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THE EFFECT OF MIDKINE ON GROWTH FACTORS AND OXIDATIVE STATUS IN AN EXPERIMENTAL WOUND MODEL IN DIABETIC AND HEALTHY RATS

Running title: Midkine affects growth factors in wound healing in rats

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Draft

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1 Abstract

2 Wound healing is important for long life. Midkine is a cytokine involved in controlling tissue
3 repair and new tissue development, and regulating inflammation. We investigated the effect
4 of midkine on wound healing in rats. In total, 108 Wistar albino rats were used: 12 as healthy
5 and diabetic controls; 96 split into four groups: healthy, saline treated (HW); healthy, midkine
6 (10 ng/kg, 48-hourly intervals) treated (HM); diabetic, saline treated (DW); and diabetic,
7 midkine treated (DM). Following wound creation, 6 rats per group were euthanized on days 3,
8 7, 14 and 28; the wounded skin was removed. Levels of epidermal growth factor (EGF),
9 matrix metalloproteinase-8 (MMP-8), transforming growth factor beta (TGF- β), platelet
10 derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and thiobarbituric
11 acid-reactive substances (TBARS) were measured. MMP-8 and PDGF levels fluctuated in all
12 groups; TGF- β fluctuated in the diabetic groups and was significantly higher in the HM group
13 than other groups after 14 days. EGF and VEGF levels were increased in the HM group after
14 3 days. TBARS levels were highest in the diabetic groups. Macroscopically, the midkine
15 treated groups healed better. Midkine can accelerate wound healing by influencing growth
16 factors and oxidative status in wound tissues.

17 Keywords: Midkine; wound healing; growth factors.

18

19 **Introduction**

20 A wound is defined as the impairment of tissue or organ integrity. The normal wound healing
 21 process is a well-orchestrated, complex and interlinked series of different processes (Guo and
 22 DiPietro 2010). Systemic problems often impair the phases of wound healing and nonhealing
 23 wounds may herald systemic pathologies, with delayed wound healing serving as a source of
 24 morbidity and mortality in chronic diseases because of the associated tissue inflammation and
 25 infection. For years, many researchers have focused on discoveries that could support the
 26 healing of skin wounds. A variety of chemicals and drugs have been tested to improve the
 27 quality and strength/speed of wound healing. There are no perfect drugs, although there are
 28 many that are promising (Rhett et al. 2008).

29 Macrophages, fibroblasts, platelets, endothelial cells and keratinocytes play important roles
 30 during wound healing. They provide a storehouse of growth factors, cytokines and
 31 chemoattractant molecules. Epidermal growth factor (EGF) acts in the formation of new
 32 epidermis. It increases collagen accumulation and induces proliferation of epidermal cells and
 33 keratinocyte migration, and in doing so strength/speed the healing of wounds (Kondo and
 34 Ishida 2010; Seeger and Paller 2015). Along with matrix metalloproteinase (MMP),
 35 transforming growth factor beta (TGF- β) supplies new matrix. MMP allows modulation,
 36 coordination of wound healing and organization of granulation tissue (Kondo and Ishida
 37 2010; Martins et al. 2013). It also contributes to the migration of cells/structures (Martins et
 38 al. 2013). Platelet derived growth factor (PDGF) allows chemotaxis and mitogenesis of
 39 neutrophils and macrophages, and conversion of fibroblasts to myofibroblasts and smooth
 40 muscle cells (Barrientos et al. 2008; Werner and Grose 2003). It accelerates wound healing
 41 (Judith et al. 2010). PDGF is the only FDA-approved growth factor for diabetic wound
 42 healing (Brem and Tomic-Canic 2007). It acts synergistically with TGF- β (Barrientos et al.
 43 2008; Werner and Grose 2003). TGF- β stimulates the synthesis of collagen and inhibitors of

44 MMP. It plays an important role in inflammation, angiogenesis, re-epithelialization and
45 connective tissue regeneration (Faler et al. 2006). In addition, it aids the formation of
46 granulation tissue and plays a part in all steps of wound healing (Kondo and Ishida 2010).
47 Vascular endothelial growth factor (VEGF) plays an essential role in the proliferation and
48 formation of new endothelial cells. It promotes formation of new blood vessels (Barrientos et
49 al. 2008; Kondo and Ishida 2010).

50 The oxidative state of a wound is a key determinant of its healing outcome. Levels of
51 thiobarbituric acid-reactive species (TBARS) and malondialdehyde (MDA) depend on the
52 level of free radicals. The increased accumulation of free radicals induces lipid peroxidation
53 in the wound area. Therefore, it has been shown that antioxidants may be helpful in wound
54 healing (Rasik and Shukla 2000).

55 The cytokine midkine (MK) is a heparin-binding growth factor of 13 kDa (Muramatsu 2011).
56 It promotes cell growth, cell migration, chemotaxis, and fibrinolysis, as well as
57 embryogenesis and survival by influencing the mitogen-activated protein kinase (MAPK) and
58 phosphatidyl inositol 3-kinase (PI3K)/AKT pathways (Xu et al. 2014). MK induces
59 epithelial–mesenchymal interactions, neurogenesis, angiogenesis, anti-apoptosis and
60 migration in embryogenesis (Muramatsu 2010). Because of its properties and physiological
61 functions, MK could be effective in wound treatment trials (Unoki et al. 1995). MK affects
62 regulation of inflammation, growth of fibroblasts and keratinocytes, cell proliferation,
63 inflammatory cell migration (macrophages and neutrophils, among others) and the formation
64 of the cytoskeleton, an effect it provides through the receptor tyrosine phosphatase (RPTP- β)
65 (Muramatsu 2011; Sumi et al. 2000). MK levels may be associated with the levels of the
66 growth factors that can assist healing (Kadomatsu et al. 2014). MK reduces apoptosis, and
67 increases cell viability and proliferation. It also reverses hypoxia-induced increases in
68 intracellular reactive oxygen species (Lee et al. 2012).

69 Diabetes is a chronic disease with dysregulation of glucose, which also leads to lipid and
70 protein metabolism disorders. It can cause a number of complications, including some that
71 make it harder for wounds to heal. Impaired wound healing is a frequent and very severe
72 problem in patients with diabetes mellitus. The nonenzymatic glycation of proteins,
73 generating advanced glycation end-products, acts as an active pathogenic stream that affects
74 healing (Dagogo-Jack and Alberti 2002). The excessive apoptosis, increased matrix
75 degradation and disintegration of the cell matrix connections associated with hyperglycemia
76 cause delays in wound healing (Darby et al. 1997). In addition, irregular inflammatory
77 responses, impairment of inflammatory cell migration, prolonged inflammation, impaired
78 neovascularization and unbalanced collagen expression retard wound healing in diabetic
79 patients (Ahmed 2005).

80 In this research, it has been hypothesized that effects of MK on the some inflammatory
81 biological markers which play role in the healing of wound (Muramatsu 2010) are taken into
82 consideration; MK may show some beneficial effect in the wound healing in the healthy or
83 diabetic subjects.

84 The costs of treating pathologies related to this deficit in the healing process increase the
85 importance of studies searching for new treatment approaches that are capable of interacting
86 with damaged tissues to accelerate the healing process/treatments. In this study, we aimed to
87 evaluate the effect of MK application to a full-thickness skin wound model in healthy and
88 diabetic rats.

89

90 **Materials and Methods**

91 *Animals*

A total of 108 male Wistar albino rats (10–12 weeks, 250–350 g) were housed in cages in a room with automatic ambient humidity ($55 \pm 5\%$), temperature ($22 \pm 2^\circ\text{C}$), and light–dark control (12-h light/dark cycle). The animals were obtained from the KONUDAM Experimental Medicine, Application and Research Center, Konya, Turkey. They were fed *ad libitum* with a commercial diet (Bil-Yem Feeds, Ankara, Turkey) that included the following: 88% dry matter, 23% crude protein, 7% crude fibre, 3% crude oil, 1.0–2.5% calcium, 0.9% phosphorus and 0.5–1% sodium, and had free access to water.

All experiments and manipulations were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA) and our use of animals was reviewed and approved by the appropriate animal care review committee at the Ethics Committee of Necmettin Erbakan University Kombatassan Experimental Medicine Research and Application Center, Turkey.

Induction of diabetes

Streptozotocin (STZ, $\geq 98\%$ [HPLC], Sigma-Aldrich Co., USA) dissolved in citrate buffer (pH 4.5, 20 mg/mL) was used to induce diabetes, with two consecutive daily subcutaneous (s.c.) doses of freshly prepared STZ (40 mg/kg) being given (Gelling et al. 2006). The rats were allowed to drink 5% glucose solution overnight to accomplish the drug-induced hypoglycemia (Liang et al. 2013). Rats with nonfasting blood glucose levels of ≥ 300 mg/dL 2 weeks after STZ injection were considered to be diabetic (Haidara et al. 2009).

Experimental design and full-thickness skin wound model

First, 12 Wistar albino rats were divided into two groups (6 healthy [H group] and 6 diabetic [D group] to act as the controls (Hour 0). The dorsal skin of these animals that did not undergo application of MK or saline was removed under anesthesia at 0 hour.

The remaining 96 rats were divided into four procedure groups: healthy control (HW), healthy midline (HM), diabetic control (DW), and diabetic midline (DM), giving 24 rats per group. The dorsal skins of these 96 animals were shaved and cleaned with iodine solution. With the animals under anesthesia, four full-thickness skin wounds (approximately 6 mm in diameter) were created using a 6-mm biopsy punch at 0 hour (diabetes mellitus having been previously established in the two diabetic groups).

In two groups (HW and DW), the wound area of the rats was treated with 0.9% saline solution (s.c.) at 48-hourly intervals during the study period.

In the remaining two groups (HM and DM), the wound area of the rats was treated with MK solution (10 ng/kg, s.c.; Sigma-Aldrich Co.) at 48-hourly intervals during the study period.

From each of these groups, six rats were examined at each of four time points (3, 7, 14, and 28 days). The wounded skin was collected at the appropriate sampling time, with the rats anesthetized using 20 mg/kg intraperitoneal thiopental sodium (Pental 1 g; Ulagay, Istanbul, Turkey).

Evaluation of wound healing

The wound granulation tissues were harvested at each time point. Harvested skins were stored at -80°C for further analysis. The concentrations of growth factors were measured using an ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200) and the following rat-specific ELISA kits: EGF, PDGF, TGF- β (USCN Business Co., Ltd., USA); MMP-8, VEGF (Cusabio, China); and TBARS (Cayman Chemical, USA) according to the manufacturer's protocol.

Statistical analysis

All experimental values are presented as mean $\pm$ SE. The data were analyzed by ANOVA, and the Duncan test was then used as a *post-hoc* test to analyze the difference between the groups and across the days (IBM SPSS Statistics 22.0). $P < 0.05$ was considered significant.

Results

EGF levels in HW group were lower than in all of the other groups at day 3 ($P < 0.05$), while EGF levels in the HM group were higher than all of the other groups at day 28 ($P < 0.05$). In the HM group, the levels increased steadily across the sampling days and the levels on days 14 and 28 were statistically significantly ($P < 0.05$) higher than at 0 hour (Table 1).

MMP-8 levels were highest ($P < 0.05$) on days 3 and 7 in the HW group, compared with the other groups. In the HW group, the MMP-8 level was highest on day 3, compared with the other days ($P < 0.05$), however, MMP-8 levels reduced from 0 hour in the DM group ($P < 0.05$) (Table 1).

The PDGF level was higher in the DW group than the other groups on day 3 ($P < 0.05$) and it was higher in the HM group than the HW and DW groups on day 14. In the other words, levels of PGDF were found to fluctuate across the days, with the levels being highest on days 7, 14, 3 and 14 in the HW, HM, DW and DM groups, respectively ($P < 0.05$). At 0 hour and on day 28, PDGF levels were determined to be lowest in the HW group. In contrast, the other groups were observed to be low only at 0 hour ($P < 0.05$) (Table 1).

The TGF- β level of the DW group was higher than the other groups on day 3 ($P < 0.05$). When the HM group was compared with the HW group, the TGF- β level was higher ($P < 0.05$) on days 14 and 28 and its highest level was found in the HM group on day 28. In the HM group, TGF- β level was higher at 0 hour compared with day 28 ($P < 0.05$). Fluctuations were seen in the TGF- β levels of the DW group. The TGF- β level decreased in the DW group

after day 3 ($P < 0.05$), whereas in the DM group the highest TGF- β level was detected on day 14 ($P < 0.05$) (Table 1).

VEGF levels increased in the DM and HM groups up until day 14. The VEGF level was higher in the HW group than in the DM group on day 3 ($P < 0.05$). This level was shown to be highest in the HM group on day 7 among the groups and the level of VEGF was lower in the DW group than in the HM group on day 14 (Table 1).

TBARS levels decreased in the HW group after 0 hour ($P < 0.05$). In the diabetic groups, TBARS levels were found to be very high at 0 hours, but levels reduced after the wound was created ($P < 0.05$). At 0 hours, there was a significant difference between the healthy and the diabetic groups ($P < 0.05$). In the same time, the TBARS level was highest in the DW group on day 28 ($P < 0.05$) among the groups (Table 1).

Discussion

The repair of cutaneous wounds in the adult body involves a complex series of spatially and temporally organized processes to prevent infection and restore homeostasis that last months and years. Three characteristic phases of wound repair (inflammation, proliferation including re-epithelialization, and remodeling) overlap in time and space. Extracellular matrix, inflammatory mediators and a wide range of growth factors take part in these cascades; these factors are now being increasingly recognized as an important modality for accelerating and modulating healing processes (Li et al. 2007). MK plays a part in proliferation, growth, migration and survival of different cell types. In addition, it induces synthesis of extracellular matrix proteins and regeneration of the basic structures that form the cell skeleton (Kadomatsu et al. 2014; Shirakata et al. 2005; Sumi et al. 2000). MK is a cytokine or growth factor that has a similar structure to other growth factor-related protein families, such as the

fibroblast growth factor family and neuronal growth factors (Kosugi et al. 2006). MK may have different effects in inflammatory pathologies.

Levels of EGF increased in MK-treated healthy rats (Table 1). EGF accelerates wound healing and supports skin morphogenesis and homeostasis (Barrientos et al. 2008; Kondo and Ishida 2010). The presence of keratinocytes is necessary, when normal wound healing (specifically epidermis and hairs) is required. In this case, EGF ensures regulation and migration of keratinocytes and hemostasis in the skin (Blumenberg 2013; Nanba et al. 2013). At the same time, it supplies new epithelium, promoting formation of matrix and cell proliferation (Santoro and Gaudino 2005; Shirakata et al. 2005). Hence, epithelialization is accelerated in wound areas by the migration of increased numbers of keratinocytes (Shirakata et al. 2005). Wound healing is delayed when keratinocytes and fibroblast cells are inhibited (Cho Lee et al. 2005).

MK can have influence on keratinocytes directly or can affect the synthesis of growth factors from inflammatory cells indirectly, so increasing rates of wound closure and hair growth. As a result, EGF may have increased the number of keratinocytes in the HM group, leading to the faster wound healing and hair growth that occurred in this group. However, these features were not at the desired level because of fluctuating EGF levels in the DW and DM groups. It may be that high glucose concentrations impair proliferation and differentiation in keratinocytes, and affect the growth factors released from macrophages (Maruyama et al. 2007; Spravchikov et al. 2001). For these reasons, the regenerative effect of MK on wound healing is not absolutely proven in the diabetic group because this group might have a lack of normal cells such as macrophages and keratinocytes in comparison with healthy animals. In addition, increased EGF and glucose levels may have induced excessive endogenous MK levels (Rawnaq et al. 2014). Therefore, wound healing may have been partially delayed in the DM group compared with the HM group.

It is suggested that epidermal healing and hair growth are delayed because of the fluctuations in EGF levels in the diabetic groups (Table 1). However, this may depend on increases in the number of keratinocytes as higher EGF levels can speed up hair growth and wound healing in the HM group. It is reported that EGF reduces the formation of scars, and accelerates vascularization and wound healing. In addition, it is reported to increase collagen levels (Xing et al. 2013). In the present study, increased EGF levels may have raised the amount of collagen and vascularity in the HM group, in which, scarless and early wound healing was observed.

In the present study, MMP-8 levels statistically fluctuated in the HW group, whereas, in the other groups, they were linear. However, MMP-8 levels were very low in the diabetic groups (Table 1). MMP-8 is the predominant collagenase involved in healing wounds (Nwomeh et al. 1999). MMP degrades extracellular matrix helping the migration of cells, the deposition of new matrix and the development of new tissue. MMP regulates the balance between tissue synthesis and degradation that is required for inflammation and regeneration. Tissue inhibitors of MMP (TIMP) strongly inhibit the effects of MMP (Martins et al. 2013; Xue et al. 2006). TGF- β stimulates TIMP and the protease inhibitor (Diegelmann and Evans 2004). For this reason, MK cannot affect MMP-8 levels sufficiently. Excessive degradation of the extracellular matrix due to MMP leads to increased diabetic wounds and delays in wound healing (Martins et al. 2013; Xue et al. 2006). MMP-8 derives from inflammatory cells that are necessary for normal wound healing, especially in the early phase. If these cells are insufficient, wound healing is delayed (Gutierrez-Fernandez et al. 2007).

Treatment with MK increased PDGF levels in the proliferation and remodeling phase of wound healing in the present study (Table 1). PDGF is important in various stages of wound healing. It is a chemoattractant, particularly of fibroblasts, collagen, neutrophils and monocytes. It regulates contraction of wounds and formation of granulation tissue. PDGF

levels are reported to be decreased in diabetics (Santoro and Gaudino 2005). Application of PDGF in a wound increases re-epithelization, vascularity and the maturation rate of granulation tissue (Cheng et al. 2007).

In the present study, the formation rate of granulation tissue, angiogenesis, fibroblasts and collagen may have increased because MK increased PDGF levels. Therefore, this can produce better wound healing in the HM and DM groups. However, the effect of PDGF alone may be insufficient for wound healing in the DM group.

TGF- β levels were enhanced by MK treatment in the present study, with a significant increase in the HM group (Table 1). TGF- β is released by platelets, macrophages and T lymphocytes. TGF- β is the main control signal that organizes fibroblast function and extracellular matrix deposition (Diegelmann and Evans 2004). It helps with the activation of macrophages, stimulation of angiogenesis, migration of keratinocytes, accumulation of collagen, and re-epithelization (Rhett et al. 2008; Santoro and Gaudino 2005). In this way, TGF- β may have raised own release from keratinocytes and macrophages (Kondo and Ishida 2010). As a result, decreased TGF- β levels are reported to delay wound healing (Crowe et al. 2000) and levels are insufficient in diabetics (Jude et al. 2002).

MK may enhance keratinocyte numbers because MK increases the TGF- β level and so can accelerate hair growth and wound healing. Hair growth and wound healing was slower in the DM group compared with the HM group because the TGF- β level fluctuated, particularly in the final stages of wound healing. Wound healing is delayed in diabetic rats because of the abnormal inflammatory response, such as lack of neutrophils (Komesu et al. 2004). In the present study, MK may affect inflammatory cells and the release of their growth factors (TGF- β and EGF, among others). In addition, wound healing and hair growth showed a greater improvement in healthy rats compared with diabetic rats.

The quantity and exposure time of MK is reported to result in changes in collagen synthesis and the usage of a 100 ng/mL dose in vitro has been recommended (Yamada et al. 1997). In the present study, treatment with MK may have increased collagen and accelerated wound healing because of a rise in TGF- β levels. As a result, MK may be increasing the synthesis of collagen directly and indirectly.

In the present study, VEGF levels increased in the HM group (Table 1). Angiogenesis is an essential element in normal wound healing. VEGF promotes blood vessel regeneration during repair (Diegelmann and Evans 2004). Keratinocytes and macrophages are the source for angiogenic factors (Rossiter et al. 2004). Because MK may have induced these cells, angiogenesis and rapid wound healing may have occurred.

The amount of VEGF was lower in the diabetic groups in the present study (Table 1). It is well known that collagen, granulation tissue and new vessels are insufficient in diabetics, mainly due to the lower levels of angiogenic factors (VEGF and angiopoietin 2, among others) and inflammatory cells compared with healthy subjects (Blakytyn and Jude 2006; Sharma et al. 2006). Topical VEGF application to diabetic mice contributes to blood vessel formation in the local wound environment and accelerates repair (Galiano et al. 2004). MK plays a critical role on inflammatory cells (polymorphonuclear neutrophils and macrophages, among others) during acute inflammation (Weckbach et al. 2014). MK may have induced angiogenic factors in the early phase of wound healing in healthy rats, but it had no similar effect in the diabetic rats because of an insufficiency of angiogenic and inflammatory cells. It can provide growth factors synthesis such as VEGF so for this reason may have induced angiogenic factors and epithelization in the HM group but not in other groups. Therefore, MK can accelerate angiogenesis and wound healing in healthy groups.

In the current research, the TBARS level was very high in the diabetic groups at 0 hour (Table 1). Polyunsaturated fatty acid peroxidation causes oxidative stress and damage to cells affected by certain diseases such as diabetes (Del Rio et al. 2005). Excessive oxidative stress disrupts the effects of fibroblasts and the response of fibroblasts to growth factors (Soneja et al. 2005). For this reason, the effects of fibroblasts and cells involved in wound healing are damaged in the diabetics groups. After the wound had been created, the TBARS levels were similar between the healthy and diabetic groups, because the test materials (skin samples) were developing new granulation tissue. The main molecule on assays of TBARS is MDA (Del Rio et al. 2005), which is high and leads to incomplete wound healing in diabetics compared to healthy subjects. Hyperglycemia causes apoptosis (Arya et al. 2011). Wound healing in diabetic groups with very high levels of TBARS may be delayed because of excessive apoptosis.

MK is reported to be a potent stimulator of glycosaminoglycan and collagen synthesis and it is suggested to be helpful in the treatment of delayed wound healing (Yamada et al. 1997). In one trial, an ischemic wound was found to delay healing and wound healing accelerated after day 7 because of a reduction in ischemia (Chen et al. 1999). Hence, MK can affect collagen synthesis and angiogenesis in the inflammatory phase because of its influence on inflammatory cells that release growth factors (VEGF, TGF- β , etc.), and can be protective against hypoxia. This mechanism explains the increase in angiogenesis and oxygenation because MK improves the expression of VEGF, TGF- β and insulin-like growth factor-1 (Zhao et al. 2014). MK may improve angiogenesis and wound healing because it increases levels of VEGF and TGF- β in the HM group. In addition, it partly supplied these growth factors and improved wound healing in the DM group; however, it was not as good as in the healthy rats. Wound healing is delayed because of lack of neutrophils, macrophages, pro-

inflammatory mediators and excessive apoptosis in the early phase, after which inflammation continues uncontrolled (Koh and DiPietro 2011).

Inflammatory or immune cells in nonhealing diabetic wounds cause impairments in the expression of growth factors, extracellular matrix formation, angiogenesis and re-epithelialization (Ochoa et al. 2007). MK may possibly control inflammation in the early phase of wound healing. Delayed wound healing in diabetes is a very complex situation, with uncontrolled inflammation and late angiogenesis, and other reasons. MK is known to increase synthesis of extracellular matrix molecules and it can be of use in burns and skin damage. It has greater effects in the early phase compared with the late phase in vitro (Sumi et al. 2000).

As a result, MK can show different effects in different stages. MK interacts with specific integrin molecules (Muramatsu et al. 2004), which ensure contact in the cell and movement of cells. Integrin molecules have α and β subtype. On migration of cells, endothelial cells have a high affinity to these subtypes (Falanga 2005). MK binds to integrin subtypes and activates adapter protein of paxillin in the cell, allowing the tyrosine phosphorylation of the protein. Phosphorylated paxillin increases the stimulation of growth factors (Muramatsu et al. 2004). In addition, it promotes cell motility, transformation and maturation. Macrophages, lymphocytes and smooth muscle cells have abundant paxillin (Turner 1998). To our best knowledge, there was no any research about MK relationship wound healing in diabetic and healthy subjects; hence effects of MK on the EGF, VEGF, MMP-8, TGF- β , PDGF and TBARS have not been evaluated, which are very important factors in the wound healing. Beneficial effects of MK on the wound healing may be derived from effecting of MK on cell migration and the release of growth factors by activating intracellular proteins such as paxillin. However, MK cannot affect the deficiency or disorder of the inflammatory cells in diabetics, meaning that wound healing is more regular and rapid in the HM group than in the DM group.

Conclusions and Suggestions

Wound healing depends on many factors (levels of growth factors and cytokines) and different cells (keratinocytes and endothelial cells, etc) and is different in diabetic subjects from healthy subjects. MK may show different effects in healthy and diabetic subjects because of its effects on the inflammatory cells that are present at low levels in diabetics. MK may affect many growth factors, cytokines and some other factors in cells. It may affect angiogenesis, formation of new matrix and accelerate the development of new epidermis. MK may increase hair growth and wound healing. In addition, its effect may be more powerful in the early stages of wound healing.

MK may be more effective in wound healing among healthy subjects than diabetics, but it could possibly be utilized with different drugs that are effective in diabetics, although its effects may change depending on time and affected cells. In the future, the effects of MK on wound healing should be researched in more detail and it may provide a new treatment strategy for wound healing. Further investigations or clinical studies are needed to determine the specific cellular target or other effects of MK.

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Conflict of Interest

355 The authors declare that no conflict of interests exists.

356 References

- 357 Ahmed, N. 2005. Advanced glycation endproducts--role in pathology of diabetic
358 complications. *Diabetes. Res. Clin. Pract.*, **67**(1): 3-21. doi: 10.1016/j.diabres.2004.09.004.
- 359 Arya, A.K., Pokharia, D., and Tripathi, K. 2011. Relationship between oxidative stress and
360 apoptotic markers in lymphocytes of diabetic patients with chronic non healing wound.
361 *Diabetes. Res. Clin. Pract.*, **94**(3): 377-384. doi: 10.1016/j.diabres.2011.08.004.
- 362 Barrientos, S., Stojadinovic, O., Golinko, M.S., Brem, H., and Tomic-Canic, M. 2008.
363 Growth factors and cytokines in wound healing. *Wound. Repair. Regen.*, **16**(5): 585-601. doi:
364 10.1111/j.1524-475X.2008.00410.x.
- 365 Blakytyn, R., and Jude, E. 2006. The molecular biology of chronic wounds and delayed
366 healing in diabetes. *Diabet. Med.*, **23**(6): 594-608. doi: 10.1111/j.1464-5491.2006.01773.x.
- 367 Blumenberg, M. 2013. Profiling and metaanalysis of epidermal keratinocytes responses to
368 epidermal growth factor. *BMC. Genomics.*, **14**: 85. doi: 10.1186/1471-2164-14-85.
- 369 Brem, H., and Tomic-Canic, M. 2007. Cellular and molecular basis of wound healing in
370 diabetes. *J. Clin. Invest.*, **117**(5): 1219-1222. doi: 10.1172/JCI32169.
- 371 Chen, C., Schultz, G.S., Bloch, M., Edwards, P.D., Tebes, S., and Mast, B.A. 1999. Molecular
372 and mechanistic validation of delayed healing rat wounds as a model for human chronic
373 wounds. *Wound. Repair. Regen.*, **7**(6): 486-494.
- 374 Cheng, B., Liu, H.W., Fu, X.B., Sun, T.Z., and Sheng, Z.Y. 2007. Recombinant human
375 platelet-derived growth factor enhanced dermal wound healing by a pathway involving ERK
376 and c-fos in diabetic rats. *J. Dermatol. Sci.*, **45**(3): 193-201. doi:
377 10.1016/j.jdermsci.2006.11.014.
- 378 Cho Lee, A.R., Leem, H., Lee, J., and Park, K.C. 2005. Reversal of silver sulfadiazine-
379 impaired wound healing by epidermal growth factor. *Biomaterials.*, **26**(22): 4670-4676. doi:
380 10.1016/j.biomaterials.2004.11.041.
- 381 Crowe, M.J., Doetschman, T., and Greenhalgh, D.G. 2000. Delayed wound healing in
382 immunodeficient TGF-beta 1 knockout mice. *J. Invest. Dermatol.*, **115**(1): 3-11. doi:
383 10.1046/j.1523-1747.2000.00010.x.
- 384 Dagogo-Jack, S., and Alberti, K.G.M. 2002. Management of diabetes mellitus in surgical
385 patients. *Diabetes. Spectr.*, **15**(1): 44-48.
- 386 Darby, I.A., Bisucci, T., Hewitson, T.D., and MacLellan, D.G. 1997. Apoptosis is increased
387 in a model of diabetes-impaired wound healing in genetically diabetic mice. *Int. J. Biochem.*
388 *Cell. Biol.*, **29**(1): 191-200.
- 389 Del Rio, D., Stewart, A.J., and Pellegrini, N. 2005. A review of recent studies on
390 malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr. Metab.*
391 *Cardiovasc. Dis.*, **15**(4): 316-328.

- Diegelmann, R.F., and Evans, M.C. 2004. Wound healing: An overview of acute, fibrotic and delayed healing. *Front. Biosci.*, **9**: 283-289. doi: Doi 10.2741/1184.
- Falanga, V. 2005. Wound healing and its impairment in the diabetic foot. *Lancet.*, **366**(9498): 1736-1743. doi: 10.1016/S0140-6736(05)67700-8.
- Faler, B.J., Macsata, R.A., Plummer, D., Mishra, L., and Sidawy, A.N. 2006. Transforming growth factor-beta and wound healing. *Perspect. Vasc. Surg. Endovasc. Ther.*, **18**(1): 55-62. doi: 10.1177/153100350601800123.
- Galiano, R.D., Tepper, O.M., Pelo, C.R., Bhatt, K.A., Callaghan, M., Bastidas, N., Bunting, S., Steinmetz, H.G., and Gurtner, G.C. 2004. Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. *Am. J. Pathol.*, **164**(6): 1935-1947. doi: Doi 10.1016/S0002-9440(10)63754-6.
- Gelling, R.W., Morton, G.J., Morrison, C.D., Niswender, K.D., Myers, M.G., Rhodes, C.J., and Schwartz, M.W. 2006. Insulin action in the brain contributes to glucose lowering during insulin treatment of diabetes. *Cell. Metab.*, **3**(1): 67-73.
- Guo, S., and DiPietro, L.A. 2010. Factors Affecting Wound Healing. *J. Dent. Res.*, **89**(3): 219-229. doi: Doi 10.1177/0022034509359125.
- Gutierrez-Fernandez, A., Inada, M., Balbin, M., Fueyo, A., Pitiot, A.S., Astudillo, A., Hirose, K., Hirata, M., Shapiro, S.D., Noel, A., Werb, Z., Krane, S.M., Lopez-Otin, C., and Puente, X.S. 2007. Increased inflammation delays wound healing in mice deficient in collagenase-2 (MMP-8). *FASEB. J.*, **21**(10): 2580-2591. doi: 10.1096/fj.06-7860com.
- Haidara, M.A., Mikhailidis, D.P., Rateb, M.A., Ahmed, Z.A., Yassin, H.Z., Ibrahim, I.M., and Rashed, L.A. 2009. Evaluation of the effect of oxidative stress and vitamin E supplementation on renal function in rats with streptozotocin-induced Type 1 diabetes. *J. Diabetes. Complications.*, **23**(2): 130-136.
- Jude, E.B., Blakytyn, R., Bulmer, J., Boulton, A.J., and Ferguson, M.W. 2002. Transforming growth factor-beta 1, 2, 3 and receptor type I and II in diabetic foot ulcers. *Diabet. Med.*, **19**(6): 440-447.
- Judith, R., Nithya, M., Rose, C., and Mandal, A.B. 2010. Application of a PDGF-containing novel gel for cutaneous wound healing. *Life. Sci.*, **87**(1-2): 1-8. doi: 10.1016/j.lfs.2010.05.003.
- Kadomatsu, K., Bencsik, P., Gorbe, A., Csonka, C., Sakamoto, K., Kishida, S., and Ferdinandy, P. 2014. Therapeutic potential of midkine in cardiovascular disease. *Br. J. Pharmacol.*, **171**(4): 936-944. doi: 10.1111/bph.12537.
- Koh, T.J., and DiPietro, L.A. 2011. Inflammation and wound healing: the role of the macrophage. *Expert. Rev. Mol. Med.*, **13**: e23. doi: 10.1017/S1462399411001943.
- Komesu, M.C., Tanga, M.B., Buttros, K.R., and Nakao, C. 2004. Effects of acute diabetes on rat cutaneous wound healing. *Pathophysiology.*, **11**(2): 63-67. doi: 10.1016/j.pathophys.2004.02.002.
- Kondo, T., and Ishida, Y. 2010. Molecular pathology of wound healing. *Forensic. Sci. Int.*, **203**(1): 93-98.

- 433 Kosugi, T., Yuzawa, Y., Sato, W., Kawai, H., Matsuo, S., Takei, Y., Muramatsu, T., and
434 Kadomatsu, K. 2006. Growth factor midkine is involved in the pathogenesis of diabetic
435 nephropathy. *Am. J. Pathol.*, **168**(1): 9-19.
- 436 Lee, S.H., Suh, H.N., Lee, Y.J., Seo, B.N., Ha, J.W., and Han, H.J. 2012. Midkine prevented
437 hypoxic injury of mouse embryonic stem cells through activation of Akt and HIF-1 α via low-
438 density lipoprotein receptor-related protein-1. *J. Cell. Physiol.*, **227**(4): 1731-1739.
- 439 Li, J., Chen, J., and Kirsner, R. 2007. Pathophysiology of acute wound healing. *Clin.*
440 *Dermatol.*, **25**(1): 9-18. doi: 10.1016/j.clindermatol.2006.09.007.
- 441 Liang, B., Guo, Z.D., Xie, F., and Zhao, A.N. 2013. Antihyperglycemic and
442 antihyperlipidemic activities of aqueous extract of *Hericium erinaceus* in experimental
443 diabetic rats. *BMC Complement. Altern. Med.*, **13** (253): 1-7. doi: 10.1186/1472-6882-13-
444 253.
- 445 Martins, V.L., Caley, M., and O'Toole, E.A. 2013. Matrix metalloproteinases and epidermal
446 wound repair. *Cell. Tissue. Res.*, **351**(2): 255-268. doi: 10.1007/s00441-012-1410-z.
- 447 Maruyama, K., Asai, J., Ii, M., Thorne, T., Losordo, D.W., and D'Amore, P.A. 2007.
448 Decreased macrophage number and activation lead to reduced lymphatic vessel formation and
449 contribute to impaired diabetic wound healing. *Am. J. Pathol.*, **170**(4): 1178-1191. doi:
450 10.2353/ajpath.2007.060018.
- 451 Muramatsu, H., Zou, P., Suzuki, H., Oda, Y., Chen, G.-Y., Sakaguchi, N., Sakuma, S.,
452 Maeda, N., Noda, M., and Takada, Y. 2004. $\alpha 4\beta 1$ - and $\alpha 6\beta 1$ -integrins are functional receptors
453 for midkine, a heparin-binding growth factor. *J. Cell. Sci.*, **117**(22): 5405-5415.
- 454 Muramatsu, T. 2010. Midkine, a heparin-binding cytokine with multiple roles in
455 development, repair and diseases. *Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci.*, **86**(4): 410-425.
456 doi: 10.2183/pjab.86.410.
- 457 Muramatsu, T. 2011. Midkine: a promising molecule for drug development to treat diseases
458 of the central nervous system. *Curr. Pharm. Des.*, **17**(5): 410.
- 459 Nanba, D., Toki, F., Barrandon, Y., and Higashiyama, S. 2013. Recent advances in the
460 epidermal growth factor receptor/ligand system biology on skin homeostasis and keratinocyte
461 stem cell regulation. *J. Dermatol. Sci.*, **72**(2): 81-86. doi: 10.1016/j.jdermsci.2013.05.009.
- 462 Nwomeh, B.C., Liang, H.X., Cohen, I.K., and Yager, D.R. 1999. MMP-8 is the predominant
463 collagenase in healing wounds and nonhealing ulcers. *J. Surg. Res.*, **81**(2): 189-195. doi: DOI
464 10.1006/jsre.1998.5495.
- 465 Ochoa, O., Torres, F.M., and Shireman, P.K. 2007. Chemokines and diabetic wound healing.
466 *Vascular.*, **15**(6): 350-355.
- 467 Rasik, A.M., and Shukla, A. 2000. Antioxidant status in delayed healing type of wounds. *Int.*
468 *J. Exp. Pathol.*, **81**(4): 257-263.
- 469 Rawnaq, T., Dietrich, L., Wolters-Eisfeld, G., Uzunoglu, F.G., Vashist, Y.K., Bachmann, K.,
470 Simon, R., Izbicki, J.R., Bockhorn, M., and Güngör, C. 2014. The multifunctional growth
471 factor midkine promotes proliferation and migration in pancreatic cancer. *Mol. Cancer Res.*,
472 **12**(5): 670-680.

- 473 Rhett, J.M., Ghatnekar, G.S., Palatinus, J.A., O'Quinn, M., Yost, M.J., and Gourdie, R.G.
474 2008. Novel therapies for scar reduction and regenerative healing of skin wounds. *Trends.*
475 *Biotechnol.*, **26**(4): 173-180.
- 476 Rossiter, H., Barresi, C., Pammer, J., Rendl, M., Haigh, J., Wagner, E.F., and Tschachler, E.
477 2004. Loss of vascular endothelial growth factor activity in murine epidermal keratinocytes
478 delays wound healing and inhibits tumor formation. *Cancer. Res.*, **64**(10): 3508-3516.
- 479 Santoro, M.M., and Gaudino, G. 2005. Cellular and molecular facets of keratinocyte
480 reepithelization during wound healing. *Exp. Cell. Res.*, **304**(1): 274-286. doi:
481 10.1016/j.yexcr.2004.10.033.
- 482 Seeger, M.A., and Paller, A.S. 2015. The Roles of Growth Factors in Keratinocyte Migration.
483 *Adv. Wound. Care (New Rochelle)*, **4**(4): 213-224. doi: 10.1089/wound.2014.0540.
- 484 Sharma, A., Singh, A.K., Warren, J., Thangapazham, R.L., and Maheshwari, R.K. 2006.
485 Differential regulation of angiogenic genes in diabetic wound healing. *J. Invest. Dermatol.*,
486 **126**(10): 2323-2331. doi: 10.1038/sj.jid.5700428.
- 487 Shirakata, Y., Kimura, R., Nanba, D., Iwamoto, R., Tokumaru, S., Morimoto, C., Yokota, K.,
488 Nakamura, M., Sayama, K., and Mekada, E. 2005. Heparin-binding EGF-like growth factor
489 accelerates keratinocyte migration and skin wound healing. *J. Cell. Sci.*, **118**(11): 2363-2370.
- 490 Soneja, A., Drews, M., and Malinski, T. 2005. Role of nitric oxide, nitroxidative and
491 oxidative stress in wound healing. *Pharmacol. Rep.*, **57**: 108-119.
- 492 Spravchikov, N., Sizyakov, G., Gartsbein, M., Accili, D., Tennenbaum, T., and Wertheimer,
493 E. 2001. Glucose effects on skin keratinocytes implications for diabetes skin complications.
494 *Diabetes.*, **50**(7): 1627-1635.
- 495 Sumi, Y., Muramatsu, H., Hata, K., Ueda, M., and Muramatsu, T. 2000. Midkine enhances
496 early stages of collagen gel contraction. *J. Biochem.*, **127**(2): 247-251.
- 497 Turner, C.E. 1998. Molecules in focus Paxillin. *Int. J. Biochem. Cell. Biol.*, **30**(9): 955-959.
- 498 Unoki, K., Okubo, A., Arimura, H., Uehara, F., Muramatsu, H., Kadomatsu, K., and
499 Muramatsu, T. 1995. [Beneficial effect of a retinoic acid responsive gene product, midkine,
500 on constant light-induced retinal damage in albino mice]. *Nippon. Ganka. Gakkai. Zasshi.*,
501 **99**(6): 636-641.
- 502 Weckbach, L.T., Gola, A., Winkelmann, M., Jakob, S.M., Groesser, L., Borgolte, J., Pogoda,
503 F., Pick, R., Pruenster, M., and Müller-Höcker, J. 2014. The cytokine midkine supports
504 neutrophil trafficking during acute inflammation by promoting adhesion via $\beta 2$ integrins
505 (CD11/CD18). *Blood.*, **123**(12): 1887-1896.
- 506 Werner, S., and Grose, R. 2003. Regulation of wound healing by growth factors and
507 cytokines. *Physiol. Rev.*, **83**(3): 835-870. doi: 10.1152/physrev.00031.2002.
- 508 Xing, B., Wu, F., Li, T., Qi, S., Xie, J., and Ye, Z. 2013. Experimental study of comparing
509 rhEGF with rh β FGF on improving the quality of wound healing. *Int. J. Clin. Exp. Med.*, **6**(8):
510 655.
- 511 Xu, C., Zhu, S., Wu, M., Han, W., and Yu, Y. 2014. Functional receptors and intracellular
512 signal pathways of midkine (MK) and pleiotrophin (PTN). *Biol. Pharm. Bull.*, **37**(4): 511-520.

- 513 Xue, M., Le, N.T., and Jackson, C.J. 2006. Targeting matrix metalloproteases to improve
514 cutaneous wound healing. *Expert. Opin. Ther. Targets.*, **10**(1): 143-155. doi:
515 10.1517/14728222.10.1.143.
- 516 Yamada, H., Inazumi, T., Tajima, S., Muramatsu, H., and Muramatsu, T. 1997. Stimulation of
517 collagen expression and glycosaminoglycan synthesis by midkine in human skin fibroblasts.
518 *Arch. Dermatol. Res.*, **289**(7): 429-433.
- 519 Zhao, S.L., Zhang, Y.J., Li, M.H., Zhang, X.L., and Chen, S.L. 2014. Mesenchymal stem
520 cells with overexpression of midkine enhance cell survival and attenuate cardiac dysfunction
521 in a rat model of myocardial infarction. *Stem. Cell. Res. Ther.*, **5**(2): 37. doi: 10.1186/scrt425.

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Figure Legends

Table 1. Growth factors and TBARS levels in the wound areas of healthy and diabetic rats on sacrificed times (mean $\pm$ SE).

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Table 1. Growth factors and TBARS levels in the wound areas of healthy and diabetic rats on sacrificed times (mean ± SE).

Parameters	Groups	Hour 0	Day 3	Day 7	Day 14	Day 28
EGF (ng/100 mg tissue)	Healthy (control) (HW)	1.72±0.40 ^{A.a}	1.63±0.13 ^{A.b}	1.99±0.23 ^{A.a}	2.48±0.66 ^{A.a}	2.5±0.22 ^{A.b}
	Healthy + Midkine (HM)	1.72±0.40 ^{C.a}	2.57±0.29 ^{BC.a}	2.83±0.31 ^{BC.a}	3.67±0.29 ^{AB.a}	4.19±0.55 ^{A.a}
	Diabetic (control) (DW)	1.88±0.28 ^{B.a}	2.60±0.25 ^{AB.a}	2.79±0.28 ^{A.a}	2.01±0.29 ^{AB.a}	2.69±0.22 ^{AB.b}
	Diabetic + Midkine (DM)	1.88±0.28 ^{A.a}	2.19±0.43 ^{A.ab}	2.54±0.43 ^{A.a}	2.77±0.98 ^{A.a}	2.4±0.43 ^{A.b}
MMP-8 (ng/100 mg tissue)	Healthy (control) (HW)	70.20±53.00 ^{B.a}	314.9±78.97 ^{A.a}	92.23±43.56 ^{B.a}	59.59±40.68 ^{B.a}	25.39±12.35 ^{B.a}
	Healthy + Midkine (HM)	70.20±53.00 ^{A.a}	67.72±26.73 ^{A.b}	8.65±1.33 ^{A.b}	59.60±51.81 ^{A.a}	22.89±14.04 ^{A.a}
	Diabetic (control) (DW)	29.90±19.32 ^{A.a}	3.67±1.70 ^{A.b}	19.97±14.44 ^{A.b}	3.48±3.33 ^{A.a}	3.17±1.36 ^{A.a}
	Diabetic + Midkine (DM)	29.90±19.32 ^{A.a}	6.85±2.77 ^{B.b}	1.22±0.46 ^{B.b}	0.91±0.16 ^{B.a}	0.66±0.48 ^{B.a}
PDGF (ng/100 mg tissue)	Healthy (control) (HW)	1.72±0.62 ^{C.a}	15.82±2.06 ^{AB.b}	17.98±3.68 ^{A.a}	7.92±4.27 ^{BC.c}	6.08±1.13 ^{C.a}
	Healthy + Midkine (HM)	1.72±0.62 ^{C.a}	15.1±1.87 ^{B.b}	18.74±2.36 ^{B.a}	26.02±2.73 ^{A.a}	14.24±2.80 ^{B.a}
	Diabetic (control) (DW)	1.81±0.93 ^{C.a}	25.29±1.52 ^{A.a}	18.07±2.02 ^{B.a}	14.40±1.98 ^{B.bc}	16.12±3.38 ^{B.a}
	Diabetic + Midkine (DM)	1.81±0.93 ^{C.a}	16.37±1.07 ^{AB.b}	17.95±2.23 ^{AB.a}	24.14±4.46 ^{A.ab}	11.50±5.65 ^{BC.a}
TGF-β (pg/100 mg tissue)	Healthy (control) (HW)	8.45±2.85 ^{A.a}	5.30±0.69 ^{A.b}	8.45±0.78 ^{A.a}	6.49±1.85 ^{A.b}	9.93±1.61 ^{A.b}
	Healthy + Midkine (HM)	8.45±2.85 ^{B.a}	6.58±1.32 ^{B.b}	10.24±2.42 ^{AB.a}	13.00±2.21 ^{AB.a}	14.24±2.80 ^{A.a}
	Diabetic (control) (DW)	7.49±0.56 ^{B.a}	11.73±1.19 ^{A.a}	7.68±1.46 ^{B.a}	7.55±1.63 ^{B.ab}	8.82±0.92 ^{AB.b}
	Diabetic + Midkine (DM)	7.49±0.56 ^{AB.a}	6.06±0.56 ^{B.b}	9.28±1.45 ^{AB.a}	10.29±1.16 ^{A.ab}	8.98±1.78 ^{AB.b}
VEGF (pg/100 mg tissue)	Healthy (control) (HW)	206.63±56.08 ^{A.a}	473.55±92.31 ^{A.a}	225.84±50.41 ^{A.b}	405.45±244.36 ^{A.ab}	185.97±63.98 ^{A.a}
	Healthy + Midkine (HM)	206.63±56.08 ^{C.a}	323.13±154.65 ^{C.ab}	793.45±109.94 ^{AB.a}	922.79±193.16 ^{A.a}	454.83±123.19 ^{BC.a}
	Diabetic (control) (DW)	166.82±65.20 ^{A.a}	193.75±100.73 ^{A.ab}	206.79±84.85 ^{A.b}	47.34±10.04 ^{A.b}	310.91±153.14 ^{A.a}
	Diabetic + Midkine (DM)	166.82±65.20 ^{B.a}	89.70±42.11 ^{B.b}	83.11±24.90 ^{B.b}	435.26±141.58 ^{A.ab}	130.68±29.48 ^{B.a}
TBARS (μM/25 mg tissue)	Healthy (control) (HW)	1.99±0.46 ^{A.b}	0.88±0.12 ^{B.a}	0.82±0.10 ^{B.a}	1.06±0.36 ^{B.a}	1.04±0.17 ^{B.b}
	Healthy + Midkine (HM)	1.99±0.46 ^{A.b}	2.16±0.97 ^{A.a}	1.79±0.85 ^{A.a}	2.25±1.10 ^{A.a}	0.73±0.03 ^{A.b}
	Diabetic (control) (DW)	25.45±8.72 ^{A.a}	0.97±0.16 ^{B.a}	1.11±0.16 ^{B.a}	1.40±0.60 ^{B.a}	1.49±0.19 ^{B.a}
	Diabetic + Midkine (DM)	25.45±8.72 ^{A.a}	2.32±1.45 ^{B.a}	0.91±0.8 ^{B.a}	0.79±0.05 ^{B.a}	0.84±0.13 ^{B.b}

Different letters in the same column (^{a,b,c}), and line (^{A,B,C}) are statistically significant (P<0,05).

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