HAYATI Journal of Biosciences 24 (2017) 187–194

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Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Original Research Article

# Intravenous Versus Intraarterial Transplantation of Human Umbilical Cord Blood Mononuclear Cells for Brain Ischemia in Rats

Yetty Ramli,[1†] Ahmad Sulaiman Alwahdy,[1*†] Mohammad Kurniawan,[1] Berry Juliandi,[2] Puspita Eka Wuyung,[3] Yayi Dwina Bilianti Susanto[3]

[1] *Neurology Department, Medical Faculty University of Indonesia/Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.*
[2] *Department of Biology, Bogor Agricultural University (IPB), Bogor, Indonesia.*
[3] *Pathology Anatomy Department, University of Indonesia/Cipto Mangunkusumo National Hospital, Jakarta, Indonesia.*

ARTICLE INFO

*Article history:*
Received 27 August 2017
Received in revised form
7 October 2017
Accepted 3 November 2017
Available online 28 November 2017

*KEYWORDS:*
human umbilical cord blood,
middle cerebral artery occlusion,
monofilament,
mononuclear cells,
rats

ABSTRACT

Cerebral ischemia is among the most common type of stroke seen in patient. Regeneration of death neurons remains questionable. Human umbilical cord blood mononuclear cell (cbMNC) is one of the treatment options for ischemia stroke through their various advantages; availability, pluripotency, and immaturity. One group of healthy rats and three groups ($n = 6$ per group) of male Wistar rats undergone permanent middle cerebral artery occlusion (MCAO). Rats were allowed to recover for 7 days before intraarterial and intravenous injection of $1 \times 10^6$ cells/kg of human cbMNC. Behavioral tests were performed before the MCAO, 1 week after MCAO, and at 3, 9, and 14 days after cbMNC injection. Brain infarct area and neurons in hippocampus were evaluated. Spontaneous activity was much significantly improved compared with the placebo group ($p < 0.05$). Comparing the neuron cells in hippocampus, intraarterial and intravenous have more changes in neurons morphology. No effect of cbMNC implantation in decreasing infarct area. Safety of xenogenic was confirmed by this study when the dosage of $1 \times 10^6$ cells/kg was used and showed their beneficial effects.


## 1. Introduction

Stroke is one of the commonest diseases with high mortality and morbidity. Recently, the only treatment approved by the food drug administration for acute ischemic stroke is thrombolysis, even though this treatment has limited golden period. Thrombolysis intravenously can be performed only in ischemic stroke where the occlusion is not in the middle cerebral artery, and regeneration therapy of death cells, unfortunately, remains questionable (Wei *et al.* 2013; Shinozuka *et al.* 2013; Brouns *et al.* 2009).

Many studies have been conducted to show the effectiveness of cell-based therapy by differentiating the route (intraarterial (IA), intravenous (IV), and intraparenchyme) with result intraarterially is more superior and safer compared with the other route. This hypothesis arised because IA route showed larger amount of cells that injected to the site of injury compared with IV route where the possibility of cells to enterapt in the lung was higher and they must be passing through peripheral organs, and the cell amount would be less in the site of injury as consequences (Guzman *et al.* 2008).

From previous studies, cell-based therapy for ischemic stroke, where cord blood mononuclear cell (cbMNC) was used, showed positive results in functional assessment by decreasing apoptosis, inflammation in periinfarct area, and stimulate angiogenesis, whether it was given intraarterially, intravenously, or intraparenchymally. First and second phase of the study have shown that the injection of stem cells for acute and subacute ischemic stroke was safe (Fruchtman *et al.* 2004).

The source of cell itself is another problem to be solved. The past two decades have shown significant progress in basic understanding of adult stem cells biology. Cells derived from the human umbilical cord have been successfully used in the clinic for almost two decades (Hows *et al.*1992; Gluckman *et al.* 1989; Locatelli *et al.* 2003; Rocha *et al.* 2001). Their simple and economic retrieval, enrichment for hematopoietic progenitors, enhanced proliferation rate, expansion potential (Lewis *et al.* 2000; Ringden *et al.* 2008), and low incidence of graft-versus-host disease (Harris *et al.* 2009; Rocha *et al.* 2004) make them a promising cell treatment for

* Corresponding author.
*E-mail address:* ule842002@yahoo.com (A.S. Alwahdy).
Peer review under responsibility of Institut Pertanian Bogor.
† Ramli Yetty and Alwahdy Ahmad Sulaiman contributed equally to this work.

https://doi.org/10.1016/j.hjb.2017.11.002

neurological diseases. Although their therapeutic benefits were initially thought to be limited to hematopoietic disorders, several recent studies have shown the potential of these human umbilical cord–derived cells to enhance regeneration and tissue repair in various pathological disorders, including neurologic diseases (McGuckin *et al.* 2006; Rosenkranz *et al.* 2011).

Various study methodology play an important role because of different route, dosage, time to inject, and the origin stem cells whether from human cord matrix or cord blood are used could make different results. The other advantage of cbMNC is their pluripotency by having a heterogenous mix of immature lymphocyte, monocyte (Sorg *et al.* 2001; Yang *et al.* 2010), hematopoietic (Mayani *et al.* 1998), endothelial (Ingram *et al.* 2004), and mesenchymal (Erices *et al.* 2000; Flynn *et al.* 2007; Lee *et al.* 2004; Secco *et al.* 2008) stem/progenitor cells.

All the cells derived from cbMNC have their contribution to the neurogenesis and angiogenesis. Neurogenesis could be stimulated by inducing endogenous stem cells in subgranular zone, subventricular zone, and subcortical area (Altman *et al.* 1963; Eriksson *et al.* 1998; Kokaia *et al.* 2013). Angiogenesis and neurogenesis is something that could not be separated, where angiogenesis could stimulate neurogenesis and helping migration of progenitor cells to the periinfarct site (Kojima *et al.* 2010).

## 2. Materials and Methods

### 2.1. Permanent middle cerebral artery occlusion model

One group of healthy rats and three groups ($n = 6$ per group) of 250–300 g of male Wistar rats undergone permanent middle cerebral artery occlusion (MCAO), where group 2 was treated with physiological fluid intraarterially, group 3 with cbMNC intraarterially, and group 4 with cbMNC intravenously. Behavioral tests were performed before the MCAO, 1 week after MCAO, and at 3, 9, and 14 days after cbMNC injection. Brain infarct area, neurogenesis in hippocampus, and neovascularization in infarct area were evaluated. One week after occlusion, rats were injected by cbMNC with $1 \times 10^6$ cells/kg that has been characterized by cd34+ (7%) intraarterially and intravenously. Two weeks after implantation, all rats were euthanized and functional assessment in day 3, 9, and 14 after implantation were evaluated. Histopathology confirmation by hematoxylin eosin (H&E) and cresyl violet staining were marked (Hunter *et al.* 2000; Lubjuhn *et al.* 2009; Rosell *et al.* 2013) (Figure 1).

### 2.2. cbMNC isolation

Cord blood sample obtained from cryopreservation was not used by the Cellsafe International Corporation. All cord blood units tested negative for human immunodeficiency virus, hepatitis C virus, hepatitis B virus, human T-cell lymphotropic virus, and syphilis. Cord blood suspension was processed using gradient centrifugation method as follows: Use aseptic technique procedures and biosafety cabinet operation. Cryopreserved cord blood samples were thawed and washed using PBS and centrifuged at 1500 rpm for 10 minutes. Washing was done two times. Ficoll-Paque solution was put in 15 mL tube. Pipette carefully the cleaning results of cord blood into a tube containing Ficoll-Paque. The volume ratio of bone marrow suspension: Ficoll-Paque = 1:1. Centrifugation was performed at 2200 rpm for 10 minutes at 20°C, centrifugation termination did not use brakes (to prevent disorganization of fractions of separate components). Buffy coat layer (the layer contains nucleated cells) was taken using a pipette slowly and transferred to a 15-mL centrifuge tube. Clean the buffy coat of erythrocytes using lysis buffer as much as 3 mL. Clean the buffy coat of lysis buffer using NaCl as much as 4 mL, and the number of cells was counted with a counting chamber and trypan blue staining.

### 2.3. Implantation of cbMNC procedures

On the 7th day after ischemia condition, the experimental animals were randomly assigned and received a transplant of $1 \times 10^6$ cells/kg of cbMNC in 1 mL of fluid. In the group treated with implantation intravenously, cbMNC was inserted slowly through the tail vein of rats. In the group treated with implantation

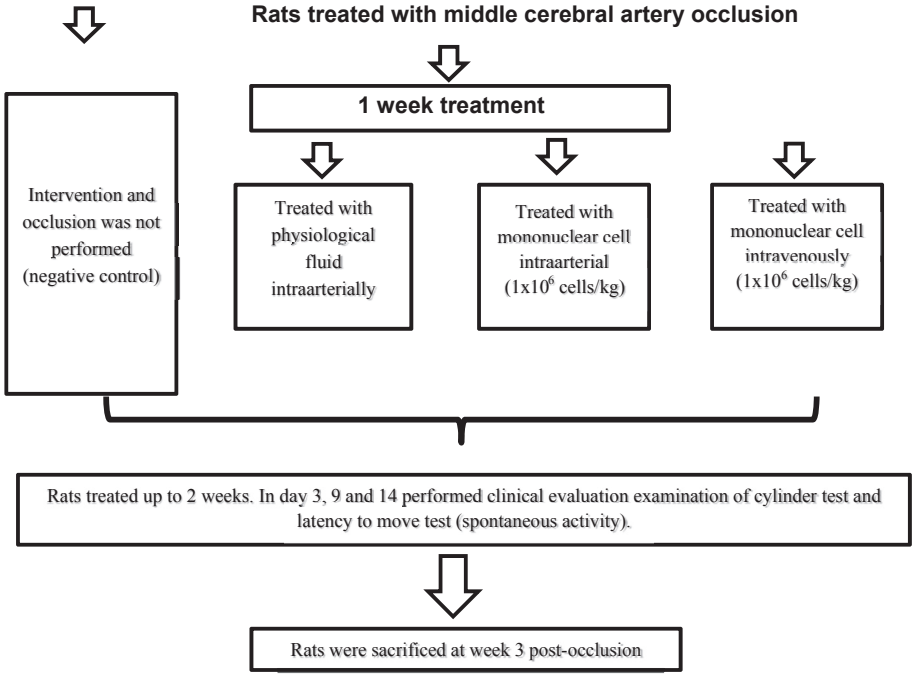


**Figure 1.** Study design.