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Mesenchymal Stem Cell Secretome for Ischemic Stroke: CD31 and VEGF Expression

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Abstract

Standard treatments for ischemic stroke are intravenous thrombolysis and endovascular recanalization. In the acute phase (<4.5 hours) only 3.2% to 5.2% of ischemic stroke patients are eligible for intravenous thrombolysis. Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into different types of cells that produce potential regenerative therapeutics in stroke patients. MSCs will secrete secretomes that contain growth factors, chemokines, cytokines, metabolites and bioactive lipids. Secretome promote production of CD31 and Vascular Endothelial Growth Factor (VEGF). Neurogenesis and angiogenesis effect from CD31 and VEGF cause brain cell regeneration and neurological improvement. This study analyzes the effect of 150 µl SH-MSCs injection toward CD31 and VEGF expression in rats with ischemic stroke. The method used was laboratory true experimental post-test with only control group design and sample was taken with consecutive sampling. It uses 18 Rattus norvegicus and divided into sham, control and P1 (MCAO + 150 ul secretome) groups. Control and P1 group were made in stroke condition with Middle Cerebral Artery Occlusion methods by clamping Common Carotid Artery. Modified Neurological Severity Score (mNSS) used to measure neurological function improvement. Mean value of VEGF and CD31 expression in P1 higher than control group and mNSS in P1 lower than control group. Through the increases of VEGF and CD31 expressions, SH-MSCs can drive cell proliferation, neuron cell survival, angiogenesis, neurogenesis and blood-brain-barrier integrity recovery in the rats' brains, so it improves clinical outcomes and neurological function in rats with ischemic stroke.

Keywords: CD31, VEGF, Secretome, MSC, Ischemic Stroke.

Introduction

A stroke is an urgent medical illness marked by an acute vascular or cerebral perfusion deficit. About 85% of strokes are caused by ischemia. At least 5 million individuals worldwide die from strokes, and millions more suffer permanent disabilities[1]. The standard treatments for ischemic stroke in the acute phase (less than 4.5 hours) are endovascular recanalization and intravenous thrombolysis. However, the recanalization procedure is challenging, and only between 3.2% and 5.2% of ischemic stroke patients are eligible for intravenous thrombolysis[2]. Stem cell-based therapy has emerged as a new approach for ischemic stroke treatment strategy[3].

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Stem cells are found in all multicellular organisms. They can regenerate and differentiate into different types of specialized cells[4,5]. Mesenchymal stem cells (MSCs) are an adult stem cell type that can be culture-developed and they can regenerate themselves. They were taking from multiple sources, including bone marrow, adipose tissue, the central cord, tooth pulp, olfactorius mucosa, and other tissues that have the same characteristics as mesenchymal tissue [6,7]. Secretome, a collection of bioactive substances, includes proteins, nucleic acids, proteasomes, exosomes, microRNAs, and vesicle membranes. These are all produced by MSC, which is one of the extracellular components and cytokines that can be metabolically secreted by mesenchymal stem cells[8]. Secretome contains growth factors, chemokines, cytokines, metabolites, bioactive lipids, and other factors (soluble proteins, free nucleic acids, lipids and extra-cellular vesicles) that are secreted into the extracellular space. They mechanize the surrounding cells interaction by microenvironment and regulate the cells with autocrine or paracrine signalling[9]. Secretome is more superior than single MSC therapy because it does not have tumor-genetic characteristics and immune compatibility, and has a lower risk of infection and embolism compared to living and proliferating cell transplantation. MSC secretome has potential to develop into brain cells with a low risk of immunodeficiency, making it a suitable therapeutic agent for ischemic strokes. Secretomes under the hypoxia condition (5% oxygen) induce the increasing of cell migration, proliferation, viability and in-vitro angiogenesis. Growth factors such as thrombocyte-derived growth factors, hepatocyte growth factor, placenta growth factor and vascular endothelial derivative growth factors are regulated under hypoxia condition[10]. Secretom can increase vascular endothelial growth factor (VEGF) and CD31 expression. Vascular Endothelial Growth Factor (currently known as VEGF-A) is a member of protein groups that include VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF) which plays an important role in angiogenesis as well as the growth of nervous system functions[11]. VEGF will strongly induce the cell regeneration systems, mediating angiogenesis, neurogenesis and synaptic functions[12]. *Platelet Endothelial Cell Adhesion Molecule 1* (PECAM-1) or CD31 is an adhesive cell. It has 130-kDa molecular weight that is highly expressed on the surface of endothelial cells and on hematopoiesis cells such as platelets, monocytes, neutrophils and T-cells[13]. CD31, which is expressed in the endothelial blood vessels, is often used as an index for the angiogenesis process. The significantly increasing of CD31 in the cortex triggers the enhancement of the formation of new blood vessels in the injury area. The purpose of this study is to analyze the effect of 150 µl SH-MSCs injection toward CD31 and VEGF expression in Rattus norvegicus or rats (Wistar) with ischemic stroke. modified Neurological Severity Score (mNSS) used to measure neurological function improvement in rats (Wistar).

Materials and Methods

Study design, setting and sampling

This study uses a post-test only control group design and a true experimental laboratory, while the object research is rats Wistar (Rattus norvegicus) in non-probability principal of consecutive sampling. Conducted at the Stem Cell and Cancer Research Laboratory (SCCR) and the Animal House Integrated Biomedical Laboratory facility, Medical Faculty of Sultan Agung Islamic University, Semarang. To complete this study starting from preparation to the report takes 12 months and the total sample of this study was 18 adult male rats Wistar (Rattus norvegicus) weighing 200-250g, aged 12-16 weeks, healthy and active.

MSC isolation, secretome preparation and content analysis

Twenty-one-day pregnant rats were anesthetized using lethal dosage anesthesia with 10 mL of cocktails using Ketamine 50 mg/kgBB, Xylazine 10 mg/ kgBB and Acepromazine 2 mg/ KgBB injected intramuscularly[14]. Umbilical cords were collected and stored in a petri cup containing 0.9% NaCl using a tweezer. Firstly, put the umbilical cord into the petri dish and wash it until clean with PBS (Gibco BRL, Grand Island, NY, USA). Secondly, cut the umbilical cords into a smaller piece (1mm), and put it on a T75 flask (Corning, Life Sciences, USA). Complete mediums consisting of DMEM (Gibco BRL, Grand Island, NY, USA), fungizon, penstrep (antibiotic), and 10% Fetal Bovine Serum (FBS) (Givco BLL, Grand Island, New York, USA) were added to cover tissues of about 3 mL, then, incubated

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in incubators at 37^0C and 5% CO2. Cell maintenance is carried out until the cell reaches 80% confluency[15].

Successfully cultivated cells from umbilical cords are validated and characterized according to secretome MSC. Secretome MSC was validated using flow cytometry techniques with CD44, CD73, CD 90 and CD 105 markers. In order to validate the flow cytometry method, 1x107 cells/ml are released from the flask using BDTM Accutase TM Cell Detachment Solution. Next, Phosphate Saline Buffer (PBS) cells are washed and placed in a 5 mL Falcon tube. Finally, flow cytometrics is read in using 1–5 tubes as a control to set up the cytometer as a compensation.

The T75 flask contains fourth passage secretome MSC with a 95% confluence placed on the hypoxia chamber. The hypoxia chamber is supplied with CO2 gas and the O2 content is measured using a DO meter until it reaches 5%. The secretome MSC in the hypoxia chamber is incubated in the incubator at a temperature of 37ºC for 24 hours. The secretome hypoxia MSC is taken and inserted into a 50 mL conical tube for filtering. After that secretome hypoxia, MSC was then stored at -80 ºC before analyzing the contents.

Animal model and sample

The sample consists of 18 adult male rats (Wistar) weighing 200-250g, aged 12-16 weeks, healthy and active, without dropping out during this study. Male rats (Wistar) were acclimatized for seven days. Rats (Wistar) were adapted for seven days with standard AIN-76A feed and water provided ad libitum. The food was calculated at around 15-20% of their body weight, which amounts to 100-150 grams per cage per day. The surgery was performed on rats by clamping the CCA (Carotid Communis Artery) to induce the MCAO (Middle Artery Occlusion). After the MCAO condition, randomization is performed by dividing the rats into 3 groups: the sham group (healthy rats), the positive control group (MCAO), and the P1 group (MCAOs + 150 µl SH-MSC). Each rat will be monitored, and at day eight, rats will be given SH-MSC for the P1 group. On the twelfth day, rats' neurological behavior will be measured with mNSS. On the fifteenth day, rats were killed and their brain tissue was examined using H&E staining to evaluate VEGF and CD31 expression and continue with the data processing and data analysis.

RNA isolation and RT-PCR analysis

To determine the mRNA expression of VEGF from the brain, we use real-time reverse-transcription polymerase chain reaction (RT-PCR). The total RNA is extracted from the cells treated with CL, PN, and their combinations use TRIzol (Invitrogen, Thermo Fisher Scientific, Inc.) according to the factory protocol. 25µl reaction volume consists of 12.5µl PCR buffer 2x for KOD FX (PCR amplification enzyme), 5µl 2 mM dNTPs, 2µl primary, 0.1µl KOD, 2.4µl water and 1µl DNA. The standard conditions for PCR are as follows: 95°C for 2 minutes, followed by 40 cycles at 95 °C for 30 seconds, 62°C in 1 minute, and the final extension at 72°C during 5 minutes. The $2-\Delta\Delta Ct$ method is used to demonstrate the relationship between the target gene expression in the experimental group and the target gene expression of the control group. The primer sequences of VEGF that were used in this study are as follows in Table 1[16].

Table 1. Primer sequences for Vascular Endothelial Growth Factor (VEGF).

Gene	Primer sequence
VEGF	F 5'-GTACCTCCACCATGCCAAGT-3'
	R 5'-AATAGCTGCGCTGGTAGACG-3'

Clinical measurement

To evaluate neurological behavior in rats with MCAO, this study uses *modified Neurologic Severity Scores* (mNSS). The mNSS scores are a frequent and easy-to-apply evaluation scale of neurological tests in rats after stroke. This examination combines neurological evaluation with many aspects, including motor function, sensory function and reflex function with a total score of eighteen. Scores 1-6 indicate mild injury, scores 7-12 indicate moderate injury and scores 13-18 indicate severe injury[17].

The method of measuring mNSS is as follows:
Motoric test: Lift the rat by the tail, 15-30 cm above a flat surface. After that, observe the flexion or extension of the front limb (score 0 = extension; score 1 = flexion), observe the hind limb for flexion or extension (score 0 = extension; score 1 = flexion), observe if the head moves >10° on the vertical axis for 30 seconds (score 0 = no flexion; score 1 = flexion) and place the rat on a flat surface in a circular shape with a diameter of 50 cm. Then, observe the rat's path (score 0 = normal path; score 1 = unable to walk straight; score 2 = circling toward paretic side; score 3 = the rat falls to the paretic side). Sensoric for visual placing test: Lift the rat by the tail and slowly lower it to the edge of the table until its nose is 10 cm away from the edge, gently move the rat towards the

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edge of the table (without letting its whiskers touch the edge) and observe the rat to see if it can reach and extend its front legs towards the table (score 0 = can reach; score 1 = cannot reach); Sensoric for tactile placing test: Hold the rat's body parallel to the edge of the table, with the front legs free, slowly lowering the rat towards the edge of the table until the whiskers on one side touch the edge of the table, then, observe whether the rat immediately extends the front leg on the same side as the whiskers to the edge of the table (score 0 = extends the front leg, score 1 = does not extend the leg); Sensoric for hind leg grasp reflex: Hold the rat with one hand, using your thumb and index finger to encircle its chest below the front legs; touch the heel of your foot with the other index finger, alternating between right and left; and observe: does the rat hold their index finger? (score 0 = gripping; score 1 = not gripping). Balance test: Place the rat on the beam, allow the rat to walk on the beam and observe (score 0 = balances with steady posture; score 1 = grasps side of beam; score 2 = hugs beam and 1 limb falls down from beam; score 3 = hugs beam and 2 limbs fall down from beam, or spins on beam (60 seconds); score 4 = attempts to balance on the beam but falls (>40 seconds); score 5 = attempts to balance on the beam but falls (>20 seconds); score 6 = falls off, no attempt to balance or hang on the beam (<20 seconds). Reflexes and abnormal movements, pinna reflex: Scratch the inner ear with a cotton bud and observe if there is any ear retraction (score 0 = retraction present; score 1 = no retraction). Corneal reflex: Scratch the cornea of the mouse with a cotton bud and observe if there is any blinking (score 0 = blinking present; score 1 = no blinking). Startle Reflex: Scratch the iron rod on the cage cover and observe if the rat turns towards the sound (score 0 = turns towards the sound; score 1 = does not turn towards the sound). Abnormal movement: Observe if the rat exhibits seizures, myoclonus or myodystonia (score 0 = none; score 1 = present).

Statistical analysis

The CD31 and VEGF data from this study that have been obtained will be processed, edited, tabulated to descriptive and then its normality was tested using the Shapiro-Wilk test. If the data is normally distributed, then proceed with one-way ANOVA. Statistical significance was defined as a *p*-value < 0.05. The mNSS data use descriptive mean value. All statistical analysis was performed using SPSS Statistics version 22 (SPSS Inc., Chicago,USA).

Results

Mesenchymal Stem Cell Characterization and Secretome

The MSC specimen results after the 5th passage show that cells are attached to the base of the flask with spindle-like cell morphology under microscopic examination in Fig. 1.

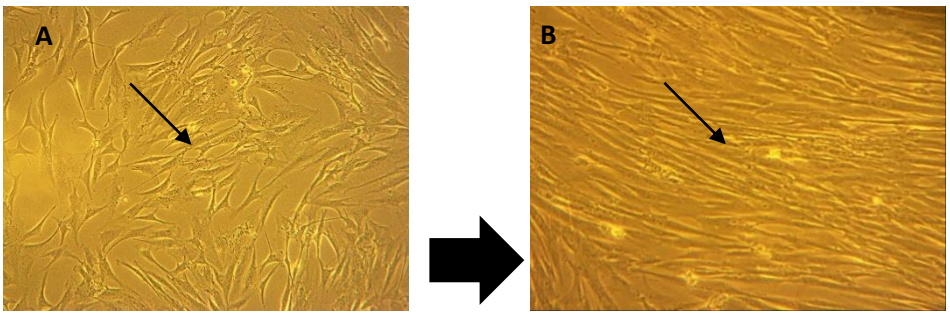

Figure 1. MSC isolation with 80% confluence. 100x magnification reveals a spindle-like shape shown by the arrow (A). Spindle-like morphology with 200x magnification (B).

MSC flow cytometry analysis showed that the cells expressed specific markers of SPM: positive expression of CD90.1 (97.6%), CD29 (97.7%), as well as negative expressions of CD45 (1.5%) and CD31 (3.2%) in Fig. 2. It's in line with the 2006 International Society Cellular Therapy standards.

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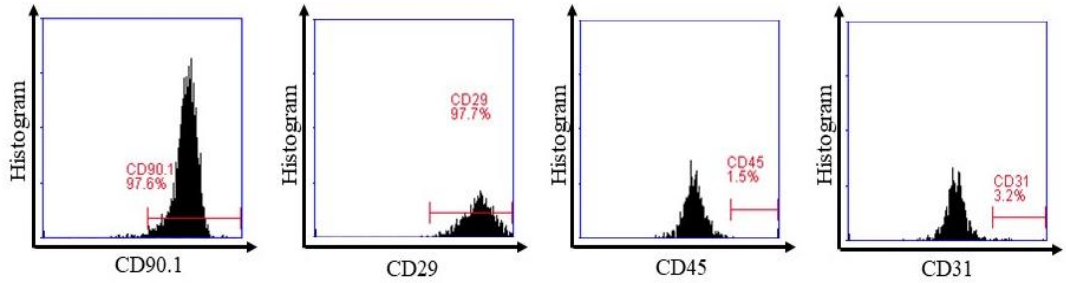

Figure 2. MSC expressed CD90.1, CD29 and negative expression of CD45 and CD31 according to flow cytometry analysis.

Secretome Content Analysis

After 24 hours, the culture media is taken and filtered using tangential flow filtration (TFF) to obtain SH-MSC. Here are the results of the Secretome Hypoxia Mesenchymal Stem Cell (SH-MSC) biomolecular content profiles in Table 2.

Table 2. The value of soluble molecules hypoxic MSC secretome.

Molecules	SH-MSCs ± SE (pg/mL)
VEGF	1228,86 ± 27,71
PDGF	1043,06 ± 24,49
FGF	1085,34 ± 28,92
IL-10	415,02 ± 12,14
TGF-β	282,83 ± 9,28
IL-6	323,99 ± 10,04

CD31 Expression in Rats Stroke Ischemic Model

The expression of CD31 was obtained and tested on the 15th day using the RT-PCR method with $p < 0,05$ as follows in Table 3.

Table 3. CD31 expression.

Group	n	CD31 (x ± SD) (%)	P value
Sham	6	11,61 ± 4,69	0,012
Control	6	5,58 ± 1,51	
P1	6	7,05 ± 2,82	

Control (MCAO) and P1 (MCAO + SH-MSC 150µl)

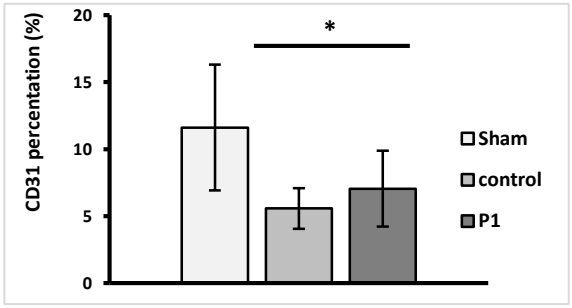

Figure 3. Graphic mean value of CD31 expression.

Based on Table 3, CD31 expressions in P1 (7,05 ± 2,82) % were higher than in the control group. Control group has the lowest levels (5,58 ± 1,51) % and sham group has the highest levels (11,61 ± 4,69) %. This study shows the increasing expression of CD31 in the p1 groups compared with the increasing in the control group.

VEGF Expression in Rats Stroke Ischemic Model

The expression of VEGF was obtained and tested on the day 15th using the RT-PCR method with $p < 0,05$ as follows in Table 4.

Table 4. VEGF expression.

Group	n	VEGF (x ± SD) (ng/ml)	P value
Sham	6	1,00 ± 0,14	
Control	6	0,46 ± 0,21	0,001
P1	6	3,37 ± 1,94	

Control (MCAO) and P1 (MCAO + SH-MSC 150µl)

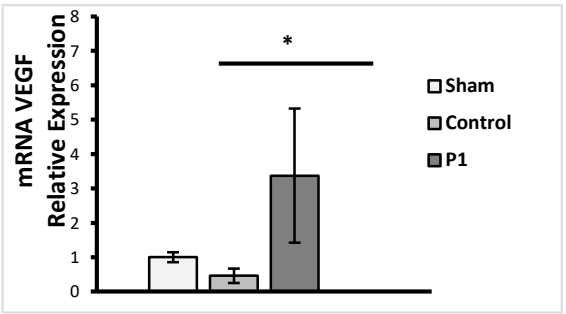

Figure 4. Graphic mean value of VEGF expression.

Based on Table 4, VEGF expressions in P1 (3,37 ± 1,94 ng/ml) were higher than expression in the control group. Control group has the lowest levels (0,46 ± 0,21 ng/ ml). This study shows the increasing expression of VEGF in the p1 groups.

mNSS in Rats Stroke Ischemic Model

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After SH-MSC injection in rats with MCAO, on day 12th, the neurological behavioral function tested on uses the mNSS method as follows below in Table 5.

Table 5. mNSS descriptive mean value.

	Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval	
MNSS Day 12th						Lower	Upper
	Sham	6	0,000	0,000	0,000	0,000	0,000
	Control	6	8,333	1,751	0,715	6,495	10,171
	P1	6	2,500	0,547	0,223	1,925	3,074

Control (MCAO) and P1 (MCAO + SH-MSC 150µl)
Based on Table 5, mean value of mNSS in P1 (2,50 ± 0,54) was lower than mean value of mNSS in the control group (8,33 ± 1,75). This study shows the improvement of neurological behavior / function in P1.

Discussion

The primary goal of successful stroke therapy is long-term recovery from neurological deficits. Cell-repair capabilities after ischemic stroke are often limited and incomplete. Spontaneous recovery to support functional cell recovery depends on the therapeutic approach of ischemic stroke[18]. In ischemic stroke, blood flow blockage to the intracranial artery leads to the reduction of oxygen and nutrients and causes changes in metabolism around cells that trigger abnormal mitochondrial activity, inflammation, damage to the blood-brain barrier and the death of astrocytes (glial cells) in the central nervous system[19]. The BBB leakage leads to peripheral immune infiltration into the brain and secondary inflammation. Astrocyte death also stimulates microglia to cleanse dead cell debris and triggers neuroinflammatory reactions through the secretion of cytokines pro inflammation such as Tumor Necrotizing Factor alfa (TNF-α), Interferon gamma (IFN-γ) Interleukin 6 (IL-6), interferon alfa- and interleukin 1 beta (IL-1). IFN-γ will activate NF-kB, IL-6 and IFN-γ will give the P13K signal activation path. The activation JAK 2 signal (Janus Kinase) and STAT 3 signal (Signal Transducer and Activator of Transcription) pathways cause mRNA transcription to occur in cells as a positive effect of the inflammatory process. mRNA transcription will enhance the Slit-RoBo (roundabout) signal. Using the slit-robo pathway by new cells or new neurons to migrate to the damaged areas, enhance the smad2/3 (self-renewal) signal, and increase the Focal Adhesion Kinase (FAK) and P13K signal. Slit Robo will activate the Extracellular Signal Regulated Kinase 1⁄2 (ERK1/2). The activation of the Phosphatidylinositol 3-kinase (P13K) / AKT and Nitric Oxide Synthase (NOS) pathway will increase the expression of CD31 cells in the endothelium to initiate the natural angiogenesis process[20,21]. However, uncontrolled ischemic conditions will increase glucose catabolism that causes depression and depolarization of the peri-infarct area and activates the biochemical pathway, thus expanding the infarction volume.

Secretomes, which are a group of molecules released by MSCs, are now considered to be multipotent cells that can differentiate into various cells such as adipocytes, chondrocytes, osteoblasts and neurons. The stem cells transmigration to the injury focus occurs throughout the endothelium through leukocyte-like pathways involving vascular cell adhesion molecule 1 (VCAM-1) and G-protein-coupled receptor signaling[22]. Secretome MSC also expresses TLR 3 and CXCR3-R receptors that are capable of detecting SDF-1, TNF- and IFN- molecules released in the inflammation area. In the injured brain cells, the stem cells will differentiate into astrocytes (glial cells) in the central nervous system and differentiate into neurons (self-renewal). MSC secretions in the brain injury area will control the inflammatory cells by polarizing the macrophages. Thus, the M1 pro-inflammatory macrophage changes to anti-inflammatory M2 (IL-4, IL-10). Inflammation occurring in the area of the injuries can alleviate/reduce chronic inflammations that can reduce the fibrosis risk in the neurons and trigger various cell repair mechanisms[23]. MSC secretome induces differentiation into endothelial cells characterized by increased expression of CDff and VEGF.

In this study, after the administration of hypoxia secretome, CD31 increased in the ischemic areas of the brain, in line with the result of a previous study that CD31 was significantly increased in patients with Peripheral Artery Disease (PAD)

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injected with 200 µl and 400 µl of MSC secretome compared to the control group. The group given a dose of 400 µl of the MSC secretome showed a higher increase in CD31 compared with the group given the dose of 200 µl of the MSC secretome[24]. This indicates that new vascular density from angiogenesis process spreads evenly in the group with MSC secretome. Another study also showed a significant increase of CD31 expression in the cortex (p=0,039) compared to that in control group[25]. It triggers the increase of new blood vessels formation in the injury area. CD31 that migrates to peri-infarct area not only secretes neurotropic factor and helps the migration process and proliferation of endogenous NPC in SVZ (Subventricular Zone), but it also increases the expression of VEGF from SHSY5Y chemotactic response initiated by CD31, so that resulted in the improvement in the brain cells of the ischemic model from the VEGF role in neurogenesis, vasculogenesis and neuroprotective effect. VEGF binds to the local vascular endothelial receptor and it triggers the angiogenesis, decreases the cleaved caspase-3immunopositive cell in the peri-infarct area and brings out the anti-apoptotic effect of VEGF in the SHSY5Y neuronal brain cell[26].

This study showed an increase in VEGF expression in the P1 group compared to in the control group. The MSC secretion called secretome or *small molecule* includes *Vascular Endothelial Factor* (VEGF), *insulin-like growth factor* (IGF-1), bFGF, TGF-β1, *nerve growth factor* (NGF), *placental growth factor* (PGF), *stromal-derived growth factor* (SDF-1/CXCL12), *monocyte chemo attractant protein-1* (MCP-1/CCL2), IL-6, IL-8, IL-10 and IL-13. These are all important for vascularization, apoptosis inhibition, survival improvement, and angiogenesis stimulation of endothelial cells under hypoxia conditions[27,28]. To initiate angiogenesis, Ang-1 will enhance endothelial mechanisms, matrix interactions and stabilize junctions as well as astrocytes by pulling the endothelium migration, promoting proliferation and gradating basal cell membranes (peptides and hyaluronic fibrines), and lumen formation. Ang-1 will also create a new endothelium cell membrane (ECM), preceded by matrix connections as well as integrin 1 adhesion and the formation of tight junctions[29]. The endothelial junction will have a penetration of pericytes and myofibroblasts into the lumen (intussusception and angiogenesis), resulting in vascular fusion and stabilization of smooth muscles and pericytes, as well as the initiation of blood flow[30]. Previous studies showed that MSC increases the VEGF neurotropic values and Ang1/Tie2 expression, which causes the increasing of capillary formation of endothelial cells in the rat's brain, thus triggering an angiogenesis response to accelerate tissue repair processes[31]. VEGF expression is increased by the induction of MSC and VEGF also affects the regulation of proliferation, migration and endothelial cell formation in blood vessels. The inhibition of apoptosis triggered by VEGF also accommodates the neuroblasts migration to the infarction zone. The MSC secretome through VEGF increases the BrdU+ value in the microvesel, which functions to trigger angiogenesis and neurogenesis in the peri-infarctic cortex, thereby improving neurological function (motor disability is improved) so that the mNSS score in the P1 group improves. In this study, the control group has decreased neurological functions marked by the increases of mNSS scores, while the P1 group has an improvement in neurological function marked by the decreases in mNSS values. This, because the SH-MSC secretes the *Growth Factor* biomolecules like VEGF, PDGF, bFGF, IL-10, TGF-β and NGF. They can trigger neuron survival, cell proliferation, angiogenesis, neurogenesis and BBB repair integrity, so that it improves the clinical rat's neurobehavior with stroke measured by a decreasing of MnSS values[32]. This result is in line with another study that showed sensorimotor improvement after MSC administration. Sensorimotor improvement can be obtained through an increase of neural stem cells and PDGFR (Oligodendrocyte Progenitor Cell / OPC) triggered by MSC. Therefore, there is the reduction of immature neuron production within 24 hours[33], but there is also an increase of mature oligodendrocyte myelin production that triggers the decreasing of GFAP (astrocyte marker) and neurofilament-L (neuron marker). The explanation above maximizes the neurogenesis process, thus affecting the improvement of neuron/sensor-motor functions evaluated through mNSS. A previous study showed the mNSS measurement results on days first, third and seventh are significantly improved, especially its cognitive improvement after administration of MSC ($p < 0.05$) compared to the control group[34]. According to this study, MSC secretome decreases the apoptosis that occurs in brain cells by increasing VEGF, the cellular neurotropic factor and BDNF that contribute to vascular repair that triggers neurological repair after stroke.

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This study strengthens how the SH-MSC therapy was applied and observed in an ischemic rat model to assess neuronal behavior using the mNSS score and measure VEGF and CD31 expression by RT-PCR. However, this study does not evaluate the levels of other inflammatory substances, which is a limitation to angiogenesis and neurogenesis significances related to neuronal survival in ischemic strokes. Moreover, this study also did not give repeated doses to reach maximum neurological improvement, hoping to address this limitation in our future study.

Conclusion

The result of this study is different from the other study because this study showed the potential of secretome hypoxia mesenchymal stem cells, while the other study shows just single mesenchymal effects. Secretome is more superior than single MSC therapy because it does not have tumor-genetic characteristics, immune compatibility, or lower risk of infection and embolism compared to living and proliferating cell transplantation[10]. Secretome mesenchymal stem cells under hypoxia conditions in this study show the higher biomolecular content of growth factors, especially VEGF. MSCs secretome under hypoxia condition can induce cell proliferation, neuron cell survival, angiogenesis, vascularization, neurogenesis and blood-brain-barrier integrity recovery in rats' brains through the increases of VEGF and CD31 expressions and lead to neurological function and clinical improvement in ischemic stroke in rats; it has proven the decreasing of mNSS score.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- No animal studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Sumatera Utara, Medan, Indonesia (No. 69/KEPK/USU/2024).

Authors' Contribution Statement

S.S., I.J., M.R., R.S.D., A.P., I.M., D.M.D., S.S., Y.A. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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سيكريتوم الخلايا الجذعية الوسيطة للسكتة الدماغية الإقفارية: التعبير عن CD31 وعامل نمو بطانة الأوعية الدموية

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الخلاصة

في الوقت الحالي، تتمثل العلاجات القياسية للسكتة الدماغية الإقفارية في التحلل الخثري الوريدي وإعادة التوعية الوعائية. في المرحلة الحادة (<4.5 ساعات)، فإن نسبة 3.2٪ إلى 5.2٪ فقط من مرضى السكتة الدماغية الإقفارية مؤهلون لتلقي علاج التحلل الخثري الوريدي. الخلايا الجذعية الوسيطة (MSCs) هي خلايا متعددة القدرات يمكنها التمايز إلى أنواع مختلفة من الخلايا التي تنتج علاجات تجديدية محتملة لمرضى السكتة الدماغية. تُفرز الخلايا الجذعية الوسيطة سيكريتومات تحتوي على عوامل النمو والكيموكينات والسيتوكينات والمستقلبات والدهون النشطة بيولوجياً. يعزز سيكريتوم الخلايا الجذعية الوسيطة زيادة إنتاج CD31 وعامل نمو بطانة الأوعية الدموية (VEGF). يؤدي تأثير تكوين الخلايا العصبية وتكوين الأوعية الدموية من نشاط CD31 وVEGF بواسطة الخلايا الجذعية الوسيطية المشتقة من الحبل السري (SH-MSCs) إلى تجدد خلايا الدماغ وتحسين الوظائف العصبية. تحلل هذه الدراسة تأثير حقن 150 ميكرولتر من SH-MSCs على التعبير عن CD31 وVEGF في فئران Rattus norvegicus المصابة بالسكتة الدماغية الإقفارية بشكل موضوعي. استخدمت الدراسة منهجية تجريبية حقيقية في المختبر مع تصميم اختبار بعدي فقط مع مجموعة تحكم، بينما تم أخذ العينة بأسلوب أخذ العينات غير الاحتمالي مع أخذ العينات المتتالي. تم استخدام 18 فأراً من نوع Rattus norvegicus كعينات، وتم تقسيمها إلى 3 مجموعات: شام، والضبط، وP1 (انسداد الشريان الدماغي الأوسط + 150 ميكرولتر سيكريتوم). تم إحداث حالة السكتة الدماغية في فئران مجموعتي الضبط وP1 باستخدام طريقة انسداد الشريان الدماغي الأوسط عن طريق تثبيت الشريان السباتي المشترك. تم استخدام مقياس الشدة العصبية المعدل (mNSS) لقياس تحسن الوظائف العصبية. من خلال زيادة التعبير عن VEGF وCD31، يمكن للخلايا الجذعية الوسيطية المشتقة من الحبل السري أن تحفز تكاثر الخلايا، وبقاء الخلايا العصبية، وتكوين الأوعية الدموية، والتوعية، وتكوين الخلايا العصبية، واستعادة سلامة الحاجز الدموي الدماغي في أدمغة الفئران، مما يؤدي إلى تحسين النتائج السريرية والوظائف العصبية في الفئران المصابة بالسكتة الدماغية الإقفارية.

الكلمات المفتاحية: CD31، عامل نمو بطانة الأوعية الدموية (VEGF)، السيكريتوم، الخلايا الجذعية الوسيطة، السكتة الدماغية الإقفارية.