

Research Article

Assessment of Stem Cell Profile in Ischemic Stroke

Bayu Winata Putera^{1,2*}, Andi Wijaya^{2,3}, Irawan Yusuf¹ and Aw Tar Choon^{2,4}

¹Postgraduate Program in Clinical Biochemistry, Hasanuddin University, Makassar, Indonesia

²ProSTEM (Prodia Stem cell Indonesia), Central Jakarta, Indonesia

³Prodia Clinical Laboratory, Indonesia

⁴National University of Singapore, Singapore

Abstract

Background

Ischemic stroke remains a major health problem associated with high mortality and severe morbidity. The challenge of treatment is now to understand the process leading to endogenous neurorepair. Hematopoietic stem cell, mesenchymal stem cell, neural stem cell and endothelial progenitor cell are involved in endogenous neurorepair. These cells can contribute in recovery after stroke.

Methods

We enrolled ischemic stroke patient in the central hospital of the army (RSPAD) Jakarta. We collected patient's blood. We are grouping the patients into 3 groups. Group A, patient with onset stroke less than 7 days (<7 days) (11 subjects), group B, patient with onset stroke 7-15 days (12 subjects) and group C patient with onset stroke more than 15 days (12 subjects). HSC, MSC, NSC and EPC were measured with flow cytometer.

*Corresponding author: Bayu Winata Putera, Postgraduate Program in Clinical Biochemistry, Hasanuddin University, Makassar, Indonesia, Tel: +62 411584200; E-mail: bayu.winata@prostem.co.id

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Results

Number of Mesenchymal Stem Cells (MSC) (227.89±82.17) appears highest than other population of stem cell in group A, B and C. Our study show that number of Neural Stem Cells (NSC) increasing significantly with time of onset stroke. The number of Hematopoietic Stem Cells (HSCs) (p=0.007) and Endothelial Progenitor Cells (EPC) (p=0.036) significantly different between onset group A and B. The number of HSC (p=0.000) also significantly different between onset group B and C likewise the number of MSC (p=0.007). Our study also show that number of Neural Stem Cells (NSC) (p=0.036) significantly different between onset group A and C.

Conclusion

Our study suggest that HSC, MSC, NSC and EPC are mobilized at varying degrees into peripheral circulation after stroke.

Keywords: Endothelial Progenitor Cells (EPC); Hematopoietic Stem Cells (HSC); Ischemic stroke; Mesenchymal Stem Cells (MSC); Neural Stem Cells (NSC)

Introduction

Acute ischemic stroke is a common problem that carries a significant risk of death and disability [1]. According to the World Health Organization, 15 millions people worldwide suffer a stroke event each year. Of those 5 millions pass away and another 5 millions are permanently disabled [2]. There are approximately 12,1 per 1000 people in Indonesia [3]. Last decade has witnessed mounting evidence supporting the capacity of bone marrow derived stem cells to mobilize from bone marrow to peripheral blood and finding their way to the injured brain [4]. This mechanism called homing. Bone marrow consist of heterogeneous population stem cells, with Hematopoietic Stem Cells (HSC) and Mesenchymal Stem Cells (MSC) as the two most studied bone marrow derived stem cells. Additionally, Endothelial Progenitor Cells (EPC) and Neuron Stem Cells (NSC) have been studied and involved in endogenous neurorepair process after stroke [4].

HSC are quiescent cells with self renewal capacity and the ability to generate all mature blood cells [5]. HSC normally reside in specialized niches in the bone marrow that help maintain their quiescence and long term repopulating activity. There is emerging evidence that certain cytokines induced during inflammation have significant effects on HSC in bone marrow [5]. In pathophysiology of stroke, there is inflammation process included [6]. In stroke condition, there are increase production of mature effector cell from lineage-hematopoietic stem cells and facilitate the mobilization of mature effector cells from the bone marrow to blood. Recent evidence suggests that Hematopoietic Stem Cells (HSC) also direct targets of inflammatory signaling [5]. HSC will homing to inflammation site. HSC also have ability to differentiate into immun cells which have a play role in inflammation [5]. Within 24 hours after ischemia, resident microglia become activated and migrate to the lesion site. Immune cells attraction from blood circulation toward the ischemic lesion site is mediated by chemokines released, which also play a crucial role in recruitment of bone marrow-derived stem cells, including HSC, MSC, EPC and NSC [7].

Mesenchymal Stem Cells (MSC) have ability as an injury drugs store. In stroke condition, MSC secrete many cytokine for regenerative microenvironment [8]. In animal stroke model, transplantation of MSC have good efficacy. Therapeutic effect of MSC showed that there is increasing growth factor include brain-derived neurotropic factor [9,10]. These neurotrophic cytokine have been implicated to play an important role in the process of angiogenesis and neurogenesis. Endothelial Progenitor Cells (EPC) play role in angiogenesis [11] and Neuron Stem Cells (NSC) play role in neurogenesis [12]. Clinical trials on cell therapy have been initiated in stroke patients [4-7,13]. A better understanding of mechanisms homing of the cells from peripheral to the brain is likely to aid in optimizing cell therapy for stroke [7]. The aim of our study is to observe the stem cells profile in ischemic stroke.

Materials and Methods

Subjects

Subjects in this study were part of cohort study of ischemic stroke in the central hospital of army (RSPAD) Gatot Subroto Jakarta. Diagnosis of ischemic stroke was made using clinical examination and Magnetic Resonance Imaging (MRI) interpretation by neurologist. We are grouping the subjects into 3 group. Group A, subjects with onset stroke less than 7 days (<7 days), group B, subjects with onset stroke between 7-15 days and group C subjects with onset stroke more than 15 days. This study was approved by ethics committee of Faculty of Medicine, Hasanuddin University Makassar, Indonesia with register number: UH14110578.

Blood sampling and cell isolation

10mL of blood was drawn from antecubital vein of subjects and collected in a vacutainer containing 3.8% buffered sodium heparin. Mononuclear cells (MNCs) were then isolated by density-gradient centrifugation of histopaque (Histopaque-1077, Sigma-Aldrich). Add 3mL of whole blood onto 3mL histopaque. Centrifuge for exactly 30 minutes at room temperature. After centrifugation, carefully aspirate the upper layer with a Pasteur pipette to within 0.5cm of the opaque interface containing mononuclear cells. Discard upper layer. Carefully transfer the opaque interface with a pasteur pipette into a clean conical centrifuge tube. Wash the cells by adding 10mL of isotonic Phosphate Buffered Saline (PBS) solution and mix by gently drawing in and out of the pipette. Centrifuge for 10 minutes. Aspirate the supernatant and discard. Resuspend cell pellet with 5mL of isotonic phosphate buffered saline solution and mix by gently drawing in and out of the pipette. Erythrocytes and granulocytes should pellet to the bottom of centrifuge tube. Mononuclear cells should band at the interface between the histopaque and plasma.

Flow cytometry

All samples were analyzed using a BD FACS Canto flow cytometer with 3 fluorescent parameters: Fluorescein Isothiocyanate (FITC), Phycoerythrin (PE) and Peridinin Chlorophyll Protein (PerCP). The Forward Scatter (FSC-H) and Side Scatter (SSC-H) of cells were measured using a linear scale. A stop criterion of 1 million events was used for all data acquisition. We are measured number of HSC, MSC, NSC and EPC using flow cytometer with gating strategy (Figure 1) and surface marker HSC with CD34 antibodies-FITC (130-081-001, Miltenyi Biotec GmbH) and CD45 antibodies-PerCP

(130-094-975, Miltenyi Biotec GmbH), MSC (CD105+/CD73+/CD90+/CD34-/CD45-) with human MSC analysis kit (562245, BD Biosciences), NSC with CD133 antibodies-PE (130-080-801, Miltenyi Biotec GmbH), CD34 antibodies-FITC (130-081-001, Miltenyi Biotec GmbH) and CD45 antibodies-PerCP (130-094-975 Miltenyi Biotec GmbH) and EPC with CD34+CD34 antibodies-FITC (130-081-001, Miltenyi Biotec GmbH), CD133 antibodies PE and KDR antibodies (Sigma) and gating strategy below:

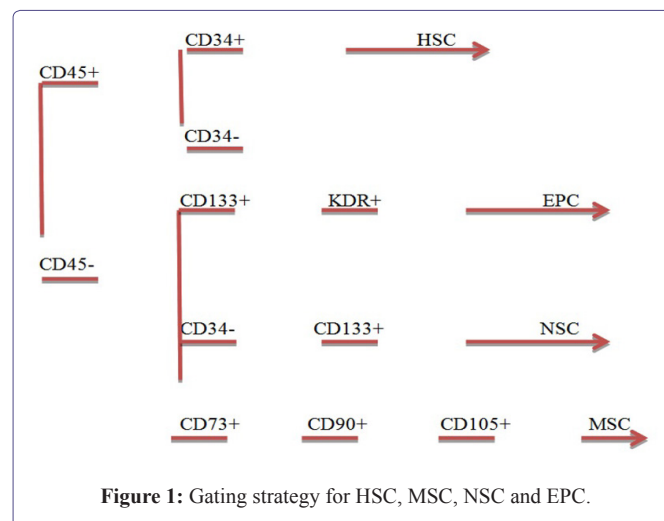


Figure 1: Gating strategy for HSC, MSC, NSC and EPC.

Results

We divided patients into 3 groups of: 11 subjects group A, 12 subjects group B and 12 subjects group C. characteristics of subjects (Tables 1 and 2).

Parameters	Group A	Group B	Group C
Age (years)	60±11.2	58±10.3	60±10.3
Sex	-	-	-
Male	9	9	9
Female	2	3	3

Table 1: General characteristics of subject.

Variable	Min	Max	Mean±SD
HSC (cells/ml)	17	332	121.74±79.23
MSC (cells/ml)	129.5	656.25	227.89±82.17
NSC (cells/ml)	1.28	956	81.94±212
EPC (cells/ml)	1	100	10±15.48

Table 2: Number of stem cells.

Note: HSC: Hematopoietic Stem Cell; MSC: Mesenchymal Stem Cell; NSC: Neural Stem Cell; EPC: Endothelial Progenitor Cell.

Discussion

Migration of stem cells from bone marrow to target organ injury is important role for regeneration and repair. Bone marrow acts as a reservoir for multiple stem cells population including Hematopoietic Stem Cells (HSC), Mesenchymal Stem Cells (MSC) Endothelial

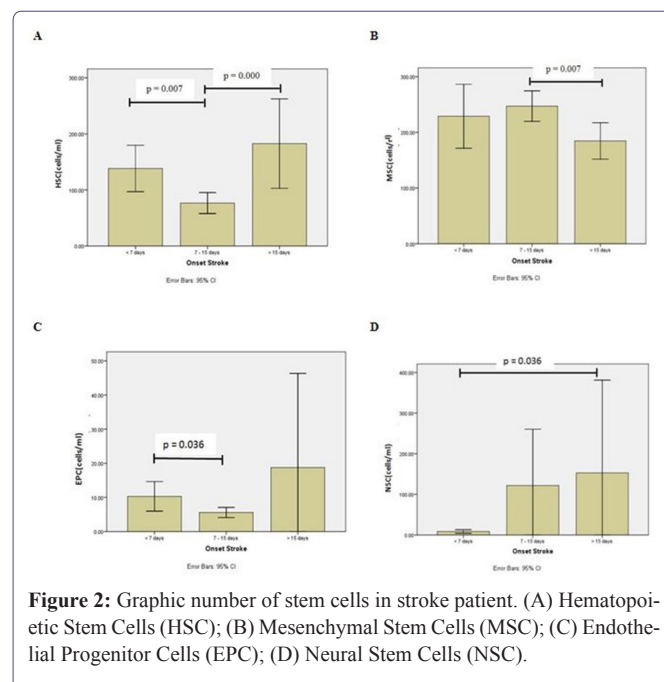
Progenitor Cells (EPC) [8]. In healthy donors, the percentage of CD34⁺ cells among circulating total nucleated cells at steady state is 0.06% [14], the percentage of MSC among circulating total nucleated cells is 0.0019% [15] and the percentage of EPC level in normal control subjects without physical or pathological stimulation is around 1.5% [16]. These cells are mobilized at varying degrees into the peripheral circulation following injury [8]. Aim of our study is to investigate stem cells profile in ischemic stroke subjects. Our study show (Figure 2A) that number of Hematopoietic Stem Cells (HSC) is the highest in group C, patients with onset stroke >15 days and the second in group A, patients with onset stroke <7 days. It indicates that HSC have play role in <7 days after stroke and >15 days after stroke. This is appropriate with Wolf and Ley study in 2015. Study Wolf and Ley [9,17] show that there is increasing number of HSCs after stroke because requirement of monocyte after stroke [9,17].

Our study showed that Mesenchymal Stem Cell (MSC) is the highest number of stem cells in all group onset stroke. Number of MSC is highest in group B, patients with 7-15 days onset of stroke (Figure 2B). Our postulate that the peak of regeneration process induces MSC occurs in 7-15 days after onset stroke. As one of potential therapeutic arms, it has been demonstrated that transplantation with bone-marrow-derived mesenchymal stem cells can promote functional recovery [18] and nervous tissue repair [19] in a vast number of previous studies associated with stroke [8,20,21]. Mechanism is proposed to explain the successful improvement of neural functional recovery through MSC administration that MSC secrete neurotrophic factors that may induce the host ischemic brain to activate endogenous repair mechanisms [4]. Chopp, et al., [9] show that MSC produce Hepatocyte Growth Factor (HGF), Vascular Endothelial Growth Factor (VEGF) [22], nerve growth factor [15], Brain-Derived Neurotrophic Factor (BDNF) [9,23,24], Basic Fibroblast Growth Factor (b-FGF) [25], Insulin Growth Factor-1 (IGF-1) [26] and Stromal Derived Factor (SDF)-1 [10,24]. These neurotrophic cytokines have been implicated to play an important role in the process of angiogenesis and neurogenesis. Besides secreting neurotropic factor, MSC also secreting exosome. Exosomes contain functional proteins, mRNAs, microRNAs, miRNA and tRNA species that play roles in intercellular communication [27]. Unfortunately, our study did not measure exosomes from MSC.

Angiogenesis is important for formation of new brain microvessels and functional recovery after ischemic stroke. Enhanced angiogenesis has been associated with function recovery following stroke [28,29]. EPC have been suggested to maintain angiogenesis and endothelial repair or protection. EPC contribute to neurovascular protection, angiogenesis and neurogenesis [30,31]. Our study demonstrated that EPC is mobilized into peripheral circulation from bone marrow after ischemic stroke. Moreover the number of EPC were higher in group C, patient with >15 days of onset stroke (Figure 2C). Our postulate is EPC play role as neurovascular protection and neurogenesis in patient with >15 days of onset stroke. The underlying mechanisms include that the regenerated blood vessels provide nutritive blood flow and EPC create a microenvironment for neural regeneration and survival, by secreting factors such as VEGF [27,32,33]. Furthermore, neuroblast migrate along these new vessels to achieve neurogenesis in peri-infarct area [34-36].

Many experimental studies have shown an increased proliferation of NSC in animal model of stroke which persist at least for four

months after ischemia [28,37]. Our study also showed that Neural Stem Cell (NSC) increasing significant with time of onset stroke (Figure 2D) and number of NSC is highest in group C, patients with onset stroke >15 days. It indicates that NSC play important role in >15 days after stroke. The mechanisms underlying the improved functional recovery of ischemic stroke animal model subjected to NSCs therapy remain unclear. NSCs were found to differentiate into neuronal and/or glial phenotypes in most animal studies [28,30].



Our study demonstrated that HSC also mobilized into peripheral circulation from bone marrow after ischemic stroke. Central Nervous System (CNS)-induced cytokine cues may serve as migratory signals to HSC. The neurotransmitter-mediated interaction between CNS and bone marrow is bidirectional, with hematopoietic signaling mechanism equally contributing to HSC regulation of CNS function [4,34]. HSC can affect the nervous system and modulate its action [4,10,38]. Clinical data show that HSC mobilized into peripheral blood after ischemic stroke and directly related to recovery of function [4,39]. Our postulate mechanisms include: HSC contribute reduce cerebral postischemic inflammation, attenuate peripheral immune activation and mediate neuroprotection after ischemic stroke [17]. The limitation of our study is that we need higher number of patients for better the error bars especially for EPC and NSC. This is a preliminary case report and we plan to expand this study to monitor the same patient over time following onset of stroke to better understand the dynamics of these parameters following onset of stroke. Such understanding of the dynamic interplay of the parameters will allow us to better predict and develop method to improve outcome in stroke patients.

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