Chula Med J Vol. 64 No. 4 October - December 2020;367-374 DOI : 10.14456/clmj.2020.46

Original article

Comparison between therapeutic effects of low-dose simvastatin and mesenchymal stem cells (MSCs) transplantation on diabetic wound healing

Supakanda Sukpat[a], Nipan Israsena[b], Suthiluk Patumraj[c,*]

[a]*Program in Medical Science (Physiology), Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
[b]*Stem Cell and Cell Therapy Research Unit, Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
[c]*Center of Excellence for Microcirculation, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Background: Non-healing diabetic ulcers are the most common cause of amputation. Several studies have reported for the therapeutic potency of simvastatin and mesenchymal stem cells (MSCs) on improving angiogenic factors and wound healing.
Objectives: This study is aimed to evaluate and compare the treatment outcomes between low-dose simvastatin and MSCs transplantation in diabetic wound healing.
Methods: Balb/c nude mice were divided into four groups: control group (CON), diabetic wounded group (DM, streptozotocin 45 mg/kg intraperitoneal daily for 5 days), diabetic wounded group with daily oral treatment of simvastatin (DM+SIM) and diabetic wounded group with implanted MSCs (DM+MSCs). Seven days before wound creation, oral simvastatin was started in DM+SIM (0.25mg/kg/day). Eleven weeks after the diabetic induction, all mice were created bilateral full-thickness excisional skin wounds on the back and received fibrin gel or MSCs into wound bed. At day 7 and 14 post wounding, the percentage of wound closure (%WC), the percentage of capillary vascularity (%CV), tissue malondialdehyde (MDA) levels, stromal cell-derived factor 1 (SDF-1) levels, neutrophil infiltration and re-epithelialization were determined by using image analysis, confocal fluorescence microscopy, TBARs assay, immunohistochemically staining and hematoxylin and eosin staining, respectively. Interleukin 6 (IL-6) levels, tissue vascular endothelial growth factor (VEGF) levels, and pAkt levels were determined by using enzyme-linked immunosorbent assay.
Results: The %WC in DM+SIM and DM+MSCs groups were significantly higher when compared to the diabetic group. This study also showed that simvastatin and MSCs could increase %CV, VEGF, pAkt and SDF-1 level. Moreover, tissue MDA, IL-6 and neutrophil infiltration in DM+SIM and DM+MSCs groups were significantly decreased when compared to the diabetic group. Furthermore, the re-epithelialization of DM+MSCs group was significantly increased when compared to the diabetic group.
Conclusion: The results showed no significant difference between groups in all parameters on day 14 post-wound creation. Therefore, low-dose simvastatin might be used as an alternative treatment for diabetic wound healing.

Keywords: Diabetic wound, simvastatin, mesenchymal stem cells (MSCs).

The International Diabetes Federation (IDF) estimated the global prevalence of diabetes to be 415 million in 2015 and predicted to rise to 642 million in 2040. [1] In particular, long-term diabetes or poor control of hyperglycemia leads to the development of severe diabetic complications in multiple organs. The most common and serious complication of diabetes mellitus is diabetic foot ulcers. Prolonged inflammatory phase can in turn disturb collagen metabolism, resulting in poor blood supply, reduced production of growth factors, and impaired angiogenesis to contribute to

***Correspondence to: Suthiluk Patumraj,** Center of Excellence for Microcirculation, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
Email: suthilukp@yahoo.com
Received: July 19, 2019
Revised: August 5, 2019
Accepted: August 16, 2019

the frequent skin lesions and poor wound healing in diabetic patients. Non-healing ulcers were the most common cause of 85.0% of amputations among diabetic patients. [2, 3]

Mesenchymal stem cells (MSCs) are non-hematopoietic stem cells with high-proliferative potency and able to differentiate into multiple lineages such as osteoblasts, chondrocytes, adipocytes, fibroblasts, endothelial cells and smooth muscle, etc. MSCs can be easily isolated from many sources such as bone marrow, adipose tissue, and umbilical cord and can be expanded in vitro.[4] However, the consequences of hyperglycemia-induced oxidative stress impair the number and functional activities of endogenous MSCs.[5] Numerous studies demonstrated that MSCs transplantation could increase angiogenic factors and stem/progenitor cell chemokines, including vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1 (SDF-1), enhance re-epithelialization and increase angiogenesis and improve wound healing. [6 - 9] Moreover, MSCs treatment are able to modulate the immune response and decrease inflammation through decreasing proinflammatory cytokines such as interleukin-1 (IL-1), and interleukin-6 (IL-6) and promoting anti-inflammatory factors. [10]

Statins are primarily used to lower circulating cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA). Besides, the mechanism of lipid lowering effect, statins also have pleiotropic effects which demonstrated the clinical benefits particular for cardiovascular system. [11, 12] The American College of Cardiology/ American Heart Association (ACC/AHA) blood cholesterol guideline in 2013 demonstrated that statin therapy reduced atherosclerotic cardiovascular risk which is recommended for individuals at increased atherosclerotic cardiovascular risk.[13] Simvastatin which is the most common derivative of statins has been reported for its pleiotropic effects on accelerated re-epithelialization, enhanced VEGF production, increased angiogenesis, and improved wound healing in diabetic and non-diabetic models.[14, 15]

However, there are no comparative studies between the effects of low-dose simvastatin and MSCs transplantation in diabetic wound healing. Therefore, the objective of this study was to compare the effects of low-dose simvastatin and MSCs transplantation in diabetic wound mouse model.

Materials and methods

Animals

Male BALB/c nude mice (7 - 8 weeks, 20 - 25 g) from the National Laboratory Animal Center, Salaya Campus, Mahidol University, Thailand were used in this study. The mice were divided into four groups as follows: control group (CON; n = 12); diabetic group (DM; n = 12); diabetic group received a daily oral treatment of simvastatin (0.25 mg/kg) (DM+SIM; n = 12); and diabetic group received an implantation of $1x10^6$ MSCs (DM+MSCs; n = 12). All procedures were conducted in accordance with guidelines for the use of experimental animals by the National Research Council of Thailand. This study has been approved by Ethics Committee, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (No. 009/ 2558).

Mesenchymal stem cells (MSCs) preparation and culture

Human mesenchymal stem cells were cultured and maintained by the Stem Cell and Cell Therapy Research Unit, Chulalongkorn University. Briefly, human MSCs were isolated from human bone marrow and were cultured in medium (5%FBS/ DMEM/1x pen-strep/L-glutamin) at 37°C 5% CO^2. The phenotypes of MSCs were positive for CD44, CD73, CD90 and CD105.

Diabetic induction and wounding protocols

In the diabetic groups, mice were induced by injection of streptozotocin (Sigma Chemical Co, USA.) in citrate buffer pH 4.5 (Sigma Chemical Co, USA.) with the dose of 45 mg/kg intraperitoneal injection daily for 5 days. The same volume of citrate buffer was injected by the same route to non-diabetic control animal. Two weeks of diabetic induction, tail-vein blood glucose levels were measured by glucometer (Advance Glucometer, Bochringer Mannheim, Germany). The inclusion criteria for diabetes is that the fasting plasma glucose concentration equals to or greater than 200 mg/dL. [16]

The wounding protocol was modified from Sivan-Loukianova E, *et al*. and Wu Y, *et al.* [6, 17] Eleven weeks of diabetic induction, the mice were anesthetized (sodium pentobarbital 55 mg/kg, i.p.) and swabbed with alcohol on dorsal-rostral back. Bilateral full-thickness excisional skin wounds (0.6 x 0.6 cm^2) were created on both left and right sides of the midline (Figure 1). A square-shaped plastic splints were secured around the perimeter of the wound to limit wound contracture. Each mouse was received fibrin gel (Shanghai RAAS blood products Co. Ltd, China) or MSCs in fibrin gel on wound bed. Then the wounds were covered by Tegaderm™ (3M Company, St. Paul, MN, USA). [6, 17] Low dose of simvastatin supplements (0.25 mg/kg BW) were given daily before wound creation for 7 days and continued to the end of the study. [18 - 20]

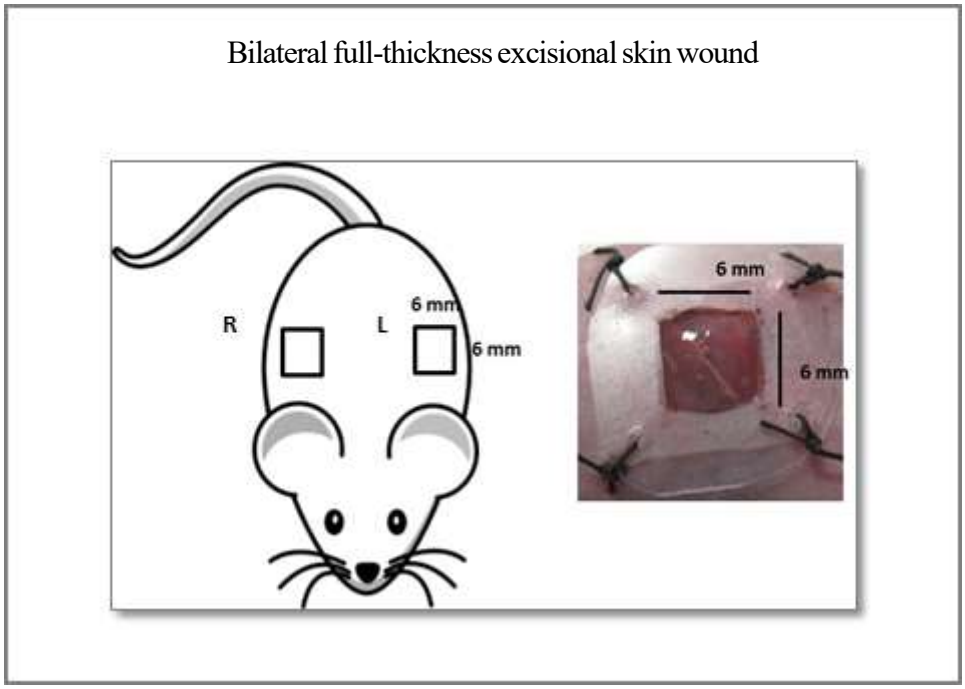

Figure 1. Bilateral full-thickness excisional skin wounds (0.6 x 0.6 cm^2) were created on both left and right sides of the midline. A square-shaped plastic splints were secured around the perimeter of the wound to limit wound contracture. The wounds were covered by Tegaderm™. [6, 17]

Wound closure (WC) measurement

This study was observed at day 7 (n = 6 in each group) and day 14 (n = 6 in each group) after wound creation. Briefly, mice were anesthetized with an intraperitoneal injection of sodium pentobarbital at a dose of 55 mg/kg. Digital photographs of wounds were taken at days 0, 7 and 14 by a digital camera (Nikon DS-L2, Japan). Areas of the wound were measured by tracing the wound margin and calculated using digital image software analysis (Image-Pro Plus II 6.1; Media Cybernetics, Bethesda, MD), and the percentage of wound closure (%WC) was evaluated using the following equation: (area of original wound area of actual wound)/area of original wound × 100.[6]

Capillary vascularity (CV) measurement

The right jugular vein of anesthetizing mouse was cannulated for injection of 0.1 ml of 5% FITC-labeled dextran. The capillary vascularity was examined using confocal fluorescence microscopy at 100x magnification (Nikon eclipse E800, Nikon, Japan). From the fluorescent photographs, the capillary diameter less than 15 μm were analyzed using Image Pro II 6.1 software. The percentage of capillary vascularity was calculated using the following equation: (number of pixels within capillaries/total number of pixels of the entire frame) × 100.[21]

Histologic examination

Mice were sacrificed at 7 and 14 days after wound creation, at which times, the left side wound sample was harvested and stored at −80°C for the measurements of tissue IL-6, tissue VEGF, tissue pAkt and tissue MDA levels. The right side wound sample was collected for the measurements of neutrophil infiltration, re-epithelialization and SDF-1 expression. Tissue specimens were fixed in 10% formaldehyde for 24 hours. Two-micrometer-thick sections were stained with hematoxylin-eosin (H&E). The H&E stained samples were used to measure the number of neutrophils infiltrating in the wound areas and re-epithelialization using light microscopy (Nikon eclipse 50i, Nikon, Japan) at 400x magnification and stereotype microscopy (Nikon SM2800, Nikon, Japan) and digital sight for microscope (Nikon DS-L2, Nikon, Japan) at 10x magnification, respectively. These results were analyzed using image Pro Plus 6.1 software and confirmed by blind assessment.[21] The percentage of re-epithelialization calculated with the following formula: (distance covered by epithelium/distance of the wound edges) x 100. [21]

As for immunohistochemistry, 2-micrometer-thick paraffin-embedded sections were cut from the paraffin-embedded tissue. Heat-induced antigen retrieval was performed in antigen retrieval solution

(pH 9, Dako, USA). Primary rabbit polyclonal SDF-1 antibody (sc-28876, Santa Cruz, USA, at 1:25 dilution) was added for incubation 1 hour at room temperature. The sections were washed with wash buffer and incubated with anti-rabbit secondary antibody (Dako, USA) for 30 min at room temperature and added with the DAB+ Substrate Chromogen System (Dako, USA) at room temperature. The sections are photographed at 400x magnification using a light microscope. Then, the images were analyzed using image software (Image-Pro Plus II 6.1). Results were confirmed by blind assessment.

VEGF, IL-6 and serine/threonine-specific protein kinase (Akt) activation immunoassay

At 7 and 14 days after wound creation, tissues from the skin wound area was harvested and stored at -80°C for analysis of VEGF, interleukin 6 (IL-6) levels and pAkt using an enzyme-linked immunosorbent assay (murine VEGF-specific ELISA kit (R&D Systems, USA), IL-6 ELISA (R&D Systems, USA) and phospho-Akt (S473) Pan Specific DuoSet IC ELISA (R&D Systems, USA) kits, based on the manufacturer's protocols. [22, 23] The amount of VEGF, IL-6 and pAkt were expressed as picograms per milligram protein unit.

Malondialdehyde (MDA) measurement

The supernatants of each tissue sample were used to analyze malondialdehyde levels (MDA; lipid peroxides parameter) by a TBARS assay kit (Cayman Chemical Co, USA). MDA was expressed as nM/mg protein unit.

Statistical analysis

The data were expressed as the means ± standard errors of mean (SEM). The differences between groups were determined by one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test using SPSS software, version 21. The comparison between groups was done by using Student's *t* - test. Differences were statistically significant when *P* - value was less than 0.05.

Results

As shown in Table 1 and Table 2, the effects of the two treatments, low-dose simvastatin and MSCs transplantation, on day 7 and 14 after wound creation, were reported and compared to those values of the DM groups. It could be summarized that in diabetic wounds, the prolonged chronic inflammatory state as represented by the levels of the extent of neutrophil infiltration and increased IL-6 were significantly different than levels of control groups. This pro-inflammatory environment caused a chronic inflammatory state with poor proliferation. Our data showed the reductions of growth factor, tissue VEGF, and SDF-1 levels were significant in the DM group than in the control group. Under these lacks of angiogenic factors and chemokines expression, the percentage of capillary vascularity (%CV) were significantly decreased in DM groups as shown in both periods of day 7 and day 14 after wound creation. In addition on day 7 and 14 post-wound creation, the percentage of wound closure (%WC) were significantly less in the DM group than in the control groups ($P < 0.05$).

Table 1. Means ± SEM of tissue malondialdehyde (MDA, nM/mg protein unit), percentage of capillary vascularity (%CV), percentage of wound closure (%WC), vascular endothelial growth factor (VEGF, picograms per milligram protein unit), percentage of SDF-1 protein expression in wound (SDF-1), phospho-Akt (pAkt, picograms per milligram protein unit), and interleukin 6 (IL-6, picograms per milligram protein unit), percentage of neutrophil infiltration, and percentage of re-epithelialization were shown for each 7-day group.

	7 days post-wounding			
	CON	**DM**	**DM + MSCs**	**DM + SIM**
MDA	3.58 ± 0.83 (n = 4)	14.88 ± 6.65 (n = 5)**	3.56 ± 0.53 (n = 5)$^{\#}$	6.01 ± 2.52 (n = 4)$^{\#}$
%CV	73.17 ± 4.21 (n = 4)	49.12 ± 4.43 (n = 3)**	68.31 ± 6.33 (n = 3)$^{\#}$	65.16 ± 5.09 (n = 3)
%WC	63.83 ± 6.36 (n = 5)	40.84 ± 2.54 (n = 6)*	71.27 ± 4.49 (n = 5)$^{\#\#}$	58.90 ± 8.95 (n = 5)$^{\#}$
VEGF	199.59 ± 34.71 (n = 6)	54.07 ± 12.99 (n = 5)**	150.29 ± 25.61 (n = 6)$^{\#}$	133.24 ± 13.22 (n = 6)$^{\#}$
SDF-1	43.89 ± 2.05 (n = 6)	28.12 ± 2.38 (n = 5)**	39.98 ± 1.61 (n = 6)$^{\#\#}$	38.05 ± 2.09 (n = 6)$^{\#\#}$
pAkt	458.44 ± 67.06 (n = 6)	137.36 ± 24.17 (n = 5)**	383.70 ± 20.61 (n = 6)$^{\#}$	429.82 ± 108.04 (n = 6)$^{\#\#}$
IL-6	38.47 ± 8.32 (n = 5)	91.70 ± 19.81 (n = 5)	70.95 ± 22.35 (n = 6)	75.30 ± 15.25 (n = 6)
Neutrophil infiltration	22.17 ± 2.77 (n = 6)	51.83 ± 7.87 (n = 6)*	26.40 ± 10.59 (n = 5)$^{\#}$	31.00 ± 7.32 (n = 6)$^{\#}$
Re-epithelialization	89.03 ± 6.78 (n = 4)	41.41 ± 6.24 (n = 5)**	78.73 ± 7.89 (n = 4)$^{\#}$	68.61 ± 18.56 (n = 4)

*$P < 0.05$ significant difference vs CON, **$P < 0.01$ significant difference vs CON
# $P < 0.05$ significant difference vs DM, ## $P < 0.01$ significant difference vs DM

Table 2. Means ± SEM of tissue malondialdehyde (MDA, nanograms per milligram protein unit), percentage of capillary vascularity (%CV), percentage of wound closure (%WC), vascular endothelial growth factor (VEGF, picograms per milligram protein unit), percentage of SDF-1 protein expression in wound (SDF-1), phospho-Akt (pAkt, picograms per milligram protein unit), and interleukin 6 (IL-6, picograms per milligram protein unit), percentage of neutrophil infiltration, and percentage of re-epithelialization were shown for each 14-day group.

	14 days post-wounding			
	CON	**DM**	**DM + MSCs**	**DM + SIM**
MDA	6.98 ± 0.91 (n = 5)	37.11 ± 19.86 (n = 5)**	10.07 ± 4.32 (n = 5)$^{\#}$	13.09 ± 7.35 (n = 4)$^{\#}$
%CV	32.81 ± 2.13 (n = 3)	21.94 ± 2.70 (n = 3)*	39.88 ± 4.61 (n = 3)$^{\#\#}$	38.17 ± 1.61 (n = 3)$^{\#\#}$
%WC	97.98 ± 1.39 (n = 5)	81.15 ± 4.28 (n = 4)*	91.23 ± 2.91 (n = 6)$^{\#}$	96.42 ± 2.22 (n = 6)$^{\#\#}$
VEGF	45.80 ± 15.71 (n = 6)	30.43 ± 4.26 (n = 5)	31.01 ± 4.23 (n = 5)	44.29 ± 7.43 (n = 6)
SDF-1	29.74 ± 2.66 (n = 5)	20.86 ± 2.54 (n = 5)*	32.25 ± 1.90 (n = 6)$^{\#\#}$	33.45 ± 2.57 (n = 6)$^{\#\#}$
pAkt	220.81 ± 48.82 (n = 4)	52.99 ± 21.71 (n = 4)	237.22 ± 85.16 (n = 5)	204.88 ± 137.09 (n = 5)
IL-6	28.34 ± 6.28 (n = 6)	76.34 ± 25.02 (n = 6)*	29.24 ± 7.29 (n = 6)$^{\#}$	24.04 ± 5.32 (n = 6)$^{\#\#}$
Neutrophil infiltration	7.80 ± 0.80 (n = 5)	23.60 ± 4.00 (n = 5)**	10.00 ± 3.10 (n = 5)$^{\#\#}$	9.60 ± 2.27 (n = 5)$^{\#}$
Re-epithelialization	100.00 ± 0.00 (n = 5)	95.46 ± 4.54 (n = 5)	100.00 ± 0.00 (n = 4)	100.00 ± 0.00 (n = 6)

*P < 0.05 significant difference vs CON, **P < 0.01 significant difference vs CON
# P < 0.05 significant difference vs DM, ## P < 0.01 significant difference vs DM

In Table 3, the comparison between groups of DM+SIM, and DM+MSCs were performed by using Student's *t* - test. The results showed no significant difference between groups in all parameters on day 14 post-wound creation. However, on days 7, the percentages of re-epithelialization and %WC in the DM+MSCs groups were significantly higher than those of the DM+SIM group.

Table 3. Students' *t test* of means ± SEM of tissue malondialdehyde (MDA), percentage of capillary vascularity (%CV), percentage of wound closure (%WC), vascular endothelial growth factor (VEGF), percentage of SDF-1 protein expression in wound (SDF-1), phospho-Akt (pAkt), and interleukin 6 (IL-6), neutrophil infiltration, and re-epithelialization were performed between two groups of 7 and 14 day DM+MSCs and 7 and 14 day DM + SIM groups.

	7 days post-wounding			14 days post-wounding		
	MSCs	**SIM**	***P* - value**	**MSCs**	**SIM**	***P* - value**
MDA	↓	↓	NS	↓	↓	NS
%CV	↑	—	NS	↑	↑	NS
%WC	↑	↑	0.005	↑	↑	NS
VEGF	↑	↑	NS	—	—	NS
SDF-1	↑	↑	NS	↑	↑	NS
pAkt	↑	↑	NS	—	—	NS
IL-6	—	—	NS	↓	↓	NS
Neutrophil infiltration	↓	—	NS	↓	↓	NS
Re-epithelialization	↑	—	0.006	—	—	NS

Discussion

Hyperglycemia induced highly oxidative microenvironment, resulting in the impairment of various cell types, including MSCs that typically required for wound healing process. Diabetic wounds are characterized by prolonged chronic inflammatory state, impaired cellular defense mechanisms and induced prolonged elevation of pro-inflammatory mediators including TNF-α, IL-6 and IL-1. This pro-inflammatory environment induces a low-grade inflammation, leading to a chronic inflammatory state. [24, 25] Moreover, reduction of growth factors, angiogenesis and abnormal blood flow cause prolonged and incomplete diabetic wound healing. [26] In this study we found that the pleiotropic effect of low dose simvastatins could improve wound healing in diabetic mice compatible to MSCs transplanation treatment. The results of wound closure (%WC) and capillary vascularity (%CV) were no significant difference between the groups of DM+SIM, and DM+MSCs after 14 days after wound creation.

In our previous study, by using daily oral feeding of simvastatin (0.25 mg/kg BW/day) in diabetic mouse model, the pleiotropic effects of simvastatin; including reducing neutrophil infiltration, enhancing VEGF production, increasing angiogenesis, and improving wound healing were demonstrated. [27] A number of studies also support this findings, particularly the increased circulating endothelial progenitor cells (EPCs), increased angiogenesis, and improved wound healing were demonstrated in both diabetic and non-diabetic models. [15, 28 - 31]

Considering these previous findings, we hypothesized that the anti-oxidative stress and anti-inflammation effects of low-dose simvastatin supplementation could improve diabetic wound healing comparable to MSCs transplantation. By comparing the groups of DM+SIM, and DM+MSCs, the results showed that on day 7; DM+MSCs could significantly increase re-epithelialization in diabetic wound compared to DM+SIM. Whereas, all other measured parameters including MDA, %CV, VEGF, SDF-1, pAkt, IL-6, and neutrophil infiltration, showed no significant differrence. A number of research findings indicated that MSCs could promote all wound healing processes simultaneously and described by its immunomodulatory and anti-inflammatory effects, promoting growth factor secretion and stimulating proliferation and differentiation for regeneration of injury tissues. [32] Whereas the pleiotropic effects of simvastatin in diabetic wound healing were mostly referred to enhancing VEGF production, increased angiogenesis and reducing oxidative stress. [15, 28]

In case of anti-oxidative agent, the previous study showed that simvastatin could act as a radical scavenger and decrease the generation of ROS. [33] By using the model of vascular injury, simvastatin was shown to increase VEGF and SDF-1 secretion and lead to promote the regeneration of endothelial cells via the activation of the Akt-mediated signaling pathway. [30, 34 - 35] SDF-1 is a chemotactic factor regulating the migration of stem cells. However, the decreased EPCs and MSCs recruitments and functions have been implicated in the diabetic wound healing impairment.[36]

Moreover, simvastatin could reduce neutrophil infiltration in skeletal muscle reperfusion injury model and wound model. The stabilizing effect of simvastatin upon eNOS could enhance NO production during the early phase of reperfusion.[37, 38] And this might be implied to our finding that simvastatin could also decrease the number of neutrophils infiltrating diabetic skin wound.

IL-6 plays roles in recruitment of leukocytes by stimulating IL-8 and monocyte chemoattractant protein-1 (MCP-1) secretion from endothelial cells. [39] The study of Rezaie-Majd demonstrated that simvastatin had anti-inflammatory effects through the down regulation and production of proinflammatory cytokines including IL-6 and IL-8 in the endothelium and leukocytes. [40]

The results of this study demonstrated the benefit effects of pleiotropic mechanism of low dose simvastatin that could improve wound healing in diabetic mice similar to the effects of MSCs transplanation. Therefore, in the future it might be helpful to improve the clinical benefit and preventing diabetes amputation. However, this finding still need further clinical research to determine the proper dose of simvastatin for diabetic patients.

These findings support the idea that pleiotropic effects of low dose simvastatin could improve wound healing in diabetic mice similar to the benefit effects of MSCs transplanation. Their similar processes are partly mediated by the reductions of oxidative stress, IL-6, and neutrophil infiltration, and then result to the next phase of enhancing proliferations of both vascular structure and epithelial cells in diabetic wound.

Conclusion

These results may be the first *in vivo* evidence that shows the comparative effects of simvastatin and MSCs on the enhancement of angiogenesis and wound closure in diabetic skin wound model.

Acknowledgements

This study was supported by the Ratchadapisek-sompotch Fund of the Faculty of Medicine, the 90th Anniversary Research Fund, the Graduate School, Chulalongkorn University, Thailand.

Conflict of interest

The authors, hereby, declare no conflict of interest.

References

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract 2017;128:40-50.
2. Schreiber J, Efron PA, Park JE, Moldawer LL, Barbul A. Adenoviral gene transfer of an NF-kappaB super-repressor increases collagen deposition in rodent cutaneous wound healing. Surgery 2005;138:940-6.
3. Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. Diabetes Care 1990;13:513-21.
4. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: Revisiting history, concepts, and assays. Cell Stem Cell 2008;2:313-9.
5. Yan J, Tie G, Wang S, Messina KE, DiDato S, Guo S, et al. Type 2 diabetes restricts multipotency of mesenchymal stem cells and impairs their capacity to augment postischemic neovascularization in db/db mice. J Am Heart Assoc 2012;1:e002238.
6. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 2007;25: 2648-59.
7. Al-Khaldi A, Al-Sabti H, Galipeau J, Lachapelle K. Therapeutic angiogenesis using autologous bone marrow stromal cells: Improved blood flow in a chronic limb ischemia model. Ann Thorac Surg 2003;75:204-9.
8. Tang YL, Zhao Q, Zhang YC, Cheng L, Liu M, Shi J, et al. Autologous mesenchymal stem cell transplan tation induce VEGF and neovascularization in ischemic myocardium. Regul Pept 2004;117:3-10.
9. Liang X, Su YP, Kong PY, Zeng DF, Chen XH, Peng XG, et al. Human bone marrow mesenchymal stem cells expressing SDF-1 promote hematopoietic stem cell function of human mobilised peripheral blood CD34+ cells in vivo and in vitro. Int J Radiat Biol 2010; 86:230-7.
10. Prockop DJ, Oh JY. Mesenchymal stem/stromal cells (mscs): Role as guardians of inflammation. Mol Ther 2012;20:14-20.
11. Kavalipati N, Shah J, Ramakrishan A, Vasnawala H. Pleiotropic effects of statins. Indian J Endocrinol Metab 2015;19:554-62.
12. Bonetti PO, Lerman LO, Napoli C, Lerman A. Statin effects beyond lipid lowering—are they clinically relevant? Eur Heart J 2003;24:225-48.
13. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 acc/aha guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: A report of the american college of cardiology/american heart association task force on practice guidelines. Circulation 2014;129:S1-45.
14. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, et al. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. Nat Med 2000;6:1004-10.
15. Bitto A, Minutoli L, Altavilla D, Polito F, Fiumara T, Marini H, et al. Simvastatin enhances VEGF production and ameliorates impaired wound healing in experimental diabetes. Pharmacol Res 2008;57:159-69.
16. Zhou B, Cao XC, Fang ZH, Zheng CL, Han ZB, Ren H, et al. Prevention of diabetic microangiopathy by prophylactic transplant of mobilized peripheral blood mononuclear cells. Acta Pharmacol Sin 2007;28:89-97.
17. Sivan-Loukianova E, Awad OA, Stepanovic V, Bickenbach J, Schatteman GC. Cd34+ blood cells accelerate vascularization and healing of diabetic mouse skin wounds. J Vasc Res 2003;40:368-77.
18. Yang YJ, Qian HY, Huang J, Li JJ, Gao RL, Dou KF, et al. Combined therapy with simvastatin and bone marrow-derived mesenchymal stem cells increases benefits in infarcted swine hearts. Arterioscler Thromb Vasc Biol 2009;29:2076-82.
19. Greer JJ, Kakkar AK, Elrod JW, Watson LJ, Jones SP, Lefer DJ. Low-dose simvastatin improves survival and ventricular function via enos in congestive heart failure. Am J Physiol Heart Circ Physiol 2006;291: H2743-51.
20. Marketou ME, Zacharis EA, Nikitovic D, Ganotakis ES, Parthenakis FI, Maliaraki N, et al. Early effects of simvastatin versus atorvastatin on oxidative stress

and proinflammatory cytokines in hyperlipidemic subjects. Angiology 2006;57:211-8.
21. Somchaichana J, Bunaprasert T, Patumraj S. Acanthus ebracteatus vahl. Ethanol extract enhancement of the efficacy of the collagen scaffold in wound closure: A study in a full-thickness-wound mouse model. J Biomed Biotechnol 2012;2012:754527.
22. Badr G, Badr BM, Mahmoud MH, Mohany M, Rabah DM, Garraud O. Treatment of diabetic mice with undenatured whey protein accelerates the wound healing process by enhancing the expression of MIP-1α, MIP-2, KC, CX3CL1 and TGF-β in wounded tissue. BMC Immunol 2012;13:32.
23. Yeh CC, Li H, Malhotra D, Turcato S, Nicholas S, Tu R, et al. Distinctive ERK and p38 signaling in remote and infarcted myocardium during post-MI remodeling in the mouse. J Cell Biochem 2010;109: 1185-91.
24. Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH. Diabetic nephropathy is associated with low-grade inflammation in type 1 diabetic patients. Diabetologia 2003;46:1402-7.
25. Garcia C, Feve B, Ferre P, Halimi S, Baizri H, Bordier L, et al. Diabetes and inflammation: Fundamental aspects and clinical implications. Diabetes Metab 2010;36:327-38.
26. Falanga V. Wound healing and its impairment in the diabetic foot. Lancet 2005;366:1736-43.
27. Sukpat S, Israsena N, Patumraj S. Pleiotropic effects of simvastatin on wound healing in diabetic mice. J Med Assoc Thai 2016;99:213-9.
28. Rego AC, Araujo Filho I, Damasceno BP, Egito ES, Silveira IA, Brandao-Neto J, et al. Simvastatin improves the healing of infected skin wounds of rats. Acta Cir Bras 2007;22 Suppl 1:57-63.
29. Asai J, Takenaka H, Hirakawa S, Sakabe J, Hagura A, Kishimoto S, et al. Topical simvastatin accelerates wound healing in diabetes by enhancing angiogenesis and lymphangiogenesis. Am J Pathol 2012;181: 2217-24.
30. Matsuno H, Takei M, Hayashi H, Nakajima K, Ishisaki A, Kozawa O. Simvastatin enhances the regeneration of endothelial cells via VEGF secretion in injured arteries. J Cardiovasc Pharmacol 2004;43: 333-40.
31. Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T, et al. Statin therapy accelerates reendothelialization: A novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation 2002;105: 3017-24.
32. Volarevic V, Arsenijevic N, Lukic ML, Stojkovic M. Concise review: Mesenchymal stem cell treatment of the complications of diabetes mellitus. Stem Cells 2011;29:5-10.
33. Parizadeh SM, Azarpazhooh MR, Moohebati M, Nematy M, Ghayour-Mobarhan M, Tavallaie S, et al. Simvastatin therapy reduces prooxidant-antioxidant balance: Results of a placebo-controlled cross-over trial. Lipids 2011;46:333-40.
34. Yang D, Han Y, Zhang J, Chopp M, Seyfried DM. Statins enhance expression of growth factors and activate the PI3K/Akt -mediated signaling pathway after experimental intracerebral hemorrhage. World J Neurosci 2012;2:74-80.
35. Cui X, Chopp M, Zacharek A, Roberts C, Lu M, Savant-Bhonsale S, et al. Chemokine, vascular and therapeutic effects of combination simvastatin and BMSC treatment of stroke. Neurobiol Dis 2009;36: 35-41.
36. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, et al. Endothelial progenitor cell dysfunction: A novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes 2004;53:195-9.
37. Cowled PA, Khanna A, Laws PE, Field JB, Varelias A, Fitridge RA. Statins inhibit neutrophil infiltration in skeletal muscle reperfusion injury. J Surg Res 2007;141: 267-76.
38. Karadeniz Cakmak G, Irkorucu O, Ucan BH, Emre AU, Bahadir B, Demirtas C, et al. Simvastatin improves wound strength after intestinal anastomosis in the rat. J Gastrointest Surg 2009;13:1707-16.
39. Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, et al. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity 1997;6:315-25.
40. Rezaie-Majd A, Maca T, Bucek RA, Valent P, Muller MR, Husslein P, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2002;22:1194-9.