Pleiotropic Effects of Simvastatin on Wound Healing in Diabetic Mice

Supakanda Sukpat MSc*,
Nipan Israsena MD, PhD**, Suthiluk Patumraj PhD***

** PhD Program in Medical Science, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
*** Stem Cell and Cell Therapy Research Unit, Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
**** Center of Excellence for Microcirculation, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Objective: *To evaluate the effects of pre-treatment with low-dose simvastatin on angiogenesis and wound healing in a diabetic mouse model.*
Material and methods: *Balb/c nude mice were divided into three groups, including control (CON), diabetic (DM), and diabetic pre-treated with low-dose simvastatin (DM + SIM). Seven days prior to wounding, the DM + SIM group was started on oral simvastatin (0.25 mg/kg/day). Eleven weeks after diabetes was induced, all mice were subjected to a bilateral full-thickness excisional skin wound on the back (0.6x0.6 cm^2). On day 14 after wounding, percentage of wound closure (%WC), percentage of capillary vascularity (%CV), and neutrophil infiltration were determined using Image Pro-Plus, confocal fluorescence microscopy, and hematoxylin and eosin (H&E) staining, respectively. Tissue vascular endothelial growth factor (VEGF) was detected by ELISA at days 7 and 14, post-wounding.*
Results: *On day 14, %WC and %CV in CON and DM + SIM groups were significantly increased, with no significant change observed in the DM group. Neutrophil infiltration in the CON and DM + SIM groups was significantly lower than that of the DM group. VEGF levels in the CON and DM+SIM groups were significantly higher than levels in the DM group on day 7, but not different among groups on day 14.*
Conclusion: *The present study demonstrated that pre-treatment with low-dose simvastatin could increase angiogenesis, reduce inflammation, and improve wound healing in diabetic mice.*

Keywords: *Capillary vascularity, Diabetic wound, Simvastatin, Wound closure*

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Diabetes mellitus is a common metabolic disease with a high and growing prevalence. According to the International Diabetes Federation (IDF), 285 million adults had diabetes mellitus (DM) in 2010, with that number expected to rise to 439 million adults in 2030[1]. Non-healing diabetic wounds are the most common cause of amputation[2].

Hyperglycemia leads to an increase in superoxide anions and reactive oxygen species (ROS), which results in a number of cellular and/or humoral deleterious effects on immune defense mechanisms. In addition, hyperglycemia causes reduced chemotactic ability to recruit inflammatory cells into injured tissue, which leads to prolonged inflammation and reduced production of growth factors. Dysfunction of endothelial progenitor cells (EPCs) and endothelial cells delay the angiogenic process. Abnormal granulation tissue and collagen formation causes failure in fibroblast, collagen synthesis. As such, there are many factors that contribute to poor and prolonged wound healing in diabetic patients[2-5].

Statins are used primarily to lower circulating cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA). Recent studies have demonstrated that statins also have non-lipid-lowering or pleiotropic effects. These pleiotropic effects have been found to include anti-oxidant effects, anti-inflammatory effects, protection of endothelial function, increased nitric oxide bioavailability, and reduction of atherosclerotic plaques[6-8]. However, the dose-response relationship between statins and their pleiotropic effects is still being debated and requires further clarification.

Correspondence to:
Patumraj S, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
Phone: +66-2-2564454, Fax: +66-2-2522702 ext. 14
E-mail: suthilukp@yahoo.com

Simvastatin, one of the most common lipophilic statins, has been demonstrated to accelerate re-endothelialization, enhance vascular endothelial growth factor (VEGF) production, increase angiogenesis, increase the number of EPCs, reduce inflammation, and improve wound healing[9-12]. However and based on our review of the literature, no previous studies have investigated the effects of pre-treatment with low-dose simvastatin on wound healing in patients with diabetes mellitus. Accordingly, the objective of this study was to evaluate the effects of pre-treatment with low-dose simvastatin on angiogenesis and wound healing in a diabetic mouse model.

Material and Method

Animal preparation

Male BALB/c nude mice weighing 20-25 g were procured from the National Laboratory Animal Center, Salaya Campus, Mahidol University, Thailand. All experiments were conducted in accordance with guidelines dictating the use of experimental animals as set forth by The National Research Council of Thailand. The protocol for this study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Animals were housed one animal per cage in a 25±3°C pathogen-limited room on a 12-hour light and 12-hour dark schedule. Animals were given free access to sterilized water and standard laboratory feed.

Mice were divided into three groups, as follows: Group 1-control group (CON; n = 12); Group 2-diabetic group (DM; n = 12); and, Group 3-diabetic group pre-treated with daily oral simvastatin (0.25 mg/kg) (DM + SIM; n = 12).

Induction of diabetes mellitus

Diabetes was induced by intraperitoneal injection of streptozotocin 45 mg/kg daily for 5 days (STZ; Sigma-Aldrich Corp., St. Louis, MO, USA). The streptozotocin was dissolved in citrate buffer (pH 4.5). The same volume of citrate buffer (pH 4.5) was injected by the same route in non-diabetic control animals. Two weeks after streptozotocin injection, a glucometer was used to measure plasma glucose level of a blood sample taken from the tail vein. Diabetic condition was defined as a plasma glucose concentration equal to or greater than 200 mg/dL[13].

Simvastatin supplementation protocol

Daily simvastatin supplements of 0.25 mg/kg were given orally, starting 7 days before wound creation and continuing until the end of the study[15].

Wounding protocols

Eleven weeks after the last day of STZ injection, mice were anesthetized by intraperitoneal injection of sodium pentobarbital 55 mg/kg. A bilateral full-thickness excisional skin wound measuring 0.6x0.6 cm^2 was created on the dorsal-rostral back of each mouse. Fibrin gel (Shanghai RAAS blood products Co. Ltd, China) was implanted in the wound bed and the wound was covered with Tegaderm™ (3M Company, St. Paul, MN, USA)[14].

Measurement of wound closure

Digital photographs of wounds were taken at day 0. Fourteen days after wounding, mice were anesthetized by intraperitoneal injection of sodium pentobarbital 55 mg/kg and digital photographs of wounds were again taken. Area of the wound was analyzed by digital image software analysis (Image-Pro Plus 6.1; Media Cybernetics, Inc., Bethesda, MD, USA). Percentage of wound closure (%WC) was evaluated using the following equation[14]:

$$\%WC = \frac{(\text{Area of original wound} - \text{Area of actual wound}) \times 100}{\text{Area of original wound}}$$

Measurement of capillary vascularity (CV)

Jugular veins of anesthetized mice were cannulated for injection of 0.1 ml of 5% FITC-labeled dextran. Percentage of capillary vascularity was examined using confocal fluorescence microscopy (Eclipse C1 Plus, Nikon, Japan). From fluorescent photographs, percentage of capillary vascularity was analyzed using Image Pro II 6.1 software (Media Cybernetics, Inc., Bethesda, MD, USA). Capillary diameter was less than 15 μm. The number of pixels within the capillary area and the total number of pixels within the studied frame were obtained from the software. With this information, capillary vascularity was then calculated using the following equation:

$$\%\ \text{capillary vascularity} = \frac{\text{Area within capillaries} \times 100}{\text{Total area of the entire frame}}$$

Measurement of neutrophil infiltration

Fourteen days after wounding, the mice were sacrificed and 0.6x0.6 cm^2 wound samples were harvested. Tissue specimens were fixed in 10% formaldehyde for 24 hours. A tissue sample from the center of each harvested wound area was cut and

Table 1. Physiological characteristics of each group on day 14, post-wounding. Mean ± SEM for body weight, blood glucose, hemoglobin A1c (HbA1c), and cholesterol levels in control group (CON), diabetic group (DM), and diabetic group pre-treated with simvastatin (DM + SIM)

	CON	DM	DM+SIM
Body weight (g)	23.44±1.84 (n = 6)	22.51±2.41 (n = 5)	22.02±1.05 (n = 5)
Blood glucose (mg/dl)	109.17±5.20 (n = 6)	339.50±36.15** (n = 4)	391.33±61.34** (n = 6)
Hemoglobin A1c (HbA1c; %)	5.28±0.15 (n = 5)	10.38±1.82** (n = 3)	12.16±2.62* (n = 4)
Cholesterol (mg/dl)	64.00±11.20 (n = 4)	104.00±8.85* (n = 3)	110.00±5.29* (n = 3)

* $p<0.05$ significant difference, as compared to CON group
** $p<0.01$ significant difference, as compared to CON group

embedded in paraffin. Four-micrometer-thick sections were stained with hematoxylin-eosin (H&E). Neutrophil infiltration into the wound area was then counted by microscope (Nikon E50i, Japan) and analyzed by Image Pro II 6.1 software (Media Cybernetics, Inc., Bethesda, MD, USA)[16].

Determination of tissue VEGF level

At 7 and 14 days after wounding, wound tissue samples were harvested from each mouse and stored at -80°C until VEGF analysis was performed. The amount of VEGF was determined by enzyme-linked immunosorbent assay (ELISA). Briefly, 50 mg tissue samples in 50 ml RIPA lysis buffer (Cell Signaling Technology, Inc., Danvers, MA, USA) with protease inhibitor cocktail (Sigma-Aldrich Corp., St. Louis, MO, USA) were homogenized by sonicator for 15 seconds over ice. Homogenates were then centrifuged at 10,000 rpm for 10 min; after which supernatants of each tissue sample were collected. Supernatants were then used to analyze tissue VEGF level and total tissue protein by commercially available murine VEGF-specific ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) and BCA Protein Assay Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA), respectively. The amount of VEGF was expressed as picograms per milligram protein unit.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Differences between groups were determined by one-way analysis of variance (ANOVA), followed by a Bonferroni post hoc test. The *p*-values less than 0.05 were considered statistically significant.

Results

Effect of simvastatin on physiological characteristics

Body weight, blood glucose, hemoglobin A1c (HbA1c), and cholesterol levels in the CON, DM, and DM+SIM groups are summarized in Table 1. On day 14, blood glucose and HbA1c in the DM and DM + SIM groups were significantly higher than that of the CON group. There was no significant difference in cholesterol levels between the DM and DM + SIM groups at day 14.

Effect of simvastatin on wound closure

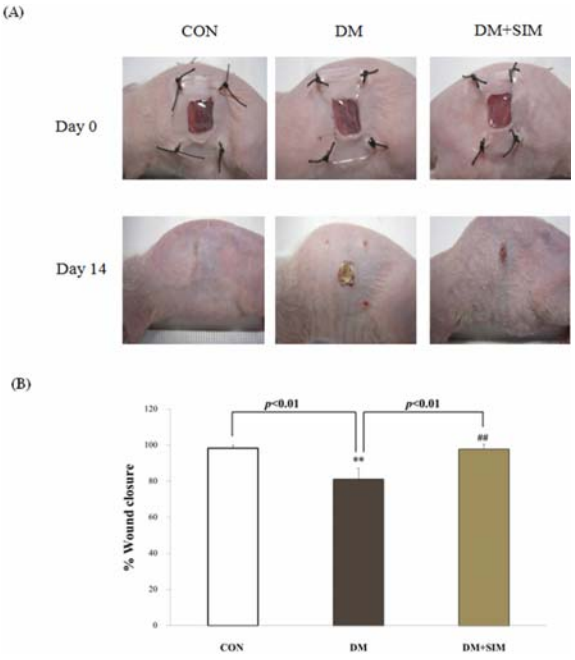
On day 14 after wounding, percentage of wound closure (%WC) in the CON and DM + SIM groups was significantly higher than that of the DM group ($p<0.01$ and $p<0.01$, respectively) (Fig. 1).

Effect of simvastatin on angiogenesis

Percentage of capillary vascularity (%CV) in the wound area of each group is shown in Fig. 2. On day 14, %CV in the CON and DM+SIM groups was significantly higher than that of the DM group ($p<0.05$ and $p<0.01$, respectively) (Fig. 2).

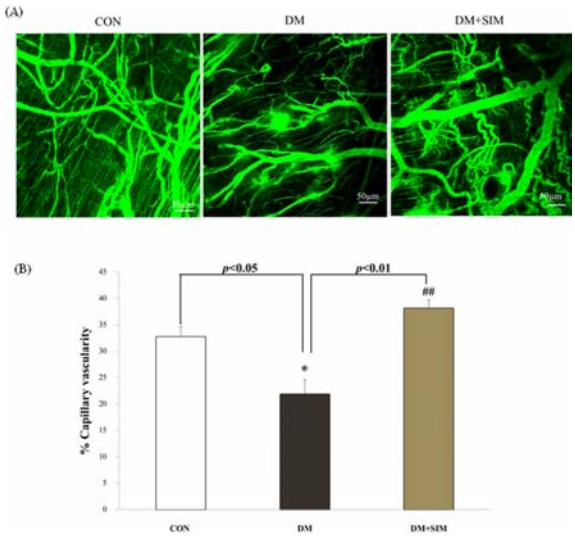
Effect of simvastatin on tissue VEGF level

Tissue VEGF levels in the CON and DM + SIM groups were significantly higher than that of DM group only at day 7 ($p<0.01$ and $p<0.05$, respectively) (Fig. 3). There was no significant difference in tissue VEGF levels among the three groups at day 14.



** $p<0.01$ significant difference, as compared to CON group
$p<0.01$ significant difference, as compared to DM group

Fig. 1 Effect of simvastatin on wound closure: (A) Images of control group (CON), diabetic group (DM), and diabetic group pre-treated with simvastatin (DM+SIM) wounds on day 14; (B) Percentage of wound closure in each group on day 14. Data presented as mean ± SEM.



* $p<0.05$ significant difference, as compared to CON group
$p<0.01$ significant difference, as compared to DM group

Fig. 2 Effect of simvastatin on percentage of capillary vascularity: (A) Confocal images obtained from the control group (CON), diabetic group (DM), and diabetic group pre-treated with simvastatin (DM+SIM) on day 14; (B) Percentage of capillary vascularity in each group on day 14. Data presented as mean ± SEM.

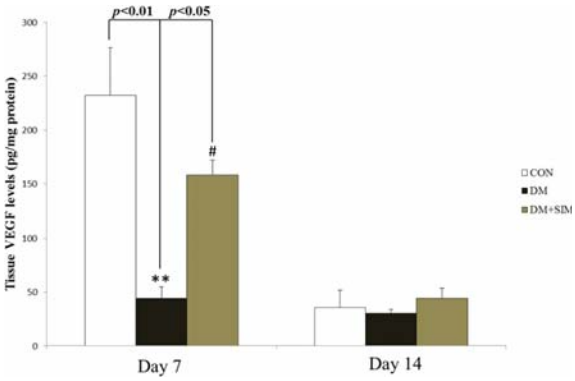
Effect of simvastatin on neutrophil infiltration

On day 14, neutrophil infiltration in the CON and DM + SIM groups was significantly lower than that of the DM group ($p<0.01$ and $p<0.05$ respectively) (Fig. 4).

Discussion

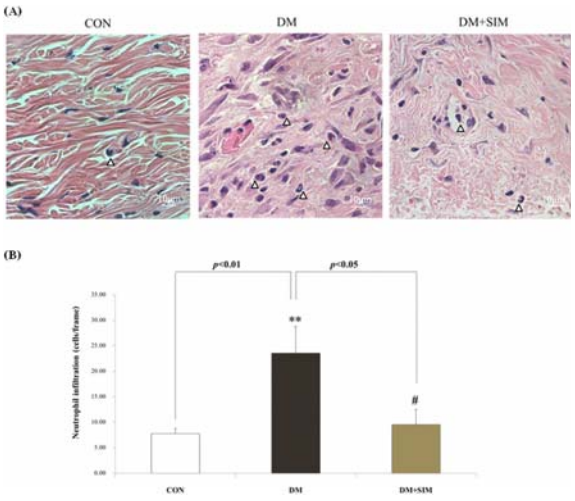
In the present study, multiple low-dose STZ injections were used to induce type 1 diabetes mellitus in male Balb/c nude mice. The toxic effect of STZ causes pancreatic β cells to become damaged by DNA fragmentation[17]. STZ administration resulted in reduced plasma insulin level and increased blood glucose level, which led to a gradual increase in glycated hemoglobin (HbA1c)[18]. Blood glucose and HbA1c in the DM and DM + SIM groups were significantly higher than in the CON group. In addition, levels of cholesterol in DM and DM + SIM were also significantly higher than CON group. These results indicate that low-dose simvastatin administration has no observable effects on blood glucose and cholesterol levels in diabetes.

From previous study, it was shown that low-dose simvastatin (0.25 mg/kg/day) could improve cell survival, preserve cardiac function, and reduce pulmonary edema in mouse model of congestive heart failure without any lipid-lowering effects[15]. Non-healing wounds in diabetes are characterized by prolonged chronic inflammatory state, disturbances in collagen metabolism, poor circulation, reduced angiogenesis, decreased nitric oxide (NO) production, and reduced production of many growth factors[19]. VEGF is an important growth factor, particularly in the angiogenic process. EPCs, which differentiate endothelial cells, have been identified for their important role in up-regulation of VEGF expression, particularly in hypoxic conditions[20]. According to a recent report, hyperglycemia leads to a reduction in both number and function of EPCs in diabetic patients[21]. As a consequence, EPCs dysfunction can cause reduction in vascular regenerative potential in diabetic patients, thereby contributing to the pathogenesis of vascular complications, particularly



** $p<0.01$ significant difference, as compared to CON group
# $p<0.05$ significant difference, as compared to DM group

Fig. 3 Effect of simvastatin on tissue VEGF level in the control group (CON), diabetic group (DM), and diabetic group pre-treated with simvastatin (DM + SIM) on day 7 and 14. Data presented as mean ± SEM.



** $p<0.01$ significant difference, as compared to CON group
# $p<0.05$ significant difference, as compared to DM group

Fig. 4 Effect of simvastatin on neutrophil infiltration: (A) Histological image of neutrophil infiltration within wound area on day 14, with arrows indicating the location of infiltrated neutrophils; (B) Mean ± SEM of infiltrating neutrophils in the control group (CON), diabetic group (DM), and diabetic group pre-treated with simvastatin (DM + SIM) on day 14.

in diabetic ulcers[21,22].

In the present study, day 7 tissue VEGF level and day 14 capillary vascularity in the DM + SIM group were significantly higher than those of the diabetic group. However, VEGF level was not significantly different between DM and DM + SIM on day 14. Consistent with the normal process of wound healing, VEGF increased to maximal levels by day 7 and then declined by day 14 after the emergence of neocapillaries[23]. Based upon these findings, we propose that pre-treatment with low-dose simvastatin can increase 7-day VEGF level and improve angiogenesis in diabetic wounds.

This study also demonstrated a significant decrease in neutrophil infiltration in the DM + SIM group, as compared to the DM group. In normal wounds, neutrophil infiltration normally declines within 3 to 4 days after wounding. However, in wounds sustained by individuals with diabetes mellitus, the inflammatory phase is prolonged due to the increased number of neutrophils in the wound, thereby causing a delay in wound healing[24]. The anti-inflammatory effect of low-dose simvastatin may be another reason for the beneficial effect of simvastatin on diabetic wound healing. Previous reports support the potential anti-inflammatory role of simvastatin in inhibiting leukocyte-endothelial cell interactions associated with decreases in intercellular adhesion molecule-1 (ICAM-1) expression, pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-α), and interleukin (IL)-1β[25,26].

In conclusion, the present study found that the pleiotropic effects of simvastatin could reduce neutrophil infiltration, enhance VEGF production, increase angiogenesis, and improve wound healing in diabetic mouse model. These findings may help to improve clinical benefit in cell therapy for delayed diabetic wound healing in the near future.

What is already known on this topic?

Simvastatin is derivative of HMG-CoA reductase inhibitor and it also has different effect beyond cholesterol reduction called "pleiotropic effects". These pleiotropic effects have been demonstrated to be able to accelerate re-endothelialization, enhance vascular endothelial growth factor (VEGF) production, increase angiogenesis, increase number of endothelial progenitor cells (EPCs), reduce inflammation, and improve wound healing, however, but still not much reports for diabetic wound.

What this study adds?

The findings of this study have shown new

knowledge for the pleiotropic effects of simvastatin on wound healing in diabetic mice model by using the 7-day pre-treatment of low-dose simvastatin supplementation before wound induction. The results suggest the benefit of low-dose simvastatin might be used as a therapeutic agent for diabetic wound healing in the future.

Acknowledgements

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Potential conflicts of interest

None.

References

1. Egede LE, Ellis C. Diabetes and depression: global perspectives. Diabetes Res ClinPract 2010; 87: 302-12.
2. Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. Diabetes Care 1990; 13: 513-21.
3. Kwon DS, Gao X, Liu YB, Dulchavsky DS, Danyluk AL, Bansal M, et al. Treatment with bone marrow-derived stromal cells accelerates wound healing in diabetic rats. Int Wound J 2008; 5: 453-63.
4. Mayfield JA, Reiber GE, Nelson RG, Greene T. Do foot examinations reduce the risk of diabetic amputation? J FamPract 2000; 49: 499-504.
5. Apelqvist J, Larsson J. What is the most effective way to reduce incidence of amputation in the diabetic foot? Diabetes Metab Res Rev 2000; 16 (Suppl 1): S75-83.
6. Omi H, Okayama N, Shimizu M, Fukutomi T, Imaeda K, Okouchi M, et al. Statins inhibit high glucose-mediated neutrophil-endothelial cell adhesion through decreasing surface expression of endothelial adhesion molecules by stimulating production of endothelial nitric oxide. Microvasc Res 2003; 65: 118-24.
7. Kinlay S. Potential vascular benefits of statins. Am J Med 2005; 118 (Suppl 12A): 62-7.
8. Laws PE, Spark JI, Cowled PA, Fitridge RA. The role of statins in vascular disease. Eur J VascEndovascSurg 2004; 27: 6-16.
9. Rego AC, AraujoFilho 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.
10. 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.
11. Veillard NR, Braunersreuther V, Arnaud C, Burger F, Pelli G, Steffens S, et al. Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranylisoprenoid pathway in human endothelial cells and macrophages. Atherosclerosis 2006; 188: 51-8.
12. Henrich D, Seebach C, Wilhelm K, Marzi I. High dosage of simvastatin reduces TNF-alpha-induced apoptosis of endothelial progenitor cells but fails to prevent apoptosis induced by IL-1beta in vitro. J Surg Res 2007; 142: 13-9.
13. 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. ActaPharmacol Sin 2007; 28: 89-97.
14. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 2007; 25: 2648-59.
15. 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 CircPhysiol 2006; 291: H2743-51.
16. Somchaichana J, Bunaprasert T, Patumraj S. Acanthus ebracteatusVahl. 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.
17. Bolzan AD, Bianchi MS. Genotoxicity of streptozotocin. Mutat Res 2002; 512: 121-34.
18. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA(1c): analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. Diabetes Care 2002; 25: 275-8.
19. 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.
20. Velazquez OC. Angiogenesis and vasculogenesis: inducing the growth of new blood vessels and

wound healing by stimulation of bone marrow-derived progenitor cell mobilization and homing. J VascSurg 2007; 45 (Suppl A): A39-47.
21. 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.
22. Imanishi T, Tsujioka H, Akasaka T. Endothelial progenitor cells dysfunction and senescence: contribution to oxidative stress. CurrCardiol Rev 2008; 4: 275-86.
23. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. ClinDermatol 2007; 25: 9-18.
24. Simpson DM, Ross R. The neutrophilicleukocyte in wound repair a study with antineutrophil serum. J Clin Invest 1972; 51: 2009-23.
25. Nezic L, Skrbic R, Dobric S, Stojiljkovic MP, Satara SS, Milovanovic ZA, et al. Effect of simvastatin on proinflammatory cytokines production during lipopolysaccharide-induced inflammation in rats. Gen PhysiolBiophys 2009; 28: 119-26.
26. Miyahara S, Kiryu J, Yamashiro K, Miyamoto K, Hirose F, Tamura H, et al. Simvastatin inhibits leukocyte accumulation and vascular permeability in the retinas of rats with streptozotocin-induced diabetes. Am J Pathol 2004; 164: 1697-706.

ผลพลีโอโทรฟิคของซิมวาสแตตินต่อการหายของแผลในหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวาน

ศุภกานดา สุขแพทย์, นิพัญจน์ อิศรเสนา ณ อยุธยา, สุทธิลักษณ์ ปทุมราช

วัตถุประสงค์: *เพื่อศึกษาผลของซิมวาสแตตินในขนาดที่ต่ำต่อการหายของแผลและต่อการเกิดหลอดเลือดใหม่ในหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวาน*

วัสดุและวิธีการ: *แบ่งหนูไมซ์ออกเป็น 3 กลุ่ม คือ กลุ่มควบคุม (CON) กลุ่มเบาหวาน (DM) และกลุ่มเบาหวานที่ได้รับซิมวาสแตติน (DM + SIM) ก่อนทำให้เกิดแผล 7 วัน โดยจะทำการป้อนซิมวาสแตติน 0.25 มก./กก. ทุกวันเมื่อครบ 11 สัปดาห์ของการเหนี่ยวนำให้เป็นเบาหวาน จึงทำให้เกิดบาดแผลโดยการตัดผิวหนังบริเวณหลังแบบ Bilateral full thickness excisional wound ขนาด 0.6x0.6 ตร.ซม. หลังการเกิดแผลครบ 14 วัน จึงทำการวัดขนาดของแผลด้วยโปรแกรม Image Pro-Plus ตรวจหาร้อยละของการเกิดหลอดเลือดใหม่ด้วย confocal fluorescence microscopy และดูการบุกรุกของนิวโทรฟิวล์ด้วยการย้อม H&E ทำการวิเคราะห์หาปริมาณของวาสคูลาเอ็นโดธีเลียลโกรทแฟคเตอร์ (VEGF) ในวันที่ 7 และ 14 หลังการเกิดแผลโดยวิธี ELISA*

ผลการศึกษา: *พบว่า 14 หลังการเกิดแผล กลุ่ม CON และกลุ่ม DM + SIM จะมีร้อยละการปิดของแผลและร้อยละของการเกิดหลอดเลือดใหม่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ และมีปริมาณนิวโทรฟิวล์ที่บริเวณแผลลดลงอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับกลุ่ม DM ส่วนการวัดปริมาณของ VEGF ในวันที่ 7 พบว่าในกลุ่ม CON และกลุ่ม DM + SIM มีค่าเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่ม DM*

สรุป: *การศึกษาครั้งนี้แสดงให้เห็นว่าการใช้ซิมวาสแตตินขนาดต่ำทำให้ผลลดการอักเสบเพิ่มปริมาณของ VEGF และการเกิดหลอดเลือดใหม่ ส่งผลต่อการหายของแผลเบาหวานดีขึ้น*