IBRO Neuroscience Reports 18 (2025) 619–626

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

# Effect of BMSCs overexpressing intelectin-1 on angiogenesis in rats with cerebral infarction

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ARTICLE INFO

*Keywords:*
Intelectin-1
BMSCs
Angiogenesis
Apoptosis
CI

ABSTRACT

*Background:* Cerebral infarction (CI) is a common and frequently occurring acute neurological disease in clinical practice, posing a severe threat to human health. CI results from various causes leading to local cerebral tissue ischemia and hypoxia due to vascular occlusion and impaired blood supply, which in turn leads to tissue necrosis and corresponding clinical manifestations of neurological deficits. However, to date, treatment options for cerebral infarction remain limited. Therefore, it is crucial to rapidly establish collateral circulation to compensate for the occluded vessels and restore blood flow perfusion.

*Objective:* To assess the effect of bone marrow mesenchymal cells transfected with intelectin-1 (Itln-1) gene on the angiogenesis and apoptosis of CI.

*Method:* Lentiviral-mediated transfection of the Itln-1 gene into bone marrow mesenchymal stem cells (BMSCs) was performed, followed by intravenous injection into rats with CI through the tail vein. The volume of the CI, capillary density, and apoptotic cells were detected.

*Results:* With the increase of AKT and eNOS phosphorylation levels, BMSCs with overexpression Itln-1 gene could significantly promote angiogenesis and reduce the infarct volume in the ischemic penumbra. Meanwhile, the ratio of Bcl-2/Bax increased, and apoptotic cells decreased.

*Conclusion:* The overexpression of Itln-1 can effectively promote CI angiogenesis and inhibit cell apoptosis than transplantation of Itln-1 gene or MSCS alone

## 1. Introduction

Cerebral infarction (CI),also known as ischemic stroke,is a serious medical condition caused by a blockage or reduction in blood flow to a part of the brain (Iizuka et al., 2019). It involves complex pathophysiological processes such as inflammation,oxidative stress,apoptosis,and vascular dysfunction (Surinkaew et al., 2020; Morris-Blanco et al., 2021). CI is a major cause of death and disability worldwide (Jonsson et al., 2018). Preventive measures,early detection,and effective treatment strategies are crucial in reducing the impact of CI.

At present,there are few effective treatments for cerebral infarction (CI) (Boltze et al., 2021). Recombinant tissue plasminogen activator (rt-PA) is an effective therapy,but its short treatment window limits its application (Ueno et al., 2018). The basic principle of stroke treatment is the reconstruction of the collateral channels after infarction;this reconstruction mainly depends on angiogenesis.Promoting angiogenesis in the subacute phase of CI can help maintain cerebral blood flow and supply oxygen and nutrients,which is beneficial for neural recovery (Zhao et al., 2018). However,the mechanisms regulating angiogenesis during CI are not yet fully understood.

Intelectin, a member of the lectin family,is a protein that participates in cell proliferation regulation (Elola et al., 2015). We recently discovered that intelectin-1 (Itln-1) promotes angiogenesis in the middle cerebral artery occlusion rat model (MCAO) and the oxygen-glucose deprivation cell model (OGD),inhibiting cell apoptosis and antioxidation through the PI3K/AKT signal pathway (Gu et al., 2019, 2017).

Bone marrow mesenchymal stem cells (BMSCs) play a crucial role in regenerative medicine due to their differentiation potential and self-replicating ability (Kraitchman et al., 2005). They promote angiogenesis and neurogenesis post-stroke by modulating the PI3K/AKT signaling pathway and exhibit anti-inflammatory and anti-apoptotic effects (Gu et al., 2014). BMSCs are ideal vectors for gene therapy due to their accessibility,wide availability,and their capacity to integrate with host cells across the blood-brain barrier for long-term survival (Jiang et al.,

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https://doi.org/10.1016/j.ibneur.2025.03.012
Received 21 December 2024; Received in revised form 31 March 2025; Accepted 31 March 2025
Available online 11 April 2025

2021a).

In this study,we transfected BMSCs with intelectin-1 gene and investigated their effect on promoting angiogenesis and inhibiting cell apoptosis in rats with ischemic stroke compared to intelectin-1 gene or BMSCs alone.

## 2. Material and methods

### 2.1. Animals

A total of 188 Lewis rats,aged 2–3 months,were obtained from Xi'an Jiaotong University.These rats were housed in a controlled environment with a temperature of 22 ± 2°C,a relative humidity of 50 ± 5 %,and a 12-hour light/12-hour dark cycle,with ad libitum access to water and food.All animal experiments, including the euthanasia process,were conducted in accordance with the Institutional Animal Care regulations at Xi'an Jiaotong University,and were also compliant with the guidelines of AAALAC and IACUC.

Rats were randomly assigned to four groups:①MCAO group②Itln group, injected with 5 μL of LV-Itln-1 intracerebroventricularly one day after MCAO;③MSCs group,administered 2 × 10^6 BMSCs (1 mL) intravenously via the tail vein;④Itln-MSCs group;injected with 2 × 10^6 BMSCs transfected with the Itln-1 gene (1 mL) through the tail vein one day after MCAO.

### 2.2. Resuscitation and culture of BMSCs

Bone Marrow Mesenchymal Stem Cells (BMSCs) from bone marrow were derived from the femurs and tibias of rats. Their isolation, culture, and identification have been completed in our earlier experiments and have been published in articles.BMSCs were cultured to the third generation and stored in liquid nitrogen for preservation for subsequent use (Zhen et al., 2010). Thawed cryopreserved tubes containing Bone Marrow Mesenchymal Stem Cells (BMSCs) were quickly immersed in a 37℃ water bath,mixed with pre-warmed culture medium,and centrifuged at 1000 rpm.After washing,cells were combined with fresh medium and cultured in a humidified incubator at 37℃ with 5 % CO2 and 95 % air.The medium was changed after 24 hours.BMSCs were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1 % penicillin-streptomycin and 10 % Fetal Bovine Serum (FBS),with medium changes every 3–4 days and cells passaged 2–3 times during the experiment.

### 2.3. Lentivirus transfected BMSCs

Cells (3rd passage) were subcultured into a six-well culture plate.The lentivirus used in this experiment is composed of four packaging plasmids: pRsv-REV, pMDlg-pRRE, pMD2G, and Lenti-GFP-Itln-1. Among these, the Lenti-GFP-Itln-1 plasmid is responsible for expressing green fluorescent protein (GFP). The plasmids pRsv-REV, pMDlg-pRRE, and pMD2G contain the essential elements required for virus packaging (Zhen et al., 2010). After 24 hours, cells were mixed with lentivirus stock solution containing intelectin-1 gene (Gu et al., 2017) (virus titer was $5 \times 10^8$ TU/mL,according to the multiple number of infection MOI=20, gently drop the culture solution containing 20 μl of virus solution onto the cell, and gently mix it.The following day,replaced the old medium with a fresh complete medium and maintained the cell culture for a duration of 5 days.Change in the fluid was assessed every 24 hours,and the expression of GFP was analyzed using a fluorescence microscope after 5 days.

### 2.4. Middle cerebral artery occlusion

The transient MCAO (tMCAO) procedure was performed as previously described (Longa et al., 1989). Rats were fasted for 1 hour prior to the operation but had free access to water.The neck tissues were dissected to expose the left common carotid artery and the external carotid artery (ECA),followed by the stripping of the left internal carotid artery (ICA) down to the pterygopalatine artery level.The ECA was then ligated distally with a small proximal incision made.A 25 mm long nylon suture (4−0) was inserted,using a silicone rubber tip with a rounded end and a diameter of 300–340 μm,carefully threaded through the ICA until slight resistance was felt.After a 2-hour period, the nylon suture was removed from the ICA.Subsequently,blood flow restoration was confirmed using laser Doppler flowmetry.

After 24 hours of modeling,the rats were scored using the 5-grade scale introduced by Longa et al. (1989), which includes:Grade 0: No impairment in nerve function.Grade I:Mild focal neurological deficit. Grade II:Moderate focal neurological deficit. Grade III:Significant focal neurological impairment.Grade IV:Difficulty walking without spontaneous movement and falling into a stupor.Experimental subjects were selected among rats with neurological function scores ranging from Grade I to III.

### 2.5. qRT-esCR

At 7 days post-MCAO,Seven days after MCAO, the rats were sacrificed and their brains were extracted. The brain tissue supplied by the left middle cerebral artery was carefully dissected layer by layer. Starting approximately 2 mm lateral to the longitudinal fissure, the central brain tissue was cautiously removed from posterior to anterior. Then, about 2 mm of the frontal lobe was excised anteriorly, followed by the removal of an additional 5 mm of excess tissue through the anterior fontanelle towards the posterior. The remaining section constituted the ischemic penumbra and surrounding tissues. The tissue was divided into smaller pieces for the extraction of RNA.First-strand cDNA was synthesized using the PrimeScript RT reagent kit,and assessed the levels of IL-1 and TNF-α.Primer sequences:

Itln-1F:5′-TGACAATGGTCCAGCATTACC-3′,
Itln-1R:5′-ACGGGGTTACCTTCTGGGA-3′.
TNF-αF:5′-CCTGTAGCCCACGTCGTAGCAAA-3′,
TNF-αR:5′-AGCGCTGAGTTGGTCCCCCT-3′,
IL-1F:5′-AGCTGGAGAGTGTGGATCCCAAGC-3′,
IL-1R:5′-AGCGACCTGTCTTGGCCGAGG-3′.

### 2.6. Immunohistochemical analysis was conducted on CD34 cells located in the penumbra region of ischemic brain tissue

At 7 days post-MCAO,samples from each group were fixed with 4 % paraformaldehyde-PBS for 12 hours,sectioned,and embedded in paraffin.The sections were then cut into 5-micron slices for immunohistochemistry using the streptavidin-biotin-peroxidase complex method (Zheng et al., 2008). The slices were incubated with anti-CD34 monoclonal antibody (1:200, Santa Cruz, CA, USA)) at 4°C for 12 hours, followed by incubation with peroxidase-conjugated goat anti-mouse IgG (1:1000, Beijing, China) at 37°C for 30 minutes.CD34 cell distribution was observed under a light microscope(BH-2; Tokyo Olympus,Japan), and calculated the number of CD34 positive cells per unit area.

### 2.7. Utilizing laser scanning confocal microscopy (LSCM) in combination with Doppler laser assay

Seven days post-MCAO,1 mL of 50 mg/mL fluorescein isothiocyanate (FITC)-dextran was administered via the femoral vein.One minute later, the rats were euthanized,and tissue from the ischemic penumbra was collected.Samples were fixed in a 4 % formaldehyde solution for 48 hours and then sectioned into 20-micrometer-thick coronal slices. Microvascular formation was assessed using laser scanning confocal microscopy (LSCM) and 3D Doctor 3.5 software.Quantitative evaluation of angiogenesis was conducted by analyzing five visual fields from slices of five randomly selected rats. Mean values were also calculated.

One week after MCAO,the skull was secured in a stereotactic frame,

and a small enclosed cranial window was created on the left hemisphere to measure local cerebral blood flow (lCBF) using a Doppler laser blood flow detector (Peri Flux 5000, Perimed AB,Beijing).The standard cellular nucleus appeared blue,while the nucleus of apoptotic cells showed a deeper color due to dye uptake and a duller fluorescence from the DNA fluorochromes.

### 2.8. TTC staining and evaluation of infarction volume

One week after MCAO,the brains were extracted and rapidly sectioned into 2-mm thick coronal slices.These sections were stained with 2 % 2,3,5-triphenyltetrazolium chloride (TTC) for 30 minutes in the dark at 37°C.The areas of infarction and the hemispheres were traced and quantified using an Imaging-Pro-Plus image analysis system from Olympus.Corrections were made for brain edema by comparing the volume of the contralateral hemisphere to the unaffected ipsilateral hemisphere,with the infarcted volume expressed as a percentage relative to the contralateral hemisphere.

### 2.9. In situ apoptosis detection (TUNEL staining)

After dewaxing and dehydration,tissue sections were treated with proteinase K (20 mg/mL in Tris/HCl,pH 7.4–8.0) at room temperature for 15 minutes,followed by three washes with PBS.The sections were then incubated with an endogenous peroxidase blocker and washed again with PBS.Subsequently,the sections were incubated with TUNEL reagent in a humidified chamber at 37°C for 60 minutes,washed with PBS,and treated with converter-peroxidase for 30 minutes.Finally,the sections were stained with DAB and hematoxylin,dehydrated,and prepared for examination under a light microscope.

### 2.10. Western blot of Bax, Bcl-2, AKT, p-AKT, eNOS, and p-eNOS proteins

After 7 days post-MCAO,rat brain tissues from the ischemic penumbra were collected and the expression of intelectin-1,Bcl-2,p-AKT, and p-eNOS proteins was assessed using Western blot.Brain tissues (n = 8 per group) were washed with cold PBS and lysed in a protein extraction reagent containing PMSF.After 15 minutes on ice, the lysate supernatants were centrifuged at 15,000 g for 15 minutes.Fifty micrograms of protein were separated by 10 % SDS-PAGE and transferred to a nitrocellulose membrane,which was then blocked with 5 % skim milk in TBST for 1 hour at room temperature.The membrane was incubated with rabbit polyclonal anti-Bcl-2, Bax, AKT, p-AKT, eNOS, p-eNOS (1:200,Santa Cruz,America), and β-actin antibodies(1:5000, Santa Cruz, America), followed by incubation with a secondary antibody(1:1000, Santa Cruz, America) conjugated to horseradish peroxidase at 4°C for 12–16 hours. After washing with TBST,detection was performed using an ECL detection kit. Protein expression was quantified using ImageJ software and normalized against tubulin.

### 2.11. Statistical analysis

Data were analyzed using the SPSS 20.0 software (SPSS, Chicago, IL, USA). Data were presented as means ± SEM.Statistical differences between the various groups were assessed by one-way ANOVA followed by the SNK-q test.A value of $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Following the transplantation of BMSCs-Itln-1, a significant increase in Intelectin-1 expression

At 7 days post-MCAO,Itln group and Itln-MSCs group showed significantly higher Itln-1 mRNA expression compared to MCAO and MSCs groups ($P < 0.0001$; Fig. 1B).There was no significant difference between Itln and Itln-MSCs groups ($P > 0.05$; Fig. 1B).In the MCAO group (Fig. 1Da) and MSCs group (Fig. 1Db), there was no GFP expression. However, in the Itln-1 group (Fig. 1Dc) and Itln1-MSCs group (Fig. 1Dd), fluorescence was strongly expressed throughout the left cerebral cortex.This indicates that BMSCs can successfully deliver genes to the ischemic penumbra,with an effect similar to that of lentiviruses.

### 3.2. Transplantation of BMSCs-Itln-1 led to a decrease in the volume of CI and an increase in the regional cerebral blood flow

One week after tMCAO, infarct volume was significantly reduced in the Itln and MSCs groups after treatment with Itln-1 and BMSCs, respectively, compared to the MCAO group ($P < 0.0001$; Fig. 1A),with no significant difference between the Itln and MSCs groups ($P > 0.05$; Fig. 1A).Treatment with BMSCs overexpressing Itln-1 further significantly reduced infarct volume compared to the Itln and MSCs groups ($P < 0.0001$; Fig. 1A).The study suggests that enhancing Itln-1 in BMSCs could potentially decrease infarct volume after MCAO.

Seven days after MCAO,regional cerebral blood flow was significantly higher in the Itln and MSCs groups compared to the MCAO group ($P < 0.0001$; Fig. 1C). After treatment with BMSCs overexpressing Itln-1,the Itln-MSCs group showed further increased cerebral blood flow seven days post cerebral ischemia/reperfusion injury ($P < 0.0001$; Fig. 1C).

### 3.3. BMSCs overexpressing Itln-1 promoted angiogenesis and improved neural function defects

Endogenous vascular injury and the maintenance of endothelial integrity are significantly influenced by angioblasts known as endothelial progenitor cells (EPCs). CD34 is one of its specific surface markers. Moreover, endothelial cells are generated by EPCs in response to ischemic injury.After injection of BMSCs overexpressing Itln-1,the expression of CD34 in the Itln-MSCs group was further increased ($P < 0.0001$; Fig. 2A).Treatment with BMSCs-Itln-1 further increased the number of microvessels in the ischemic boundary zone of MCAO rats after cerebral I/R injury ($P < 0.0001$; Fig. 2B), with no significant difference between the Itln and MSCs groups ($P > 0.05$; Fig. 2B).These results suggest Injection of BMSCs overexpressing Itln-1 significantly increased CD34 expression and further promoted neovascularization. Moreover,the neurological deflict score was significantly reduced in the Itln-MSCs group ($P < 0.0001$; Fig. 2C),indicating that BMSCs overexpressing Itln-1 can more effectively improve neurological function.

### 3.4. BMSCs-Itln-1 inhibited cell apoptosis in the ischemic areas

Apoptosis plays a key role in cerebral ischemia (CI).To investigate the effects of Itln-1,MSCs,and Itln-1-overexpressing BMSCs on CI-induced cell apoptosis, researchers used Western blot to measure Bcl-2 and Bax protein levels and calculated the Bcl-2/Bax ratio one week post-MCAO.The Itln and MSCs groups had higher Bcl-2 levels and lower Bax levels than the MCAO group,leading to a higher Bcl-2/Bax ratio ($P < 0.05$; Fig. 3B).The Itln-MSCs group showed the highest Bcl-2/Bax ratio and the fewest apoptotic cells as detected by the TUNEL method ($P < 0.05$; Fig. 3A).This suggests that BMSCs overexpressing Itln-1 can reduce apoptosis by modulating Bcl-2 and Bax, potentially improving outcomes in CI.

### 3.5. BMSCs-Itln-1 induced the activation of AKT and eNOS pathways

Endothelial cells enhance their survival in response to various growth factors and cytokines,with AKT playing a key role in regulating angiogenesis.Under ischemic conditions,the upregulation of Itln-1 in BMSCs enhances the phosphorylation of AKT and eNOS,which may

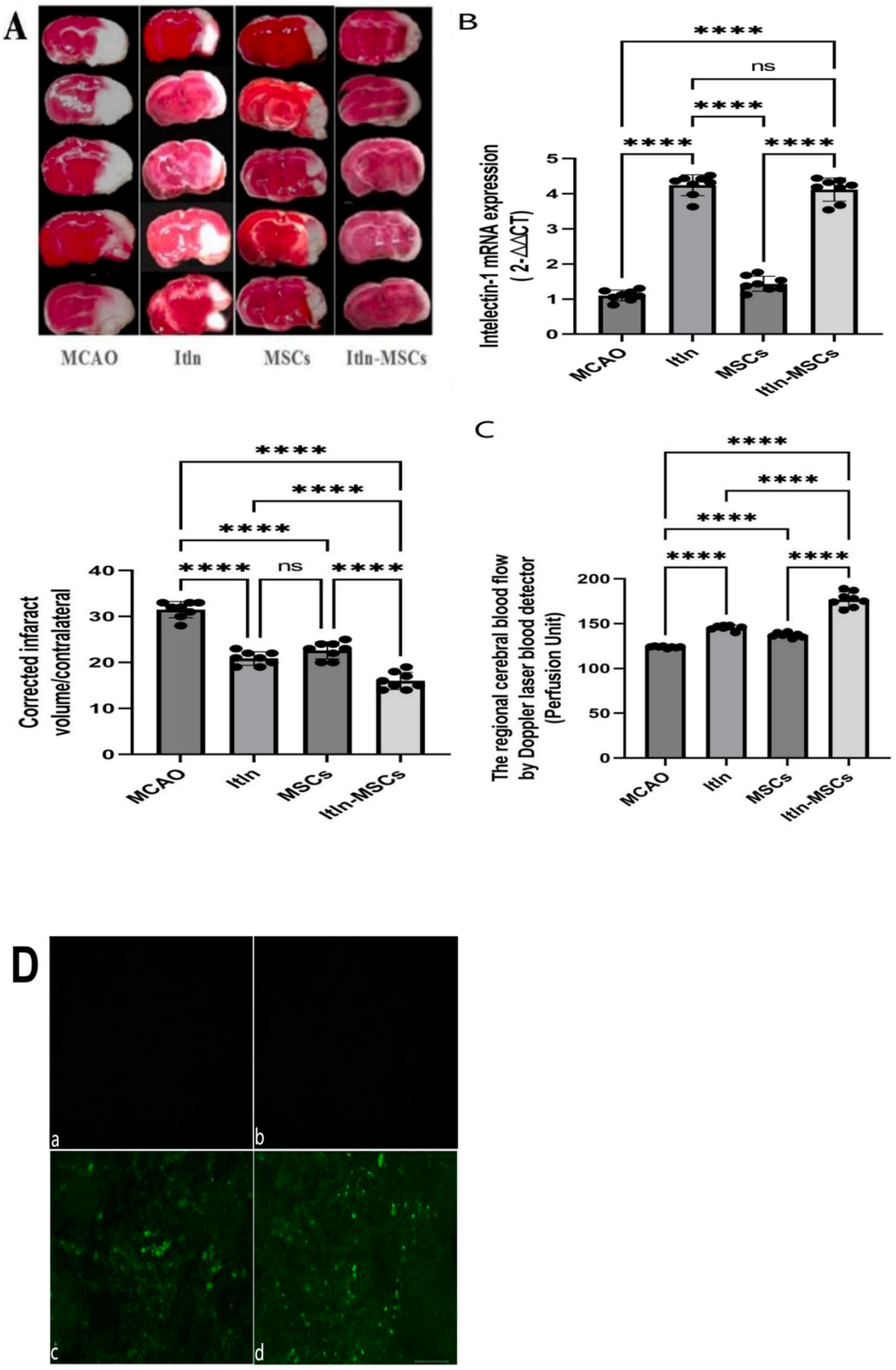


**Fig. 1.** BMSCs overexpressing Itln-1 reduced infarct volume and increased the regional cerebral blood flow (Each group n = 8):(A) CI volume in each group by TTC staining. (B) The expression of Itln-1 mRNA in the cerebral ischemic penumbra. (C) The regional cerebral blood flow (Perfusion Unit) measured by the Doppler laser bloodstream detector.(D) Immunohistochemistry of itlin-1 and GFP.$^{****}P < 0.0001$.

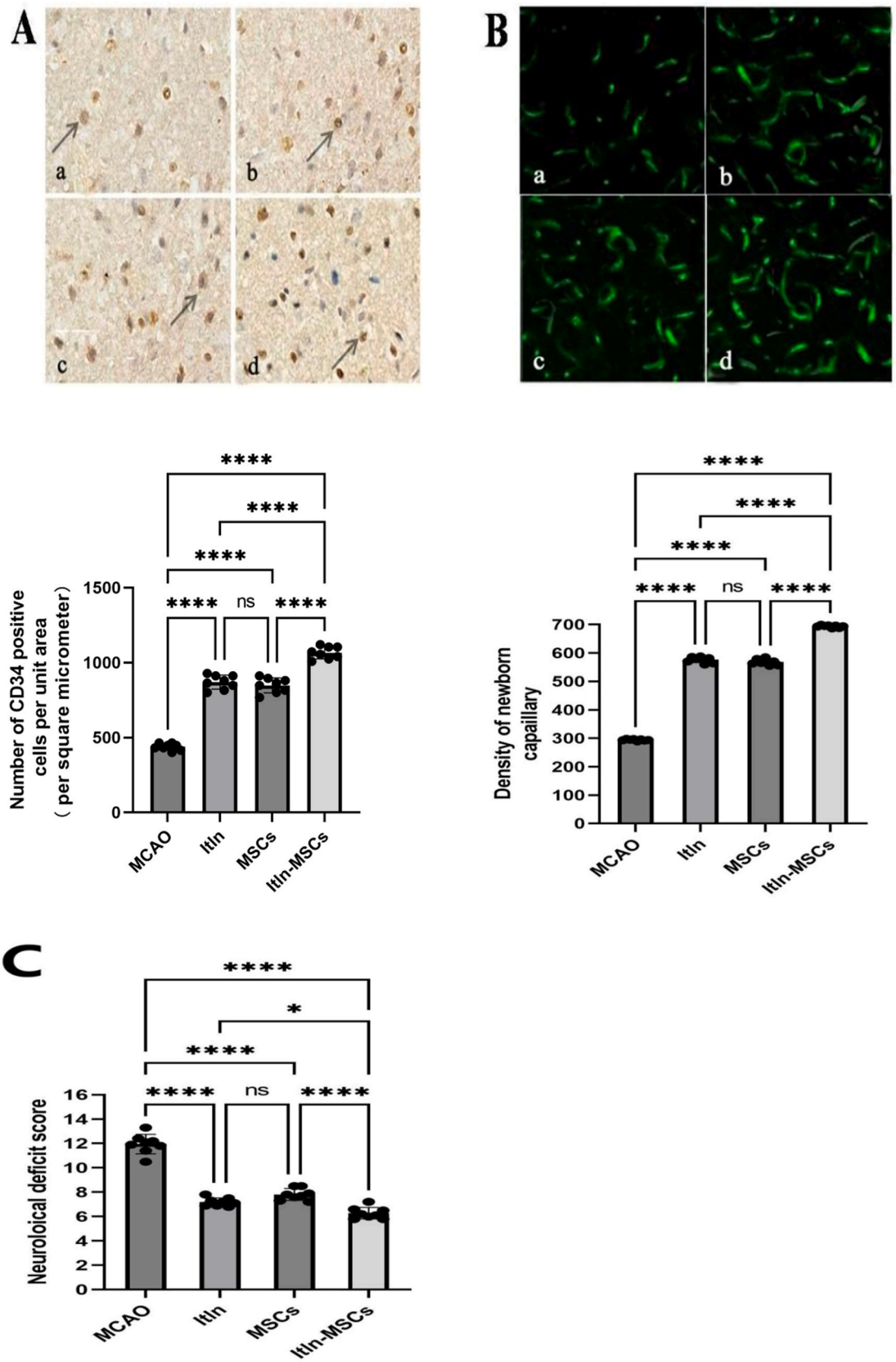


**Fig. 2.** BMSCs overexpressing Itln-1 promoted angiogenesis and improved neural function defects(Each group n = 8): (A)Expression of CD34 in the left cerebral cortex of rats.(B) The number of microvessels in the ischemic boundary zone of MCAO by 3D laser scanning confocal microscopy. (C) Neurological function defect scores of rats with CI by the Modified neurological severity score method (mNSS) in each group.$^{****}P < 0.0001$,scar bar= 50um.

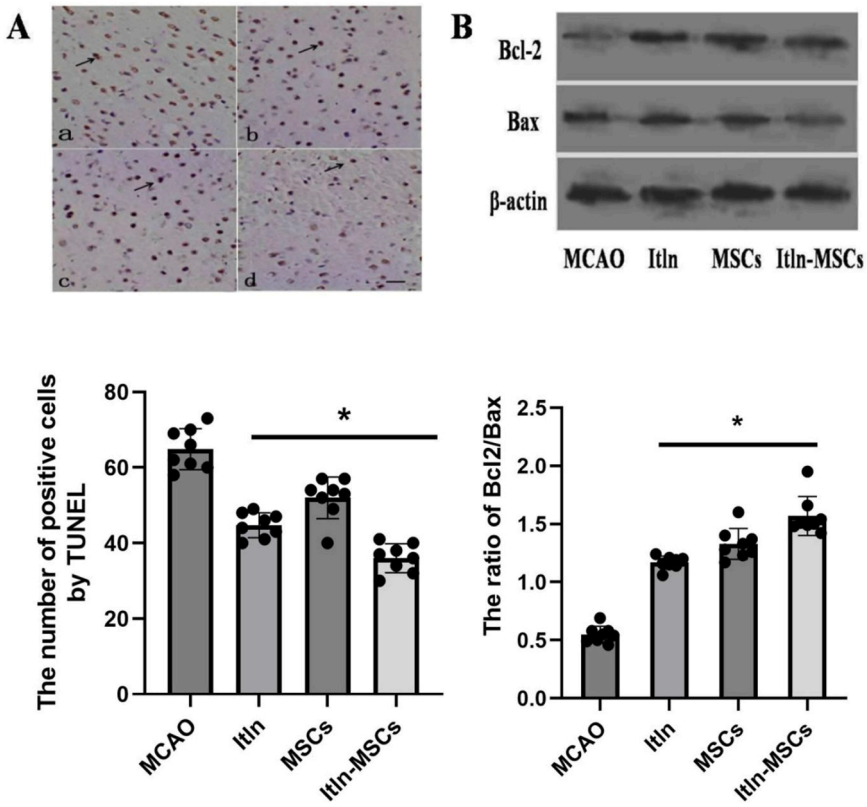


**Fig. 3.** BMSCs overexpressing *Itln-1* inhibited cell apoptosis in the ischemic areas(Each group n = 8). (A)The number of apoptotic cells (TUNEL method) in the left cerebral cortex of rats 1 week after I/R injury. (B) Expression of Bcl-2 and Bax protein (Western blot) in the left cerebral cortex of rats 1 week after I/R injury. *P < 0.05,scar bar= 50um.

promote angiogenesis and inhibit apoptosis by activating the AKT-eNOS pathway.Compared to the MCAO group,the phosphorylation levels of AKT and eNOS significantly increased in the Itln and MSCs groups,with the Itln-MSCs group showing an even greater increase (P < 0.0001; Fig. 4).

## 4. Discussion

This study investigated the effects and potential mechanisms of BMSCs overexpressing Itln-1 on angiogenesis following cerebral ischemia.Previous research used a lentivirus to overexpress the Itln-1 gene, which was found to promote angiogenesis and inhibit apoptosis via the PI3K/AKT pathway (Gu et al., 2019). However,the lentivirus, used solely for gene delivery, had limitations in capacity and technical complexity and may trigger an immune response (Azzouz et al., 2004), making it more suitable for experimental settings than clinical use.

Over the past decade,a greater understanding of the capabilities of BMSC in tissue regeneration has emerged (Samakova et al., 2019). BMSCs regulate the PI3K/AKT signal pathway to promote angiogenesis and neurogenesis through paracrine function following CI (Jiang et al., 2021b). BMSCs are considered to be ideal transgenic vector cells because of their convenient materials, wide sources,rapid expansion *in vitro* culture,low immunogenicity after subculture, ability to transport and express exogenous genes,and ability to integrate with host cells through the blood-brain barrier and survive for a long time (Carmeliet and Jain, 2011).

In this study,we found that BMSCs overexpressing Itln-1 can promote angiogenesis.Our results showed that the Itln-1 gene was effectively transduced to a large ischemic peri-focal area with sustained expression as determined by qRT-PCR. Angiogenesis,the process of forming new blood vessels,is regulated by various factors including growth factors, cytokines,adhesion molecules,chemokines,enzymes,and modulators of angiogenesis that interact with each other (Carmeliet and Jain, 2011). Preclinical studies have indicated that targeting angiogenesis may help alleviate stroke conditions (Sun et al., 2003); thus, angiogenesis is considered a promising therapeutic strategy for post-stroke recovery (Ergul et al., 2012).

Angiogenesis is regulated by the co-expression of endothelial progenitor cell (EPC) surface markers CD34,CD133,and vascular endothelial growth factor (VEGF) receptor-2.The expression of CD34, the initial marker for defining EPCs,decreases as EPCs mature into endothelial cells (Asahara et al., 1997). In this study,we treated tMCAO rats with BMSCs, a lentivirus carrying the Itln-1 gene,or BMSCs overexpressing Itln-1.We found that BMSCs overexpressing Itln-1 significantly increased CD34 expression, vessel density,and reduced infarct volume compared to the other treatments.This suggests that BMSCs overexpressing Itln-1 are more effective at promoting angiogenesis after cerebral ischemia,likely due to the combined effects of BMSCs' paracrine actions and the Itln-1 gene on angiogenesis.

Next, we assessed neurological function deficits and local cerebral blood flow using laser Doppler flowmetry (LDF).The findings showed that following the administration of BMSCs overexpressing Itln-1,there was a significant reduction in the neurological deficit score and an increase in local cerebral blood flow in the Itln-MSCs group rats.Thus, BMSCs overexpressing Itln-1 can effectively promote angiogenesis and ameliorate blood flow and neurological function deficits.

Apoptosis is a critical pathophysiological mechanism associated with ischemia/reperfusion (I/R) injury,and reperfusion can accelerate the apoptotic death process initiated by ischemia (Villa et al., 2003). Numerous studies suggest that inhibiting apoptosis is a key protective mechanism against cerebral I/R injury (Yu et al., 2020). Bcl-2 family--related proteins, including anti-apoptotic proteins (such as Bcl-2, Bcl-xL) and pro-apoptotic proteins (such as Bax, Bcl-xS),have been shown to play a significant role in the execution of apoptosis (Sergio et al., 2018).

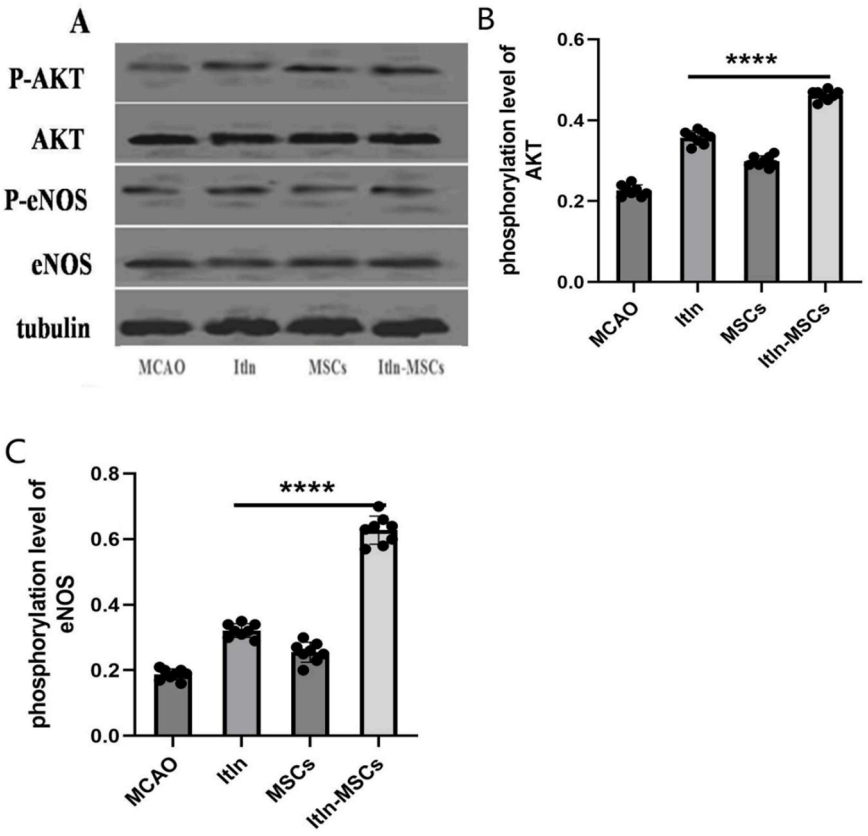


**Fig. 4.** BMSCs overexpressing *Itln-1* stimulated the phosphorylation of Akt and eNOS(Each group n = 8).(A-C)Phosphorylation levels of Akt and eNOS (Western blot) in the left cerebral cortex of rats 1 week after I/R injury. $^{****}P < 0.0001$.

Increased Bcl-2 expression and decreased Bax expression in the hippocampus following ischemia can protect against cerebral ischemic injury by reducing neuronal apoptosis (Yi et al., 2020). Consistent with previous research, we found that BMSCs overexpressing Itln-1 could increase Bcl-2 expression and decrease Bax expression (with a Bcl-2/Bax ratio greater than 1).Using the TUNEL method to observe cellular apoptosis morphologically,we discovered that BMSCs overexpressing Itln-1 can significantly reduce cell apoptosis.The results indicate that BMSCs overexpressing Itln-1 may inhibit cell apoptosis by increasing Bcl-2 expression and decreasing Bax expression.

AKT and its downstream target, eNOS, are the crucial regulators of vascular growth and function (Shiojima and Walsh, 2002). This study found that the phosphorylation levels of AKT and eNOS in rats with CI treated with BMSCs overexpressing *Itln-1* were significantly higher than those in the Itln and BMSCs groups.Furthermore,we confirmed that BMSCs could significantly promote CI angiogenesis and AKT and eNOS phosphorylation.Therefore, we conclude that BMSCs overexpressing Itln-1 promoting angiogenesis may be related to the activation of the PI3K/AKT signal pathway.

## 5. Conclusion

BMSCs overexpressing *Itln-1* could effectively promote vascular regeneration, inhibit inflammation and cell apoptosis,and improve nerve function defects and cerebral blood flow post-CI.This process may be associated with angiogenesis and anti-apoptosis,as well as the activation of the PI3K/AKT signal pathway.Also,we found that the effect of BMSCs overexpressing *Itln-1* was superior to BMSCs alone or lentivirus therapy in promoting angiogenesis post-CI.

## Abbreviations

CI, Cerebral infarction
I/R, Ischemia/reperfusion
MCAO, Middle cerebral artery occlusion
BMSCs, Bone marrow mesenchymal stem cells
Itln-1, Intelectin-1

## CRediT authorship contribution statement

**Gu Naibing:** Writing – review & editing, Funding acquisition. **Chang Le:** Investigation. **Di Zhengli:** Writing – review & editing, Project administration. **Zhao Hongwei:** Investigation. **Zha Lei:** Data curation. **Guo Kun:** Writing – original draft, Conceptualization. **Zhu Bo:** Writing – original draft, Conceptualization.

## Ethical statement

This study was conducted in accordance with bylaws of the Research Ethics Committee of Xi 'an Jiaotong University of Medicine ( No: XJTUAE20 ) .

## Funding

This study was supported by the Shaanxi Natural Science Foundation (No: 2021JM-562) and Xi 'an Science and Technology plan project (No: 2023YXYJ0036).

## Declaration of Competing Interest

The authors declare no conflict of interest.

## Acknowledgements

Bo Zhu and Kun Guo contributed equally to this work.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2025.03.012.

## Data availability

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

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