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ORIGINAL ARTICLE

Mesenchymal Stem Cell Therapy and Simvastatin Treatment in Experimental Ischemic Stroke: An Evaluation for Anatomical Repair and Community-Based Stroke Rehabilitation

ATA UR REHMAN[1], KIRAN KHAN[2], SYED ZARYAB AHMED[3], FARAZ SALEEM[4], SUHAIL IQBAL[5], NAUSHEEN HAMEED SIDDIQUI[6]
[1]*Department of Pharmacology, Shaheed Mohtarmma Benazir Bhutto Medical College Lyari, Karachi*
[2]*Department of Community Medicine, Shaheed Mohtarmma Benazir Bhutto Medical College Lyari, Karachi*
[3]*Department of Biochemistry, Ziauddin Medical College, Ziauddin University, Karachi.*
[4]*Department of Pharmacology and Therapeutics Baqai Medical College, Baqai Medical University, Karachi.*
[5]*Department of Anatomy Chandka Medical College, SMBBMU, Larkana.*
[6]*Department of Pharmacy, Benazir Bhutto Shaheed University Lyari, Karachi*
Correspondence to: Ata ur Rehman, Email: hkdr_ata@yahoo.com

ABSTRACT

Background: Ischemic stroke remains a leading cause of disability, particularly in low- and middle-income countries, where community-based rehabilitation (CBSR) faces resource constraints. Mesenchymal stem cell (MSC) therapy promotes neuroprotection and repair, while simvastatin offers pleiotropic benefits including enhanced cell homing and anti-inflammation. This preclinical study evaluated their combination in a rat MCAO model to assess implications for CBSR.

Methods: Sixty adult male Sprague-Dawley rats underwent 90-min transient MCAO and were randomized into five groups (n=12): sham, MCAO vehicle, MCAO + simvastatin (10 mg/kg IP daily ×7 days), MCAO + MSCs (1×10^6 IV at 24h), and combination. Bone marrow MSCs were isolated, characterized (CD90+/CD29+), and administered via tail vein. Outcomes at days 1-28 included Bederson score, adhesive removal/rotarod tests, infarct volume (TTC/Nissl), immunohistochemistry (NeuN/GFAP/CD31/BrdU), western blot (BDNF/VEGF), and qRT-PCR (SDF-1). Data analyzed by ANOVA/Tukey ($p<0.05$).

Results: Combination therapy reduced infarct volume by 62% (12.4±2.8% vs. 32.6±4.1% MCAO, $p<0.001$), preserved NeuN+ neurons 2.8-fold (145±18/mm²), curbed gliosis 48%, and boosted CD31+ vessels 2.1-fold versus MCAO. Behavioral recovery excelled: Bederson 1.2±0.4 (vs. 3.8±0.7), rotarod 152±22s (vs. 45±12s), adhesive 12.3±3.1s (vs. 35.4±5.6s; all $p<0.001$), surpassing monotherapies by 30-40%. BDNF/VEGF rose 3.2/2.7-fold, SDF-1 mRNA 4.1-fold.

Conclusions: Simvastatin-MSC synergy enhances neuroprotection, angiogenesis, and functional recovery beyond monotherapies, with accessible pharmacology ideal for LMIC CBSR integration. These findings support Phase I translation for scalable stroke rehab.

Keywords: Mesenchymal Stem Cells, Simvastatin, Ischemic Stroke, Community Based Stroke, Rehabilitation

INTRODUCTION

The Mesenchymal stem cell (MSC) therapy has appeared as a promising regenerative approach for ischemic stroke rehabilitation, offering neuroprotection, immunomodulation, and tissue repair through paracrine effects and differentiation potential[1]. In experimental models of ischemic stroke, MSCs reduce infarct volume, stimulate angiogenesis, and enhance functional recovery by secreting trophic substances such as BDNF and VEGF, addressing the limited endogenous repair after stroke. MSCs improve neurological scores in people who have suffered chronic strokes, despite limitations including shorter cell survival in the ischemic environment. A meta analyses have confirmed improved NIHSS and Barthel Index results for at least 24 months after MSCs transplantation[2]. Additionally, it has been documented that simvastatin, except than its well known anti-lipid activities improves MSC treatment via showing pleiotropic neuroprotective effects, endothelium protection, increased neurogenesis, and anti-inflammatory activities through NF-κB suppression[3]. Various preclinical studies have shown that by upregulating SDF-1/CXCR4 signaling and decreasing apoptosis, simvastatin enhances MSCs homing, improve survival, and success in stroke models, highlighting better behavioral recovery than monotherapy. Multiple clinical trials have highlighted that high dose of simvastatin improved outcomes three months after stroke without posing the subjects at hemorrhagic risk, that showed a synergy with MSCs treatments for rehabilitation[4]. The benefits of posed combination have been attributed to ability of simvastatin to prime MSCs, reduce oxidative stress and increase secretome-mediated healing, which is vital translating laboratory results into community rehabilitation where long term recovery is required. Recent documentations have highlighted the safety of simvastatin, and have mentioned that with minor side effects such as fever this combination supplements to standard treatment for managing the consequences of stroke for longer duration[1,3].

It has been documented that ischemic stroke affects 13 million individuals annually and causes 87% of the disability burden in low and middle income countries including Pakistan[5]. Furthermore, the stroke management requires immediate intervention and continuous rehabilitation. It has been documented that effective management decreases death rate but the patients with stroke still suffer the functional, cognitive, and motor disabilities which requires comprehensive rehabilitation to exploit their unconventionality and quality of life[6]. Community based stroke rehabilitation (CBSR) programs work by managing the stroke patients at their homes and communities furthermore, it offers multidisciplinary teams, which promotes early assisted discharge from hospitals, and self-management plans to promote healing and improve the quality of life. The CBSR program ensures the education of caregivers, promote telehealth integration, and associates fitness centers as part of the implementation process at the community level, which promises 30–40 minutes of cardiorespiratory training for 3-5 times a week[6,7].

The CBSR programs in LMICs are economical, supported by caregivers, and predominantly home based. The rehabilitation programs overcome the resource shortages and improve treatment outcomes. Recent data indicates that home based programs are better than hospital based programs for community driven short term rehabilitation[8]. However, the lack of evidence in low resource areas creates difficulty to promote specialized employment but that can be overcome by setting specific regulations and measurable telemedicine approaches. Furthermore, successful CBSR program requires strategic planning, standardizing data, and prioritizing practical therapies to bridge the rehabilitation gap[9]. It has been identified that simvastatin along with MSCs treatment in experimental ischemic stroke have important sequela for CBSR, which might translate easily available pharmacological cell hybrid therapies in near future. In comparison to monotherapies, these experimental models show synergism, improved MSC engraftment, decreased neuronal loss, and increased neurogenesis that is translated into greater motor to cognitive recovery, meeting the required demands in chronic stroke patients[10]. The importance of the study highlights the ability of simvastatin to increase MSC potency without requiring additional pre requisites which is ideal for LMICs. Additionally, this combined

approach empowers community teams to deliver regenerative adjuncts, nurturing self-management and reducing rehospitalizations. The current study aims to document fair stroke healing, combining cutting edge biology with practical community delivery for long lasting social effect.

METHODOLOGY

It was a pre-clinical experimental study, male, 8-10 weeks old, 250-300g Sprague-Dawley rats were used to identify the combined effects of MSCs therapy and simvastatin treatment in a middle cerebral artery occlusion (MCAO) model of ischemic stroke, with implications for community based rehabilitation protocols. The Sprague-Dawley rats (total n=60, calculated via power analysis with G*Power software assuming 80% power, alpha=0.05, effect size=0.8 from prior studies, yielding n=10-12 per group for behavioral/histological outcomes) were randomly divided into five groups (n=12/group), Group 1, sham-operated control (surgery without occlusion), Group 2, MCAO vehicle control (PBS post-occlusion), Group 3, MCAO + simvastatin 10 mg/kg intraperitoneal daily for 7 days, Group 4, MCAO + MSCs (1×10^6 bone marrow derived MSCs intravenously via tail vein at 24h post-occlusion, and Group 5, MCAO + simvastatin + MSCs (combination); all procedures were carried out as per institutional animal ethics guidelines. MSCs were isolated from donor rat bone marrow by flushing femurs/humeral bones with DMEM, density gradient centrifugation (Ficoll), plating at 1×10^5 cells/cm² in MSC medium (DMEM low glucose +10% FBS +1% penicillin/streptomycin), passaged at 80% confluency up to passage 3, characterized (>95% CD90+/CD29+, <2% CD45- via flow cytometry), and viability confirmed (>90% by trypan blue); simvastatin (Sigma-Aldrich) was dissolved in 0.5% DMSO for dosing. Focal ischemia was induced via transient MCAO (90-min occlusion, 24h reperfusion): rats were anesthetized (3% isoflurane induction, 1.5% maintenance), body temperature maintained at 37°C, right common/internal carotid arteries exposed, a 4-0 nylon filament (tip coated with silicone) advanced 20mm into MCA via external carotid stump under stereotaxic guidance, filament withdrawn after 90min for reperfusion, and success verified by 2,3,5-triphenyltetrazolium chloride (TTC) staining in pilot n=5 (infarct >30% hemisphere excluded). Post-treatment assessments was performed at days 1, 3, 7, 14, and 28: neurological function via modified Bederson scale (0-5 score) and adhesive removal test (time to remove bilateral stickers, 3 trials/day), motor coordination by rotarod (accelerating 4-40 rpm, latency to fall), and weekly body weight monitoring was performed. At endpoint (28th Day), rats were transcardially perfused (4% paraformaldehyde), brains extracted, sectioned coronally (30µm cryostat), and analyzed: infarct volume by TTC/Nissl staining (ImageJ, % hemisphere); immunohistochemistry for NeuN (neurons), GFAP (astrogliosis), Iba1 (microglia), CD31 (angiogenesis), BrdU (proliferation, injected 50mg/kg IP days 1-7), Ki67 (quantified as positive cells/mm² in peri-infarct/SVZ, 5 sections/rat); qRT-PCR for neuroprotective genes (GAPDH housekeeping). Data was analyzed using one-way ANOVA followed by tukey post-hoc analysis on SPSS version 27.

RESULTS

Neurological Function: The modified Bederson score improved markedly in the combination group by day 28 (mean 1.2 ± 0.4 vs. MCAO 3.8 ± 0.7, p<0.001), with adhesive removal test latency reduced by 65% (12.3 ± 3.1s vs. 35.4 ± 5.6s, p<0.001), reflecting better sensorimotor integration; simvastatin alone yielded moderate gains (2.4 ± 0.5, 22.1 ± 4.2s), while MSCs alone showed sustained but lesser effects (2.1 ± 0.6, 18.7 ± 3.9s).

Infarct Volume and Histology: Infarct volume shrank by 62% in the combination group (12.4 ± 2.8% hemisphere vs. MCAO 32.6 ± 4.1%, p<0.001), with NeuN+ neurons in peri-infarct zone up 2.8-fold (145 ± 18 cells/mm² vs. 52 ± 11, p<0.001) and GFAP+ astrogliosis down 48%; CD31+ vessels increased 2.1-fold, indicating robust angiogenesis.

Molecular Markers: Western blot revealed 3.2-fold BDNF elevation (p<0.001) and 2.7-fold VEGF in combo (vs. 1.8- and 2.0-fold singles), with qPCR showing SDF-1 upregulation (fold-change 4.1 ± 0.6), supporting paracrine-driven recovery without systemic toxicity (no weight loss differences).

Table 1: Behavioral Outcomes at Day 28 (Mean ± SD, n=10-12/group)

Group	Bederson Score (0-5)	Adhesive Removal (s, right)	Rotarod Latency (s)
Sham	0.1 ± 0.3	5.2 ± 1.4	180 ± 15
MCAO Vehicle	3.8 ± 0.7	35.4 ± 5.6	45 ± 12
MCAO + Simvastatin	2.4 ± 0.5*	22.1 ± 4.2*	98 ± 18*
MCAO + MSCs	2.1 ± 0.6*	18.7 ± 3.9*	112 ± 20*

Table 2: Histopathological Outcomes (Mean ± SD)

Group	Infarct Volume (% hemisphere)	NeuN+ Cells (peri-infarct, /mm²)	GFAP+ Area (%)	CD31+ Vessels (/mm²)
Sham	0 ± 0	210 ± 25	5.1 ± 1.2	45 ± 8
MCAO Vehicle	32.6 ± 4.1	52 ± 11	28.4 ± 4.3	22 ± 5
MCAO + Simvastatin	24.2 ± 3.7*	92 ± 14*	19.7 ± 3.2*	32 ± 6*
MCAO + MSCs	21.8 ± 3.4*	118 ± 16*	16.2 ± 2.8*	38 ± 7*
MCAO + Simva + MSCs	12.4 ± 2.8*#	145 ± 18*#	14.8 ± 2.4*#	46 ± 9*#

Table 3: Neurotrophic and Angiogenic Markers (Fold-Change vs. MCAO, Mean ± SD)

Group	BDNF	VEGF	SDF-1 mRNA	BrdU+ Cells (/mm²)
MCAO Vehicle	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	15 ± 4
MCAO + Simvastatin	1.8 ± 0.3*	1.5 ± 0.4*	2.1 ± 0.5*	28 ± 6*
MCAO + MSCs	2.4 ± 0.4*	2.0 ± 0.3*	2.8 ± 0.6*	42 ± 8*
MCAO + Simva + MSCs	3.2 ± 0.5*#	2.7 ± 0.4*#	4.1 ± 0.6*#	68 ± 10*#

DISCUSSION

Synergistic Neuroprotection and Functional Recovery: The results confirm that the therapy involving both mesenchymal stem cells (MSC) and simvastatin produces better results in experimental ischemic stroke, showing a 62% decrease in infarct size, a 2.8-fold increase in neuron preservation around the infarct, and a 65% more rapid recovery of sensorimotor function compared to MCAO controls exceeding the effectiveness of monotherapies by 30-40% across various measures[11]. This synergy corresponds with earlier MCAO rodent research where simvastatin preconditioning enhanced MSC survival through SDF-1/CXCR4 upregulation (4.1-fold in this study), reducing apoptosis in the peri-infarct area—a hypoxic environment susceptible to oxidative stress In contrast to MSCs by themselves, which perform well in paracrine VEGF/BDNF release (2.0-2.4-fold), the combination increased these to 2.7-3.2-fold, reducing GFAP+ gliosis by 48% and doubling the number of CD31+ vessels, aligning with findings of statin-boosted angiogenesis in transient ischemia models[12]. Simvastatin's pharmacological effects include not only lipid reduction through HMG-CoA reductase inhibition but also the suppression of NF-κB and modulation of ER stress, creating an anti-inflammatory environment that enhances MSC immunomodulation crucial since inflammation contributes to 70% of secondary injury after a stroke[13]. Anatomically, this is seen as maintained cortical-striatal connections, reflected in rotarod improvements (152s vs. 45s MCAO), unlike the vehicle group's penumbral degeneration; BrdU+/Ki67+ proliferation (68/mm²) in

SVZ/peri-infarct highlights neurogenesis in the subventricular zone, a preserved repair pathway enhanced by combined trophic factors. These findings exceed standalone statin studies (e.g., 25% infarct reduction) or MSC meta-analyses (20-30% recovery)[14].

Stem Cell and Anatomical Repair Mechanisms: From a stem cell perspective, our findings highlight the bystander dominance of MSCs in differentiation, with secretome-mediated repair (Table 3) reinstating the integrity of the neurovascular unit surges in VEGF/CD31 revitalizing BBB permeability compromised in MCAO, diminishing edema similar to clinical Phase I/II trials[15]. Simvastatin specifically boosts this by increasing CXCR4 expression on MSCs, which enhances tail-vein targeting to the ischemic penumbra (shown by a 2.1-fold increase in vessel density), a constraint that restricts monotherapy effectiveness to 40-50% engraftment. Anatomically, the preservation of peri-infarct NeuN (145/mm²) and the reduction of astrogliosis maintain the layered architecture of the cortex and basal ganglia circuits, essential for motor execution; this reflects human DWI-MRI patterns where penumbral survival is linked to mRS outcomes, indicating its translational significance[16].

In contrast to neural progenitors, the safety of MSCs (no tumor formation) and their scalability stand out, particularly when preconditioned—our 10mg/kg simvastatin dosage aligns with neuroprotective treatments while avoiding myopathy risks. Limitations involve the possible immunogenicity of allogeneic MSCs (reduced by low MHC-I), necessitating autologous validation; however, the 28-day endpoint encompasses chronic remodeling not seen in acute models, connecting to CBSR timelines where enduring deficits persist[17].

Pharmacological and Community Medicine Implications: Pharmacologically, the repurposing of simvastatin (US$0.05/dose) makes regenerative therapy accessible, stimulating AMPK/autophagy balance to combat ER stress-induced autosis—an innovative approach where the combination normalizes Beclin-1/LC3 flux, unlike pro-autophagic MSCs by themselves. This places it as a complement to tPA/endovascular therapy, lowering the risk of recurrent stroke (high-dose statins reduce 20% according to meta-analysis) while enhancing rehabilitation plasticity through BDNF[15,18].

In community medicine, particularly in LMICs such as Pakistan (stroke rate 350/100k/year), these results support the incorporation of affordable simva-MSC protocols into CBSR: home-administered oral simva after discharge combines with task-oriented therapy, utilizing MSCs' outpatient IV practicality (1e6 cells, approximately US$200 autologous). Our 35% higher Bederson/rotarod scores validate scalable models telehealth monitored infusions at PHC facilities, caregiver-led activities tackling the 80% rehabilitation gap as hospital availability decreases[19]. Contrary to resource-intensive neural transplants, this hybrid enables non-experts; initial CBSR trials indicate 25% ADL improvements with statins alone, further enhanced here by stem cells for fairness. Upcoming Phase II trials ought to evaluate comorbid elderly individuals; however, preclinical power (n=60, 80% power) strongly advocates for advancement, highlighting the importance of multidisciplinary pharmacology-stem cell integration for reducing global burden[17,19].

Translational Outlook and Limitations: This research innovatively explores combo therapy's multi-faceted repair (pharmacology: pleiotropy; anatomy: neurovascular; stem cells: paracrine targeting; community: accessibility), surpassing standards and addressing deficiencies in chronic MCAO data. Strengths consist of blinded multi-modal endpoints and reasonable n=12/group. Constraints: the rat model's mismatch in gyrencephaly with humans; lack of long-term (90-day) cognitive assessment (e.g., Morris water maze); no MRI for monitoring dynamic infarcts. Nevertheless, consistency with recent meta-analyses (MSC safety/efficacy OR=2.5) and statin recommendations reinforces Phase I preparedness. In LMIC CBSR, it signals "regen-rehab" models, possibly reducing disability-adjusted life years by 50% through policy adoption critical as stroke incidence rises 30% by 2030[20-21].

CONCLUSIONS

The combination of simvastatin and MSC boosts neuroprotection, angiogenesis, and functional recovery more effectively than either treatment alone, and its straightforward pharmacology is suitable for integrating CBSR in LMICs. These results endorse Phase I translation for scalable stroke rehabilitation

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