International Journal of PharmTech Research

CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.10, pp 180-189, 2015

The Effect of Granulocyte Colony Stimulating Factor Administration on Mobilization, Proliferation and Differentiation of Mesenchymal Stem Cells

Ismail Hadisoebroto Dilogo[1,2], Erica Kholinne[1]

[1]Department of Orthopaedic and Traumatology, Faculty of Medicine Universitas Indonesia, Jl. Salemba Raya 6, Jakarta, Indonesia
[2]Stem Cell Medical Technology Integrated Service Unit, CiptoMangunkusumo Central Hospital, Faculty of Medicine Universitas Indonesia, CMU 2 Building, 5th floor, Jl. Diponegoro 71, Jakarta, Indonesia

Abstract: Objectives: Mesenchymal stem cells (MSCs) are routinely harvested from bone marrow. Bone marrow derived MSCs have several drawbacks including limited volume of aspiration and donor site morbidity, therefore an alternative donor site is needed. Isolation of MSCs from peripheral blood mitigates such issues. However, the MSCs isolated from peripheral blood are significantly lower in quantity. Granulocyte colony-stimulating factor (GCSF) administration in aphaeresis technique mobilizes mononuclear cells and increases the number of isolated haematopoietic stem cells. It is also recently known that administration of GCSF PB mobilizes MSCs from the bone marrow into the peripheral blood, thus increasing the number of MSCs isolated from peripheral blood. The objective of this study is to evaluate the effect of subcutaneous administration of GCSF to both bone marrow and peripheral blood MSCs. Isolated MSCs were evaluated in terms of its proliferation and differentiation capacity. Findings from this study will evaluate the potential use of GCSF to enhance bone marrow MSCs and GCSF-mobilized peripheral blood as an effective donor site for MSCs.
Method: Fourteen male New Zealand white rabbits were randomly divided into control and treatment groups. Each bone marrow and peripheral blood sample was collected consecutively from one rabbit. Therefore, there were 7 rabbits in control groups; group 1control for bone marrow (C-BM, n=7) and group 2 control for peripheral blood (C-PB, n=7). The other 7 rabbits were in treatment group; group 3 treatment for bone marrow (T-BM, n=7) and group 4 treatment for peripheral blood (T-PB, n=7). Treated animals received subcutaneous injection of GCSF, 10 mcg/kilogram body weight/ day for 7 days prior to sample collection. Isolated samples were purified, analysed for cell expansion, differentiation capacity and time.
Results: MSCs were obtained from all groups with varying degree of isolated cell quantity and quality. Mean initial isolated cell number for C-BM3.07 x 10^6, C-PB 2.11 x 10^6, T-BM 2.89 x 10^6 and T-PB 7.35 x 10^6.($p<0.001$). Mean confluency time for C-MB 25.8 days, C-PB 35.7 days, T-BM 26 days and T-PB 19,7 days ($p<0.001$). Mean confluent cell number for C-BM: 6.54 x 10^6, C-PB: 4.61 x 10^6, T-BM: 5.94 x 10^6, and T-PB: 11.14 x 10^6 ($p<0.001$). Mean differentiation time into osteoblast for C-BM: 15.5 days, C-PB: 25.4 days, T-BM: 15.4 day, andT-PB:11.2 days ($p<0.001$). Statistically significant differences were found for mean initial isolated cell number between C-BM and T-PB ($p<0.001$), C-PB and T-PB ($p<0.001$), and T-BM and T-PB ($p<0.001$). Posthoc analysis for confluency time, confluency cell number and

differentiation time was found significantly different for all groups except group C-BM and T-BM (p=1.0).
Conclusion: This study has successfully isolated MSCs from GCSF mobilized-peripheral blood. Isolated MSCs from GCSF-mobilized peripheral blood showed typical MSC histological characteristics with enhanced proliferative and differentiation capacity compared to non-mobilized samples. GCSF-mobilized peripheral blood is a promising alternative donor site for MSCs with clinical potential.
Keywords: Granulocyte colony stimulating factor, mesenchymal stem cells, mobilization, proliferation, differentiation.

Introduction

Two of the most severe orthopaedic complications are delayed union and nonunion of fractures. In England, the incidence of non-union comprised of approximately 5 – 10 % of the total 850,000 new cases of fracture annually.[1]Nonunion increases the length of hospital stay and treatment cost. The condition restricts the patient in daily functioning and productivity, thus significantly reduce the overall quality of life of the patient.[2]The diamond concept of fracture healing emphasizes four major factors required in normal bone regeneration; osteogenic cells, osteoinductive factors, scaffold and rigid mechanical fixation.[3] Disturbances of fracture healing may occur if there is any disruption in one of the four factors. It was theorized that the osteogenic cells are lacking in atrophic nonunion and fractures with bone defect. Thus, utilizing the osteogenic capacity of mesenchymal stem cells (MSCs) is well sought for such cases.[4-6]

MSCs are isolated from various sources, including bone marrow, peripheral blood, adipose tissue, placenta and umbilical cord.[7]Bone marrow mesenchymal stem cells (BM-MSCs) were the first discovered and most extensively studied MSCs. However, it has several major drawbacks including invasive collection procedure, donor site morbidity, limited amount of aspirable bone marrow, and low stem cell yield requiring longer *in vitro* culture time.[7,8] Therefore, an alternative MSCs donor side with fewer disadvantages is preferable. A small population of MSCs was detected in the peripheral blood.[9-11]The finding sparks wave of excitements in the field of MSCs research because sample collection from peripheral blood is significantly less invasive and more convenient. However, some studies suggested that the quantity of MSCs in peripheral blood was too small for effective *in vitro* cultivation and clinical applications.[11-13]

Granulocyte colony-stimulating factor (GCSF) is routinely used for haematopoietic stem cell (H-SC) mobilization in apheresis.[14-15]It induces stem cell mobilization by decreasing stromal cell derived factor-1 alpha (SDF-1α) and up-regulating its receptor CXCR4.[16]Investigations that studied the role of GCSF in MSCs mobilization is limited, and so far had produced contradictory findings.

The main objectives of this research it to investigate the effect of GCSF on the quantity and quality of both bone marrow and peripheral blood derived MSCs. Findings from this study will help further studies to evaluate the potential bedside application of GCSF to enhance BM-MSCs and GCSF-mobilized peripheral blood as an effective donor site for MSCs.

Methods

The experiment was conducted in Primate Research Center, Bogor Agricultural Institute, Bogor, Indonesia. Animal ethics approval was obtained from the Animal Care and Use Committee (reference number: R.04-13-IR). Fourteen New Zealand white rabbits were used in this study. Sample size determination was calculated using Federer's formula: (T-1) (n-1) >15, where T equals to 4, which was the total number of group. Twenty percent mortality rate was taken into consideration, hence n ≤ 7 rabbits in each group. The two principle donor sites of MSCs were bone marrow and peripheral blood. Complying with the animal ethics regulations, each sample from both donor sites was collected from one rabbit. Fourteen rabbits were randomized into two main control and treatment groups, which were further subdivided into two different groups as follow: Group 1 (n=7) control for bone marrow (C-BM), Group 2 (n=7) control for peripheral blood (C-PB), Group 3 (n=7) treatment for bone marrow (T-BM) and Group 4 (n=7) treatment for peripheral blood (T-PB).

Animal Intervention and Sample Collection

Animals were fed *ad libitum* and acclimatized for 7 days before intervention. All rabbits in the treatment groups received subcutaneous injection of GCSF, 10 µg/kilogram body weight (kgBW)/day, for 7 consecutive days. Subcutaneous injections were given at the buttock area. All control rabbits received nothing. Prior to sample collection, all rabbits were anesthetised by a combination of ketamine (35 mg/kgBW) and xylazine (5 mg/kgBW) injected intramuscularly. After being anesthetised, injection site were cleaned using povidone iodine. For each sample collection, a sterile 10 mL - 18G syringe prefilled with 1000 Unit of heparin was used. Bone marrow aspiration was performed at the iliac crest, followed by venous blood collection from the auricular area. Aspirates were collected from each donor site into sterile 15 mL polypropylene tubes (Vacuette). After that the termination was followed by injection of overdose pentobarbital sodium (Nebutal ® Sodium Solution; Boehringer, Ingelheim, Germany).

Cell Culture

Samples were pipetted and placed in a 15ml tube with equal amount of phosphate buffer saline (PBS [Gibco, Grand Island, New York]). Ficoll gradient density technique was used. Mixed samples were layered carefully over an equal volume of 1.073 g/ml Ficoll-Paque™ according to gradient density technique and centrifuged at 900g for 30 minutes. The mononuclear cell were recovered from the gradient interphase (cloud-like layer) and washed with 5ml PBS. The samples were centrifuged at 900g for 10 minutes. Supernatant were discarded and pellets were resuspended in Dulbecco's Modified Eagles Medium (DMEM; Gibco, Grand Island, New York) containing 1 g/l of glucose (DMEM-LG) supplemented with 100 U/ml penicillin (Hyclone, Logan, UT), 100 µg/ml streptomycin (Hyclone, Logan, UT), and 25 µg/ml amphotericin B (Hyclone, Logan, UT). All samples are counted with haemocytometer chamber (Spencer Bright-Line, Improved Neubauer) for initial cells number. All cells then were plated in T 25 flask with ventilated cap(Corning, New York, USA) in 5 ml of growth medium supplemented with 20% Fetal Bovine Serum (FBS [Gibco-BRL, Carlsbad, CA]). The culture were maintained at 37°C in 5% CO_2incubator, with an initial medium change at 7 days after initial plating to remove non-adherent cells and followed by every 3 days until confluency state (80% confluent).Observation under microscope was performed to obtain a fibroblastic like plastic adherent cells. The time needed for confluency was evaluated by 2 technicians and recorded. Confluency cell number was recorded.

Confirmation of MSCs was done by characterization with cell surface antigen markers with CD 34, 73 and 90 by flowcytometry.

MSCs at third passage will be cultured at 3000 cells/cm^2 density in 8 ml osteogenic medium consist of 100nM *dexamethasone*(Sigma, St Louis, MI, USA), 10 mm *beta-glycerol phosphate* (Sigma), and 50 µm *ascorbic acid 2- phosphate* (Sigma). Afterwards, it was incubated at 20% O_2 flow, 5% CO_2 flow, 37°C. After a week, the culture was washed several times using PBS, and the medium was changed with new fresh medium culture until confluence. The cells' morphology was observed during medium culture changing using microscope (Nikon, Germany). The observed osteoblast should be cuboidal in shape and dark copper coloured. The time of differentiation is recorded and followed by Alizarin Red staining confirmation.

The incubated cell were separated from its medium, and washed by PBS. After that a monolayer cells were fixated by using *buffered formalin* (10%) solution for 30 minutes. The fixed monolayer cells were then stained using Alizarin red solution for 45 minutes at room temperature and dark exposure. Positive result will be shown as positive calcium deposits (red brick coloured nodules).

Data for initial cell number, confluency time, confluency cell number and differentiation time were analysed using one way ANOVA, continued with Bonferroni post-hoc and Tamhane analysis, using SPSSv.16.(IBM).

Results

MSCs in all groups were able to be isolated, proliferate and differentiate into osteoblast (Figure 1-4). One way ANOVA analysis are shown in table 1. The initial cell number (mean) of group 1: 3.07 x 10^6, group 2: 2.11 x 10^6, group 3: 2.89 x 10^6 and group 4: 7.35 x 10^6 ($p<0.001$). The initial cell number for T-PB group is approximately 2.3-3.4 times higher than others. Confluency time (mean) for group 1: 25.8 days, group 2: 35.7

days, group 3: 26 days, group 4: 19.7 days (p<0.001). The T-PB group achieved confluency 1.3 – 1.8 times faster than others. Confluency cell number (mean) group 1: 6.54 x 10^6, group 2: 4.61 x 10^6, group 3: 5.94 x 10^6, group 4: 11.14 x 10^6 (p<0.001). The confluency cell number for T-PB group is approximately 1.7 – 2.4 times higher than others. Differentiation time was counted as: group 1: 15.5 days, group 2: 25.4 days, group 3: 15.4 days, group 4:11.2 days (p<0.001). The T-PB Peripheral blood treatment group had the fastest time to differentiate approximately 1.6 – 2.2 times faster compared to others.

Table 1. The comparison of initial cell number, confluency time, confluency cell number, and differentiation time between groups

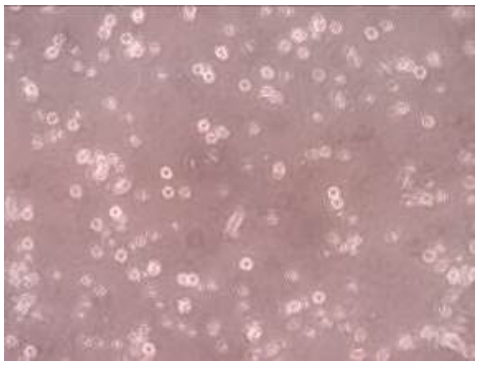
Variables	Groups	N	Mean	Standard deviation	p value
Initial cell number (10^6)	Group 1	7	3.07	0.90	< 0.001
	Group 2	7	2.11	0.46	
	Group 3	7	2.89	0.41	
	Group 4	7	7.35	1.16	
Confluency time (day)	Group 1	7	25.86	3.13	< 0.001
	Group 2	7	35.71	1.50	
	Group 3	7	26.00	3.83	
	Group 4	7	19.71	0.76	
Confluency cell number (10^6)	Group 1	7	6.54	1.01	< 0.001
	Group 2	7	4.61	0.30	
	Group 3	7	5.94	0.17	
	Group 4	7	11.14	0.43	
Differentiation time (day)	Group 1	7	15.57	1.51	< 0.001
	Group 2	7	25.43	1.90	
	Group 3	7	15.43	1.51	
	Group 4	7	11.29	1.50	

Table 2. Post hoc analysis

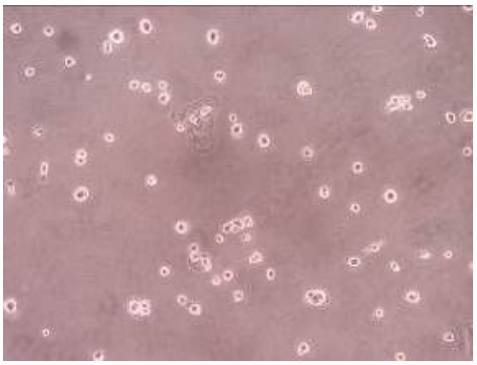
Variable	Groups		Mean difference (I-J)	95% Confidence Interval		Sig.
	I	J		Lower bound	Upper bound	
Initial cell number	Group 1	Group 2	0.96	-0.27	2.18	0.204
		Group 3	0.19	-1.04	1.41	1.000
		Group 4	-4.28	-5.50	-3.06	0.000
	Group 2	Group 3	-0.77	-1.99	0.45	0.494
		Group 4	-5.24	-6.46	-4.01	<0.001
	Group 3	Group 4	-4.46	-5.69	-3.24	<0.001
Confluency time	Group 1	Group 2	-9.86	-14.31	-5.41	<0.001
		Group 3	-0.14	-6.06	5.78	1.000
		Group 4	6.14	1.66	10.62	0.010
	Group 2	Group 3	9.71	4.29	15.14	0.002
		Group 4	16.00	13.87	18.13	<0.001
	Group 3	Group 4	6.29	0.78	11.79	0.027
Confluency cell number	Group 1	Group 2	1.93	0.49	3.36	0.011
		Group 3	0.60	-0.85	2.05	0.670
		Group 4	-4.60	-6.03	-3.17	<0.001
	Group 2	Group 3	-1.33	-1.76	-0.90	<0.001
		Group 4	-6.53	-7.16	-5.90	<0.001
	Group 3	Group 4	-5.20	-5.81	-4.59	<0.001
Differentiation time	Group 1	Group 2	-9.86	-12.34	-7.38	<0.001
		Group 3	0.14	-2.34	2.62	1.000
		Group 4	4.29	1.80	6.77	<0.001
	Group 2	Group 3	10.00	7.52	12.48	<0.001
		Group 4	14.14	11.66	16.62	<0.001
	Group 3	Group 4	4.14	1.66	6.62	<0.001

Posthocanalysis are shown in table 2. Posthoc analysis for initial cell number was found significantly different for group 1 and 4 (p=0.000), group 2 and 4 (p<0.001) and group 3 and 4 (p<0.001). Posthoc analysis for confluency time, confluency cell number and differentiation time was found significantly different for all groups except group 1 and 3 (p=1.000, 0.670, 1.000).

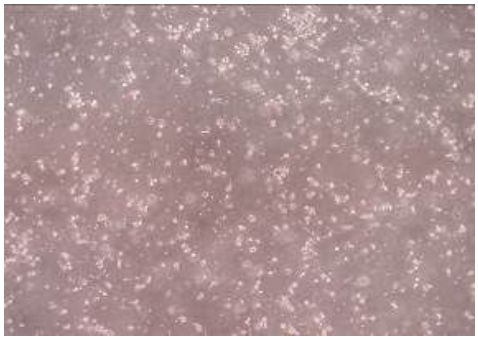
1 (a). Initial cells observation :C-BM group (160x hpf)



1 (b). Initial cells observation :C-PB group (160x hpf)



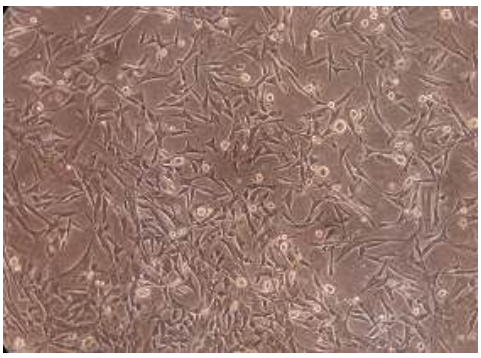
1 (c). Initial cells observation :T-BM group (80x hpf)



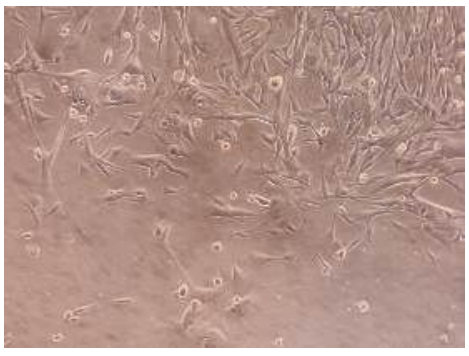
1 (d). Initial cells observation :T-PB group (80x hpf)

Figure 1. Initial cells observation

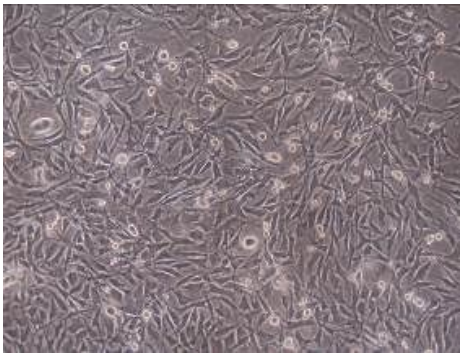
2 (a). Cells observation week 3 :C-BM group (160x hpf)



2 (b). Cells observation week 3 :C-PB group (160x hpf)



2 (c). Cells observation week 3 :T-BM group (160x hpf)



2 (d). Cells observation week 3 :T-PB group (160x hpf)

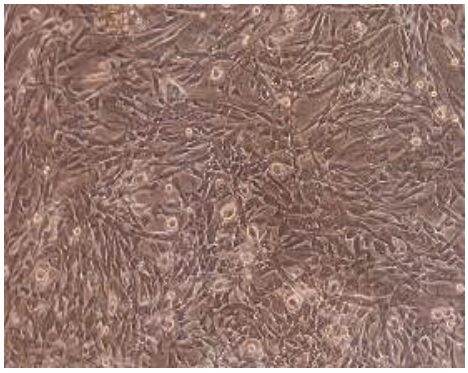


Figure 2. Cells observation week 3

3 (a). Osteoblast differentiation observation: C-BM group (160x hpf)

3 (b). Osteoblast differentiation observation: C-PB group (160x hpf)

3 (c). Osteoblast differentiation observation: T-BM group (160x hpf)

3 (d). Osteoblast differentiation observation: T-PB group (160x hpf)

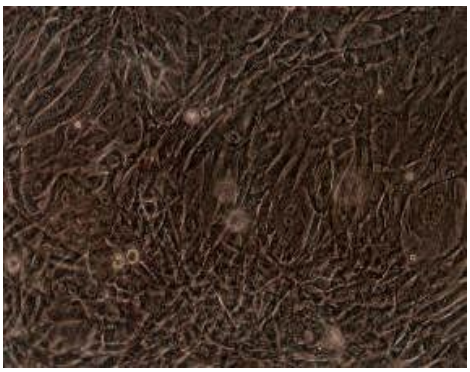
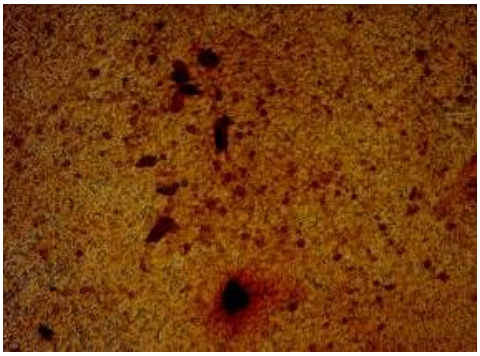
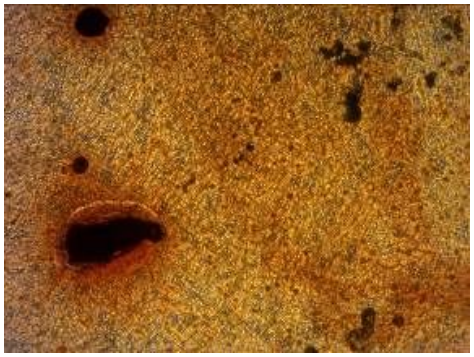


Figure 3. Osteoblast differentiation

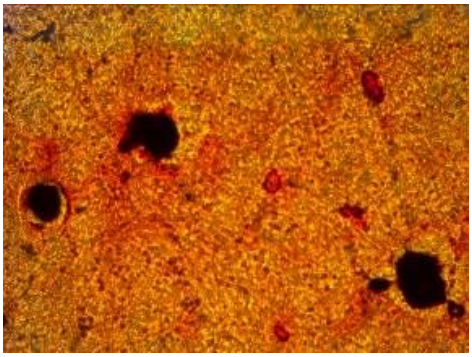
4 (a). Alizarin Red Staining :C-BM group (80x hpf)



4 (b). Alizarin Red Staining :C-PB group (80x hpf)



4 (c). Alizarin Red Staining :T-BM group (80x hpf)



4 (d). Alizarin Red Staining :T-PB group (80x hpf)

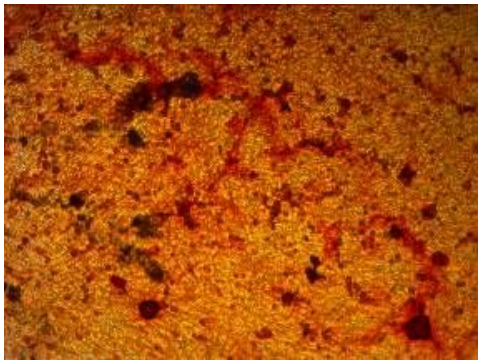


Figure 4. Alizarin Red Staining

Discussions

GCSF plays an important role in proliferation, maturation, survival, and activation of granulocytes and progenitor hematopoietic cells. GCSF is also an effective mobilizer of hematopoietic stem cells and has been widely used in marrow transplants.[17]

In this study, T-PB significantly showed the highest number in cells count when compared with other groups. We therefore conclude that GCSF may increase the number of mononuclear cells in the peripheral blood that later to be proven as MSCs.

The exact mechanism of the mobilization of MSCs to the peripheral blood during exposure of GCSF is still a mystery. This event might be due to the ability of the GSCF to stimulate the cells proliferation, hence will cause a shift of the peripheral blood cells.[18]This is the effect of a shift to left of the cells, to a more immature lineage. One of the examples of such cells is the MSCs.[19-24] Martinez et al, [25] studied in vitro models using a migration chamber system, and showed that the proportion of MSCs in marrow is reduced after GCSF were administered. The increase number of MSCs in the peripheral blood is also suspected due to the effect of the cytokines that stimulate the "shift" such as TGF-β (Transforming Growth Factor - β) , IL-1 (Interleukin – 1), TNF-α (Tumor Necrosis Factor - α), and SDF-1 (Stromal Cell-Derived Factor – 1), and GCSF. But the effects of GCSF are not elaborated in detail in this study. We can draw a conclusion that the direct evidence of the mobilization of the MSCs due to the cytokines is not complete in vivo.

Peripheral blood MSCs number will experience an increase that is thought to be attributed to the direct effect of the cell depletion from the marrow, or perhaps there are other factors from the progenitor cells in the peripheral blood cells, such as the Colony-Forming Unit Granulocyte-Macrophage (CFU-GM), and multiple

strain colonies such Colony Forming Unit-Granulocyte (CFU-G) and erythroid colonies such as Burst-Forming Unit-Erythroid (BFU-E) and primitive stem cell.[20]

Some studies found that MSCs mobilization might be the result of a quick hematopoiesis reconstitution process.[19,20]Zaucha et al,[20] have found that short term administration of GCSF can increase the general cellularity of stem cells significantly through erythropoiesis and myelopoiesis. This hypothesis is also supported by Chatta and Begley, et al[26,27] by stating that the increase in cellularity is due to the expansion of mitotic pool to accelerate transfer of mature cells to peripheral circulation. But these findings were refuted by Kovacs, et al[28] that stated an increase in marrow cellularity is also caused by leucopharesis that is caused by a compensation process, aphaeresis loss of the leucocytes and hematopoietic progenitor cells.

The ability of proliferation and differentiation of MSCs in this study was indicated by confluence time, confluence cell number, and differentiation time. The confluence time of peripheral blood treatment group was the shortest, followed by bone marrow control group. Similar result was also found in confluence cell count. It can be concluded that the MSCs proliferation ability of GCSF-induced peripheral blood is more potent.

Our study could not explain the effect of GCSF towards proliferation and differentiation of MSCs. It has been known that cytokine were able to increase and induce the cell migration and from hematopoietic stem cell. MSCs were able to express some chemokine receptors, including CXC Chemokine Receptor type 4 (CXCR4), CX3C Chemokine receptor1 (CX3CR1), CXC ligand 12 (CXCL12) and fractalkine. These chemokine are thought able to indirectly stimulate MSCs migration. GCSF administration within 24 hours were able to induced SDF-1 down regulation and CXCR4 up regulation expression.[29]

Hematopoietic stem cells which express CXCR4 and CX3CR1 were showed to have ability to proliferate and differentiate. After being mobilized, MSCs will turned into rapidly self-renewing MSC (RS-MSC) and have better in vivo engraftment ability and more potent in multi-lineage differentiation. [30]

This study also found that all aspirated cells were able to differentiate into osteoblast. Differentiation time on peripheral blood treatment group were the shortest, followed by treatment group of bone marrow, control group of bone marrow, and treatment group of peripheral blood. Therefore, it can be concluded that GCSF mobilized MSCs were able to differentiate into osteoblast, with good differentiation capability.

There are several theories on the direct and indirect GCSF effect on MSCs differentiation. Fukunaga et al and Dong et al[31,32] found that in vivo GCSF administration on acute myeloid leukemia patients acted indirectly on MSCs differentiation by sending transduction signals to differentiate on GCSF receptors. However, Tang et al[33] stated that the expression of GCSF receptor on bone marrow MSCs is very low, and concluded that indirect mechanism takes place.

We also found some limitations in our study in which only Alizarin Red staining is applied for osteoblast marker and GCSF dosage used is for human subject. The strength of our study was supported by the use of validated animal models, and it is the first and pilot study to evaluate mobilization, proliferation and differentiation of MSCs.

Conclusions

Peripheral blood MSCs areable to be isolated after GCSF induction with intact proliferation and differentiation capacity. GCSF administration increases peripheral MSCs number and shorten culture duration. Peripheral blood is a promising alternative donor site for MSCs.

Acknowledgements:

We thank Primate Research Centre, Bogor Agricultural Institute for facilitated this work and M.Sopiyudin Dahlan MD, for statistic consult. This research was supported by University of Indonesia, grant number 1084/SK/R/UI/2013 and 0095/SK/R/UI/2014.

References

1. Brighton CT, Shaman P, Heppenstall RB, Esterhai JL, Jr., Pollack SR, Friedenberg ZB. Tibial nonunion treated with direct current, capacitive coupling, or bone graft. ClinOrthopRelat Res. 1995;321:223-34.
2. Dahabreh Z, Dimitrou R, Giannoudis PV. Health economics: a cost analysis of treatment of persistent fracture non-unions using bone morphogenetic protein-7. Injury. 2007;38(3):371-7.
3. Giannoudis P, Psarakis S, Kontakis G. Can we accelerate fracture healing? A critical analysis of the literature. Injury. 2007;38Suppl 1:S81-9.
4. LeeHB, ChungYS, HeoSY, Kim NS. Augmentation of bone healing in non-union fracture using stem cell base dtissue engineeringinadog:acasereport.Veterinarni Med, 2009;54:198-203.
5. KonE,MuragliaA,CorsiA,BiancoP,MarcamLiM,MartinI,etal.Autologousbone marrow stromal cells loaded onto porous hydroxy apatite ceramic am Lelerate bone repair in critical-size defects of sheep long bones.JBiomedMaterRes.2000;49:328-37.
6. Arinzeh TL, Peter SJ, Archambault MP, van den Bos C, Gordon S, Kraus K, et al. Allogeneicmesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. J Bone J Surg (Am) 2003;85:1927-35
7. WexlerSA, Donaldson C, Denning-KendallP, RiceC, Bradley B, Hows JM. Adult bone marrow is a rich source of human mesenchymal'stem' cells butumbilicalcord and mobilized adult blood arenot. Br J Haematology 2003;121: 368–74.
8. KassisI, ZangiL, RivkinR, LevdanskyL, SamuelS, MarxG,etal. Isolation of mesenchymal stem cells from GCSF-mobilized human peripheral blood using fibrin microbeads. Bone marrow transplant 2006;37:967-76.
9. Hosing C. Hematopoietic stem cell mobilization with G-CSF. Methods Mol Biol 2012;904:37-47
10. Dreger P, Haferlach T, Eckstein V, Jacobs S, Suttorp M, Löffler H, et al. G-CSF-mobilized peripheral blood progenitor cells for allogenic transplantation: safety, kinetics of mobilization, composition of the graft. Br J Haematol 1994:87(3):609-13
11. Mielcarek M, Roecklein BA, Torok-Storb B. CD14+ Cells in Granulocyte Colony-Stimulating Factor (G-CSF)-Mobilized peripheral blood mononuclear cells induce secretion of interleukin-6 and G-CSF by marrow stroma. Blood 1996;87:574-580
12. Rauck AM, Ruymann FB, Klopfenstein K, Grossman N, Rosselet R, Grovas L, et al. Mobilization of periheral blood stem cells in children using G-CSF after carboplatin containing myelo suppressive chemotherapy. J Clin Apher 1998;13:146-54
13. Nafar M, Parvin M, Sadeghi P, Ghoraishian M, Soleimani M, Tabibi A, et al. Effects of stem cells and granulocyte colony stimulating factor in reperfusion injury. Iran J Kidney Dis. 2010;4(3):207-13
14. Kothari S, Abrahamsen MS, Cole T, Hammond WP. Expression of granulocyte colony stimulating factor (GCSF) and granulocyte/macrophage colony stimulating factor (GM-CSF) mRNA upon stimulation with phorbol ester. Blood Cells Mol Dis 1995;21(20):192-200
15. Delgaudine M, Lambermont B, Lancellotti P, Roelants V, Walrand S, Vanoverschelde JL, et al. Effects of granulocyte-colony-stimulating factor on progenitor cell mobilization and heart perfusion and function in normal mice. Cryotherapy 2011;13(2):237-47
16. Teofili L, Lovino MS, Di Mario A, Ortu La Barbera E, Pierelli L, Bussa S, et al. In vitro expansion of CD34+ cells mobilized with chemotherapy and G-CSF. Int J Artif Organs;1993;16(5):89-95.
17. Molineaux G, Foote MA, Arvedson T. Twenty Years of G-CSF: Clinical and Nonclinical Discoveries. New York: Springer; 2012. p. 15-25
18. Gyger M, Sahovic E, Aslam M. Does recombinant human granulocyte colony-stimulating factor really prime marrow stem cells in mice and humans? Blood. 1999:93:4448-4449
19. Tang FP, Wu XH, Yu XL, Yang SH, Xu WH, Jin Li. Effects of granulocyte colony-stimulating factor and stem cell factor, alone and in combination, on the biological behaviours of bone marrow mesenchymal stem cells. J Biomed Sci Eng. 2009;2:200-7
20. Zaucha JM, Postuszny WK, Bieniaszewska M, Mysliwski A, Hellman A. The Effect of Short G-CSF Administration on the Numbers and Clonogenic Efficiency of Hematopoetic Progenitor Cells in Bone Marrow and Peripheral Blood of Normal Donors. Ann Transplant. 2000;5(4):20-26
21. Pilarski LM, Pruski E, Wizniak J, Paine D, Sceberger K, Mant MJ, et al. Potential role for hyaluronan and the hyaluronan receptor RHAMM in mobilization and trafficking of hematopoietic progenitor cells. Blood. 1999;93:2918-27

22. Janowska WA, Maequez LA, Nabholtz JM, Cabuthat ML, Montano J, Chang H. Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. Blood. 1999;93:3379-90
23. Papayannopoulou T, Priestley GV, Nakamoto B. Anti-VLA4/VCAM-I-induced mobilization requires cooperative signaling through the kit/mkitligand pathway. Blood. 1998;91:2231-9
24. Prujit JF, Willemze R, Fibbe WE. Mechanism underlying hematopoietic stem cell mobilization induced by the CXC chemokine interleukin-8. CurrOpin Hematol. 1999:6:152-8
25. Martinez C, Urbano-Ispizua A, Rozman M, Rovira M, Marin P, Montfort N, et al. Effect of short-term administration of G-CSF (filgrastim) on bone marrow progenitor cells: analysis of serial marrow samples from normal donors. Bone Marrow Transplant. 1999;23:15-9
26. Begley CG, Nicola NA, Metcalf D. Proliferation of normal human promyelocytes and myelocytes after a single pulse stimulation by purified GM-CSF or G-CSF. Blood. 1988;71:640-5
27. Chatta GS, Price TH, Allen RC, Dale DC. Effects of in vivo recombinant methionyl human granulocyte colony-stimulating factor on the neutrophil response and peripheral blood colony-forming cells in healthy young and elderly adult volunteers. Blood. 1994;84:2923-2929
28. Kovacs P, Bruch C, Herbst EW, Fledner TM. Collection of in vitro colony-forming units from dogs by repeated continuous flow leukaphereses. Acta Haematol. 1978;60:172-181
29. Ripa RS, Haack-Sorensen M, Wang Y, Jorgensen E, Mortensen S, Bindslev L, et al. Bone Marrow-Derived Mesenchymal Cell Mobilization by Granulocyte-Colony Stimulating Factor After Acute Myocardial Infarction : Results From the Stem Cells in Myocardial Infarction (STEMMI) Trial. Circulation. 2007;116:I-24-I-30.
30. Pevsner-Fischer M, Zipori D. Regulatory Networks in Stem Cells: Environmental signals regulating mesenchymal progenitor cell growth and differentiation. New York: Humana Press; 2009. P.175-181
31. Fukunaga R, Ishizaka-Ikeda E, Seto Y, Nagata S. Expression cloning of a receptor for murine granulocyte colony-stimulating factor. Cell. 1990;61:341-50.
32. Dong F, van Paassen M, van Buitenen C, Joefsloot LH, Lowenberg B, Touw IP. A point mutation in the granulocyte colony-stimulating factor receptor (G-CSF-R) gene in a case of acute myeloid leukemia results in the over expression of a novel G-CSF-R isoform. Blood. 1995;85:902-11.
33. Tang FP, Wu XH, Yu XL, Yng SH, Xu WH, Li J. Effects of granulocyte colony-stimulating factor and stem cell factor, alone and in combination, on the biological behaviours of bone marrow mesenchymal stem cells. J Biomed Sci Eng. 2009;2:200-7

*****

*****