6-diamidino-2-phenylindole (DAPI; H-1200, Vector Laboratories, Inc., Burlingame, CA, USA). Five brain slices and 10× magnified images of coronal brain sections were selected for cell counting. The number of cells was calculated using i-solution (IMT i-solution Inc.) after capturing images with a fluorescence microscope (Carl Zeiss Imager M1, Carl Zeiss AG, Oberkochen, Germany).

### 10. Statistical analyses

All data analyses were performed using the SigmaPlot statistical software, version 11.2 (Systat Software, San Jose, CA, USA), and all data are presented as the standard error of the

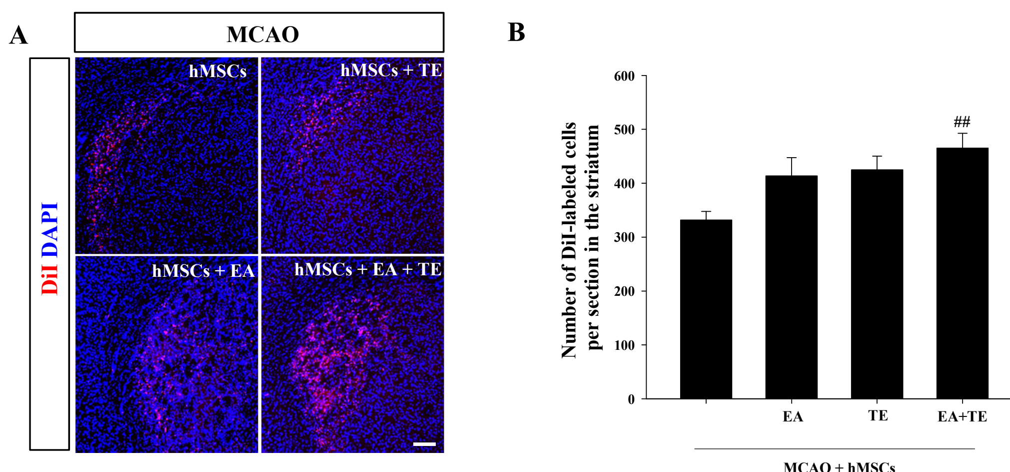


Figure 2. DiI-labelled cells in the striatum co-localized with DAPI. At day 30 following middle cerebral artery occlusion (MCAO), co-treatment of human mesenchymal stem cells (hMSCs) with electroacupuncture (EA) and tenuigenin (TE) causes an increase in hMSC migration toward the wounded locations. hMSCs that have been fluorescently labeled with CM-DiI in the contralateral striatum (A). Scale bar = 20 μm. Histogram analysis of DiI-labeled hMSCs (B; n = 5-6). Data are expressed as the mean ± SEM. ##$p < 0.01$ vs. the MCAO + hMSCs group.

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mean (SEM). When comparing more than two groups, Tukey's post-hoc test was used after a one-way or one-way repeated-measures analysis of variance (ANOVA). $p < 0.05$ was considered significant.

## RESULTS

### 1. EA and TE promote hMSC survival, migration, and differentiation in an ischemic brain

After MCAO, the hMSCs were transplanted into an ischemic penumbra (lateral 2.5 mm from bregma). On day 30 post-MCAO surgery, we detected DiI-labeled hMSCs in the ipsilateral striatum using fluorescence microscopy. The EA and TE groups demonstrated a tendency towards higher numbers of DiI-labeled cells compared to the hMSCs-only group. Specifically, the MCAO + hMSCs + EA + TE group exhibited significantly higher numbers of DiI-labeled cells compared to the MCAO + hMSCs group (Fig. 2; $p < 0.01$).

We assessed the effect of EA and TE treatment on hMSC differentiation by staining brain tissues with markers for neural stem cells (Sox2), immature neuroblasts (Dcx), mature neurons (NeuN), and astrocytes (GFAP). DiI-positive cells were represented by the number of marker-positive cells in the ipsilateral striatum (Fig. 3). Compared to the MCAO + hMSCs + EA group, both the MCAO + hMSCs + TE and MCAO + hMSCs + EA + TE groups had significantly higher numbers of Sox2/DiI-positive cells merged with DAPI (Fig. 3A, E; $p < 0.001$). There was a significant decrease in Dcx/DiI double-positive cells in the MCAO + hMSCs + TE group compared to the MCAO + hMSCs + TE + EA group (Fig. 3B, F; $p < 0.05$). In comparison to the MCAO + hMSCs group, the MCAO + hMSCs + EA + TE group had a significantly higher number of NeuN/DiI double-positive cells in the striatum ($p < 0.001$). In addition, both the MCAO + hMSCs + EA and MCAO + hMSCs + TE groups had significantly lower numbers of NeuN/DiI double-positive cells in the striatum compared to the MCAO + hMSCs + EA + TE group (Fig. 3C, G; $p < 0.01$ and $p < 0.001$). Conversely, the number of GFAP and DiI-labeled double-positive cells significantly decreased in the EA, TE, and EA + TE groups compared

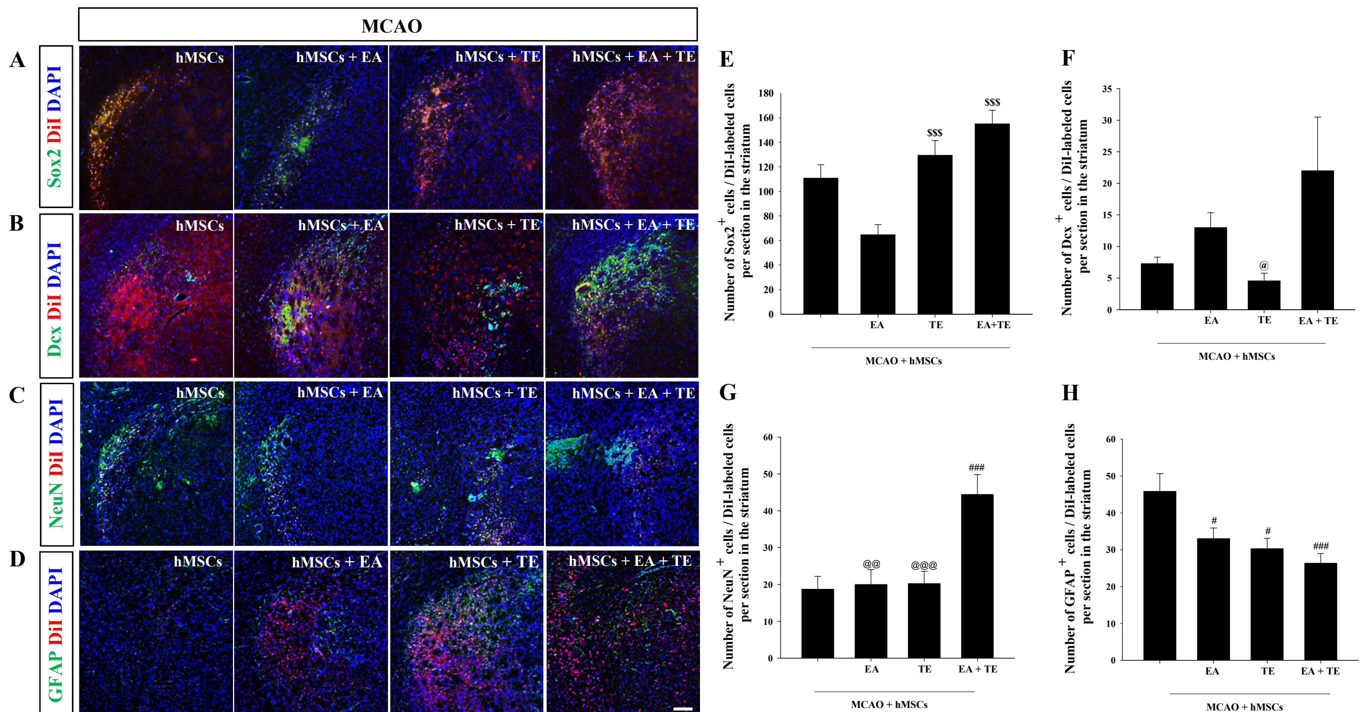


**Figure 3.** Neuronal markers with DiI-labeled cells in the striatum. Transplanted human mesenchymal stem cells (hMSCs) co-treated with electroacupuncture (EA) or tenuigenin (TE) promote the expression of various neuronal markers. Representative fluorescence microscopic images of Sox2/DiI/DAPI (A), Dcx/DiI/DAPI (B), NeuN/DiI/DAPI (C), GFAP/DiI/DAPI (D) merged cells in the ipsilateral striatum at day 30 following middle cerebral artery occlusion (MCAO). Scale bar = 20 μm Numbers of Sox2/DiI/DAPI (E), Dcx/DiI/DAPI (F), NeuN/DiI/DAPI (G), GFAP/DiI/DAPI (H) merged cells at day 30 after ischemic stroke (n = 5-7). Data are expressed as the mean ± SEM. $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ vs. the MCAO + hMSCs group; $^{\$\$\$}p < 0.001$ vs. MCAO + hMSCs + EA group; $^{@}p < 0.05$, $^{@@}p < 0.01$ and $^{@@@}p < 0.001$ vs. the MCAO + hMSCs + EA + TE group.

to the MCAO + hMSCs group (Fig. 3D, H; $p < 0.05$, $p < 0.05$, and $p < 0.001$). These findings indicate that the transplanted hMSCs primarily represented neural stem cells, mature neuron-like cells, or neuroblast-like cells at day 30 post-MCAO, with less astrogliosis in the MCAO + hMSCs + EA + TE group.

The number of BrdU/NeuN double-positive cells significantly increased in the subventricular zone in both MCAO + hMSCs and MCAO + hMSCs + EA groups compared to the MCAO-operated group (Fig. 4; $p < 0.001$).

## 2. MSC, EA, and TE improve brain function after stroke

In the corner test, stroke-affected mice displayed a left-side preference when exiting the corner due to right limb weakness post-MCAO. The MCAO + hMSCs + EA group exhibited a significant reduction in left turns on test days post-MCAO compared to the MCAO group ($p < 0.05$). However, no significant differences were observed in other treatment groups (Fig. 5A). Spontaneous asymmetry was assessed using the cylinder test. The hMSC treatment groups exhibited a reduction in left paw usage on days 21, 15, and 27 compared to the MCAO-operated group. No significant differences were observed between MCAO + hMSCs and the other treatment groups compared to the MCAO-operated group (Fig. 5B). In the rotarod test, the mice in the MCAO-operated group spent significantly less time on the rotarod compared to those in the control group ($p < 0.001$). However, no significant differences were observed between the treatment groups (Fig. 5C). In the wire grip test, the mice in the MCAO-operated group struggled to hold onto the wire compared to those in the control group ($p < 0.001$). The MCAO + hMSCs group scored significantly higher than the MCAO-operated group ($p < 0.001$), and all treatment groups displayed similarly high scores on behavioral test day 27 in the MCAO-hMSCs group ($p < 0.05$) (Fig. 5D). In the passive avoidance test, the retention latency exhibited by the MCAO-operated group was lower compared to the control group ($p < 0.05$). MCAO-operated mice treated with hMSCs, either alone or in combination with other treatments, exhibited higher retention latency compared to the MCAO-operated group (Fig. 5E; $p < 0.01$). There were no immediate behavioral improvements post-treatment, but gradual improvements in motor and cognitive functions were observed over time.

# DISCUSSION

Stroke is a devastating condition with limited treatment options [1]. This study investigated the potential therapeutic benefits of co-treatment with EA and TE following hMSC transplantation in a mouse model of ischemic stroke. Notably, the combination of EA and TE significantly enhanced hMSC survival, migration, and differentiation into neural stem cells, immature neuroblasts, and mature neurons, while concurrently suppressing astrocyte formation, resulting in improved neurogenesis. However, there was no significant improvement

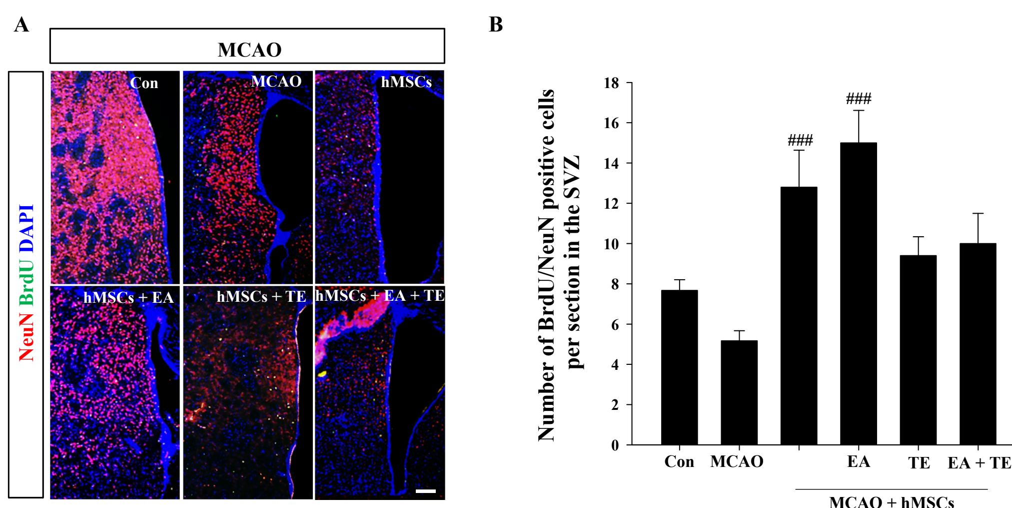


**Figure 4.** NeuN/BrdU double-positive cell co-localization with DAPI in the subventricular zone. Transplanted human mesenchymal stem cells (hMSCs) co-treated with electroacupuncture (EA) increase endogenous neurogenesis in the subventricular zone at day 30 after middle cerebral artery occlusion (MCAO). NeuN/BrdU/DAPI merged cells in the subventricular zone (A). Scale bar = 20 μm. Histogram analysis of NeuN/BrdU positive cells (B; n = 5-7). Data are expressed as the mean ± SEM. $^{###}p < 0.05$ vs. the MCAO group.

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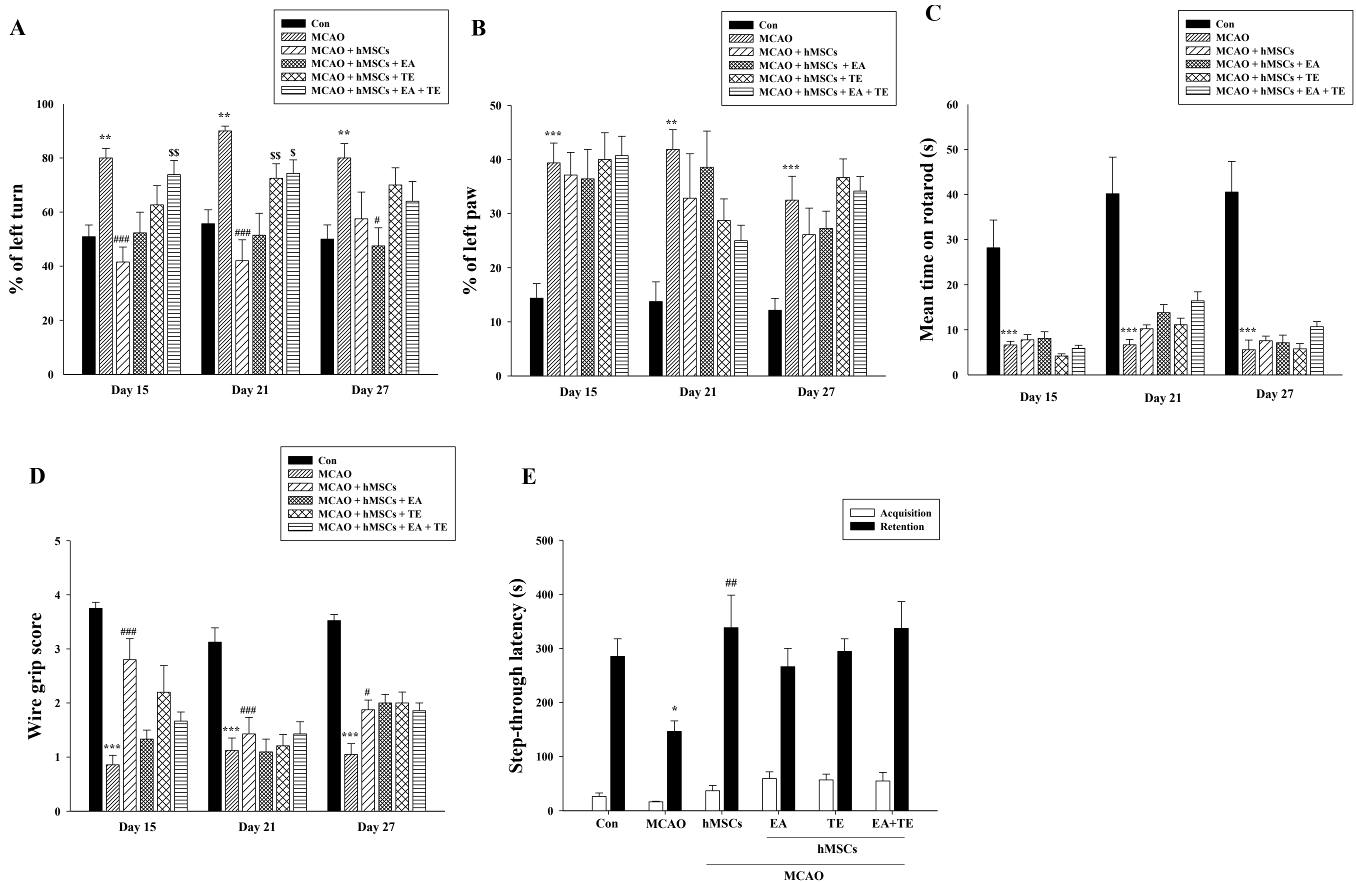


**Figure 5.** Transplanted human mesenchymal stem cells (hMSCs) improve functional outcomes after middle cerebral artery occlusion (MCAO). Transplanted hMSCs co-treated with electroacupuncture (EA) or tenuigenin (TE) attenuate functional deficits after MCAO (n = 6-8). Quantification of the corner test (A), cylinder test (B), rotarod test (C), and wire grip test (D). Quantification of the passive avoidance test (E; n = 6-8). Data are expressed as the mean ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ vs. the MCAO group; $^{\$}p < 0.05$ and $^{\$\$}p < 0.01$ vs. the MCAO + hMSCs group.

in behavioral outcomes immediately after treatment. Motor and cognitive functions did gradually improve over time. These findings provide valuable insights into the potential of this combination approach for promoting hMSC migration, differentiation, and functional recovery following stroke.

The first notable finding in this study is the enhanced survival of transplanted hMSCs in the ischemic brain when subjected to EA and TE co-treatment, suggesting that the combination of EA and TE establishes a more favorable microenvironment for the survival of transplanted cells. Increased cell survival is a crucial factor in the effectiveness of cell-based therapeutics for neurological disorders [14], and these findings are encouraging. Individual components of EA and TE treatment have been reported to influence cell survival. EA alone has demonstrated a significant ability to enhance the survival of neural progenitor cells, thus exhibiting neuroprotective effects in stroke models [15-17]. The therapeutic impact of EA is thought to be due to its modulation of neuroinflammation, enhancement of neuroprotection, and promotion of neuroplasticity [6]. By stimulating the release of neurotrophic factors such as BDNF and VEGF, EA plays a role in supporting neuronal survival, growth, and differentiation [7, 8]. Additionally, EA has the potential to influence the activation of endogenous neural stem cells, thereby amplifying the restoration processes within the injured brain [9].

Similarly, TE has demonstrated neuroprotective properties, including promoting cell survival in a model of cerebral ischemia [11]. A growing body of knowledge suggests that natural compounds derived from traditional oriental herbal remedies can enhance brain function and stimulate stem cell proliferation [10]. TE, a bioactive compound extracted from the Chinese herb *Polygala tenuifolia*, has become a focal point of research due to its potential neuroprotective and neurogenic effects.

Studies propose that TE may protect against neurodegenerative disorders due to its neuroprotective properties and ability to stimulate neurogenesis [12]. Moreover, TE has been implicated in attenuating oxidative stress and inflammation, pivotal factors in neurodegeneration [18, 19]. Huang et al.'s study [20] unveiled TE's capacity to enhance antioxidation and synaptic plasticity in the hippocampus, indicating its potential for cognitive enhancement. These findings underscore the promising potential of EA and TE combination therapy as a method to enhance the survival of transplanted hMSCs in the ischemic brain. This improved cell survival may have a substantial impact on how MSC-based therapies are used for neurological diseases.

Another critical aspect of this study is the promotion of hMSC differentiation into various neural cell types. The observed increase in the number of Sox2/DiI, Dcx/DiI, and NeuN/DiI-positive cells in the MCAO + hMSCs + EA + TE group suggests a greater propensity for transplanted hMSCs to differentiate into neural stem cells, immature neuroblasts, and mature neurons. Several studies have observed that hMSCs are inclined to differentiate into several neural cell types, including neural stem cells, immature neuroblasts, and mature neurons [10, 13, 21, 22]. These findings highlight the therapeutic potential of EA and TE co-treatment in stimulating neural regeneration following ischemic stroke.

Notably, EA and TE co-treatment also led to a significant decrease in GFAP/DiI double-positive cells, indicating reduced astrogliosis in the co-treatment group compared to hMSCs-only treatment. Astrogliosis, the proliferation of astrocytes, is a common response to brain injury. Although some degree of astrogliosis is necessary for tissue repair, excessive astrogliosis can hinder regeneration [23]. The reduction in astrogliosis observed in the co-treatment group suggests that EA and TE co-treatment might modulate the brain immune response and establish a more conducive environment for neural repair.

Although the behavioral outcomes immediately following the stroke were not significant, it is important to note that gradual improvements in motor and cognitive functions were observed, suggesting that the regenerative effects of hMSCs, possibly facilitated by EA and TE, may take time to fully manifest. This complex nature of brain repair processes and the many facets of behavioral recovery in ischemic stroke requires further investigation.

This study serves as a reference for the development of ischemic stroke treatment plans. The ability to improve hMSC differentiation into neuronal cell types, promote neurogenesis, and regulate astrocyte response has significant clinical implications for patients with stroke. However, the study reveals several areas warranting further investigation and refinement. Notably, there is a need for deeper mechanistic insights into how EA and TE exert their effects at the cellular and molecular levels. Moreover, the absence of a comparative analysis between the efficacy of EA and TE co-treatment and established stroke treatments raises questions about the treatment's relative effectiveness. The exclusive focus on ischemic stroke may limit the generalizability of the findings to other stroke types, and the lack of a dose-response analysis leaves optimal dosage information unexplored. Addressing these limitations in future research would enhance the evidence base for the therapeutic potential of EA and TE co-treatment in ischemic stroke. Additionally, exploring the possibilities of integrating these therapies with other interventions or medications may yield further insights into enhancing the overall recovery process.

## CONCLUSION

The co-treatment of EA and TE exhibits promising potential in enhancing the survival, migration, and differentiation of transplanted hMSCs after stroke. Although immediate behavioral improvements were not observed, the gradual recovery of motor and cognitive functions suggests that the treatment protocol warrants further investigation as a potential treatment modality for ischemic stroke.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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