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Small molecule inhibition of dipeptidyl peptidase-4 enhances bone marrow progenitor cell function and angiogenesis in diabetic wounds

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Abstract

In diabetes, stromal cell-derived factor-1 (SDF-1) expression and progenitor cell recruitment are reduced. Dipeptidyl peptidase-4 (DPP-4) inhibits SDF-1 expression and progenitor cell recruitment. Here we examined the impact of the DPP-4 inhibitor, MK0626, on progenitor cell kinetics in the context of wound healing. Wildtype (WT) murine fibroblasts cultured under high-glucose to reproduce a diabetic microenvironment were exposed to MK0626, glipizide, or no treatment, and SDF-1 expression was measured with ELISA. Diabetic mice received MK0626, glipizide, or no treatment for 6 weeks and then were wounded. Immunohistochemistry was used to quantify neovascularization and SDF-1 expression. Gene expression was measured at the RNA and protein level using quantitative polymerase chain reaction and ELISA, respectively. Flow cytometry was used to characterize bone marrow-derived mesenchymal progenitor cell (BMMPC) population recruitment to wounds. BM-MPC gene expression was assayed using microfluidic single cell analysis. WT murine fibroblasts exposed to MK0626 demonstrated increased SDF-1 expression. MK0626 treatment significantly accelerated wound healing and increased wound vascularity, SDF-1 expression, and dermal thickness in diabetic wounds. MK0626 treatment increased the number of BM-MPCs present in bone marrow and in diabetic wounds. MK0626 had no effect on BM-MPC population dynamics. BMMPCs harvested from MK0626-treated mice exhibited increased chemotaxis in response to SDF-1 when compared to diabetic controls. Treatment with a DPP-4 inhibitor significantly improved wound healing, angiogenesis, and endogenous progenitor cell recruitment in the setting of diabetes.

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AUTHOR CONTRIBUTIONS

A.J.W. and Z.N.M. contributed to the idea generation, design, and completion of this work. A.J.W., Z.N. M., D.D., J.A.B., M.S.H., L.H.F., S.K., V.W.W., G.G. W., F.G., M.J., M.B., and M.T.L. contributed to the experimental work and manuscript preparation. G.C.G. guided the idea generation, experimental work, and manuscript revision.

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SUPPLEMENTARY MATERIALS

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INTRODUCTION

Diabetes mellitus is a major public health problem, costing more than \$200 billion and accounting for more than 20% of total health care dollars spent in the United States (US) annually^{1,2}. Clinically, diabetes is characterized by vascular abnormalities of the retina and kidneys³, as well as global impairment in the neovascular response to tissue hypoxia⁴. Nonhealing wounds, resulting from impaired blood vessel formation, are a major cause of diabetes-associated disability². Nonhealing diabetic wounds often require limb amputation, thus exacerbating an already significant burden on the quality of life of patients and their care providers. With the prevalence of diabetes among US adults expected to rise to nearly 1 in 3 by the year 2050⁵, new treatment options are needed to both prevent and treat diabetes-associated disability.

Our laboratory has previously explored the mechanisms by which diabetes impairs neovascularization and wound healing. We have found that hyperglycemia hinders the activation of the transcriptional regulator hypoxia-inducible factor-1 α .⁶ Hypoxia-inducible factor-1 α is normally stabilized under low oxygen tension, allowing it to enter the nucleus and stimulate expression of several downstream signaling molecules including vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1 (SDF-1).^{4,7} These downstream effectors play key roles in normal wound healing,⁸ and the exogenous delivery of each has been shown to have beneficial effects in diabetic wounds.^{9,10}

SDF-1 in particular has attracted increased interest for its potential role in wound healing.^{10,11} This chemokine binds the CXCR4 receptor, initiating a variety of downstream effects that include the recruitment of circulating progenitor cells to areas of hypoxia.¹² Diabetes notably impairs progenitor cell adhesion and migration in response to hypoxia¹³ and disrupts bone marrow-derived mesenchymal progenitor cell (BM-MPC) kinetics by depleting pro-vasculogenic subpopulations in the bone marrow reservoir.¹⁴ In a related study, exogenous SDF-1 delivery in diabetic wounds was found to improve wound healing and restore normal progenitor cell recruitment.¹⁰ Although delivery of exogenous SDF-1a or subcutaneous administration of a peptide hormone (ie, relaxin), which can promote SDF-1a activity,¹⁵ display some beneficial effects on diabetic wound healing, delivery of therapeutic proteins and peptides can be challenging. In this respect, using small molecule inhibitors to enhance the expression of endogenous SDF-1a has an advantage over other approaches. Moreover, DPP-4 inhibitors are approved antidiabetic drugs whose use in the market has been increasing rapidly.^{16,17}

DPP-4 is a membrane-bound extracellular peptidase that has the ability to cleave the SDF-1 peptide chain resulting in significant changes in functional activity.^{18,19} To increase endogenous levels of SDF-1, we targeted its main regulator, dipeptidyl peptidase-4 (DDP-4) in hopes of increasing the functional expression of SDF-1.²⁰ DPP-4 is an endogenous serine exopeptidase that cleaves SDF-1, thereby inhibiting its chemotactic effects on circulating progenitor cells¹⁸ (Fig 1A). Thus, we hypothesize that DPP-4 inhibition may improve diabetic wound healing through enhancement of the SDF-1 signaling pathway and increased BM-MPC recruitment. We compared this to the longterm effects of glipizide treatment, a common sulfonylurea, which has been shown to be efficacious in reducing glucose levels.²¹

We used a mouse high-fat diet model of diabetes, as genetic models (eg, db/db) are less representative of the true pathophysiology of the disease.^{22,23} In this study, we focused on the potential impact of DPP-4 modulation on wound healing and BM-MPC recruitment and function.

MATERIAL AND METHODS

Animals.

Wildtype (WT) mice [C57Bl/6] (Jackson Laboratories, Bar Harbor, Maine) were used and fed a highfat diet (Research Diets, New Brunswick, NJ; Fig 1B). The diet consisted of 60% calories from fat, 20% from carbohydrate, and 20% from protein. Blood glucose levels were measured every 4 weeks (Supplemental Figure 1). There were a total of 3 groups (WT High Fat Diet, WT High Fat Diet/Glipizide, and WT High Fat Diet/MK0626) with each group containing an N = 12. The animals were kept on a high-fat diet for a total of 20 weeks.¹⁹ All animals were confirmed diabetic by Week 16. All mice were housed in the Stanford University Veterinary Service Center in accordance with NIH and institution approved animal care guidelines. All procedures were approved by the Stanford Administrative Panel on Laboratory Animal Care.

Glucose tolerance tests.

In order to track the onset of type II diabetes, glucose tolerance tests were performed at weeks 0, 4, 8, 12, and 16.²⁴ Prior to glucose administration, mice were fasted for 16 hours to achieve a fasting blood glucose level. Mice then received an intraperitoneal injection of 20% glucose at a volume of (μL) = $10 \mu\text{L} \times \text{body weight (g)}$. A small incision was created on the distal portion of the mouse's sterilized tail using a scalpel and gently squeezed to obtain 2 drops of blood, the first of which was discarded. Blood glucose levels were assessed using the minimally invasive OneTouch Ultra Blood Glucose Monitoring System (Johnson and Johnson, New Brunswick, New Jersey). Blood samples were assayed at 0, 15, 30, 60, and 120 minutes following glucose administration. The tests were performed in a blinded manner.

SDF-1 ELISA.

Fibroblasts were isolated from the dorsal skin of WT mice as previously described.²⁵ Fibroblasts were plated out on three 6-well plates (1.5×10^5 cells/well) with high glucose (4.5 g/L) Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Fibroblasts were then exposed to high glucose medium in hypoxia (37°C, 1% oxygen) for 96 hours with serial medium changes occurring every 24 hours. The cells were then exposed to either MK0626 (10 μM) or high glucose media alone. Total protein was quantified using the Quick Start Bradford Protein Assay Kit (Bio-Rad, Hercules, California). SDF-1 protein levels were measured using the Mouse CXCL12/SDF-1 alpha Quantikine ELISA Kit (R&D Systems, Minneapolis, Minnesota).

***In vivo* excisional wound model.**

Wounding was performed using a previously described murine splinted excisional wound healing model.²⁶ Using a 6-mm biopsy punch (Integra Miltex, Plainsboro, New Jersey), 2 sterile 6-mm full thickness wounds were excised from either side of the midline of the shaved dorsum of anesthetized mice. Each wound was held open by a donut-shaped silicone splint with a 10-mm diameter centered on the wound and secured to the skin using an immediate-bonding adhesive (Krazy Glue; Elmer's Inc., Columbus, Ohio) and 6-0 nylon suture (Ethicon Inc., Somerville, NJ) to simulate human wound healing.²⁶ All wounds were covered using an occlusive dressing (Tegaderm; 3M, St. Paul, Minnesota). Digital photographs were taken every other day until wound closure. The wound area was measured using ImageJ software (NIH) (n = 12 wounds per group). All measurements were performed by a blinded observer.

Histology and immunohistochemistry.

Upon wound closure, mice were euthanized and wounds were harvested with an 8 mm biopsy punch (Integra Miltex, Plainsboro, New Jersey). Skin tissues were fixed in 4% paraformaldehyde overnight followed by serial dehydration in ethanol and embedding in paraffin. In order to assess dermal thickness, 5- μ m sections were then stained for H&E and photographed at 10X magnification.

Immunofluorescence staining for CD31 and SDF-1 was then performed to assess wound vascularization. Frozen tissue samples were prepared by OCT Compound (Sakura Finetek US, Torrance, California) and cryosectioned for immunohistochemistry.²⁷ Slides underwent antigen retrieval using 0.01M sodium citrate buffer in PBS solution, followed by blocking for 1 hour with 5% goat serum in PBS. Sections were then incubated in CD31 antibody (ab28364; Abcam, Cambridge, Massachusetts) or SDF-1 antibody (ab25117; Abcam, Cambridge, Massachusetts) overnight, followed by a secondary antibody. Lastly, slides were DAPI-stained for cell nuclei and mounted. ImageJ was used to binarize immunofluorescent images taken with the same excitation, gain, and exposure settings.²⁸ Intensity threshold values were set automatically and quantification of staining was determined by pixel-positive area normalized to dermal area. All histology was conducted in quadruplicate.

BM-MPC harvest.

After euthanasia, mice femora and tibiae were harvested and washed in serial dilutions of Betadine (Purdue Pharma, Stamford, Connecticut). Epiphyses were removed and the bones flushed with 2% FBS in phosphate-buffered saline (PBS) to retrieve the marrow plugs. Plugs were then dissociated by trituration, filtered through a 100 μ m cell strainer, and pelleted by centrifugation at 300Xg for 8 minutes. Cells were then either prepared for culture or for flow cytometric analysis. Cells prepared for culture were resuspended in DMEM with 10% FBS and 1% penicillin/streptomycin (Life Technologies, Grand Island, New York) in 4.5 g/L glucose for hyperglycemic conditions. Total bone marrow was then plated at 1×10^6 cells/cm² on tissue culture plastic. Cells were subcultured at 80%–90% confluence and used after passage three.

Flow cytometric analysis.

FACS analysis was conducted on bone marrow harvested from femur and tibia, and tissue harvested from wounds. On postinjury day 20, wounds from control, glipizide, and MK0626 groups were excised using an 8 mm punch biopsy (Integra Miltex, Plainsboro, New Jersey) and digested for 1 hour in Liberase TL (0.5 mg/mL; Roche) at 37°C. Digested tissue samples and BM-MPCs were then filtered, centrifuged, and incubated with fluorescently conjugated antibodies against CD45 (PE; BD Biosciences, San Diego, California), stem cell antigen-1 (Sca-1) (FITC, BD Biosciences), cKit (PE-Cy7; BD Biosciences), and lineage markers (Lin: TER119, B220, CD4, CD8, CD11b, Gr-1; PE-Cy5; eBiosciences). Cells not stained with these antibodies were incubated with the proper controls or left unstained. Cells were washed and analyzed using FACS Aria. Data were analyzed using FlowJo digital fluorescence-activated cell sorting software by a single blinded evaluator (Tree Star Inc, Ashland, Oregon).

Chemotaxis assay.

BM-MPCs (2×10^4 cells) suspended in DMEM were seeded onto 24-well transwell inserts (8 μ m pore size; Corning Costar). At time point zero, 100 nM recombinant murine CXCL12 (R&D Systems) was added to the bottom chamber. Plates were placed in 21% oxygen and the cells were allowed to migrate for 6 hours at 37°C. Nonmigrating cells were removed from the top surface of the upper chamber using a cotton tip applicator. Migrating cells adhering to the undersurface of the upper chamber were counted by 4',6'-diamidino-2-phenylindole (DAPI; Vector) positivity. A total of 5 high-powered fields were selected randomly for counting using a Zeiss Axioplan 2 microscope at 200 $\times$ magnification.

Quantitative polymerase chain reaction analysis.

Day 7 wounds were snap frozen on dry ice and stored at -80°C. Taqman Gene Expression Assays (Applied Biosystems, Foster City, California) were used for in vitro transcriptional analyses. Primers used were *tgfb1*, *tgfb3*, *vegfa*, *mcp1*, and *mmp9*.

Microfluidic single cell analysis.

Fresh BM-MPCs (Lin-/CD45-/Sca-1+) were sorted as single cells into 10 μ L of lysis buffer using a Becton Dickinson FACS Aria flow cytometer. Using low cycle preamplification, cDNA was obtained using Cells Direct (Life Technologies) with Taqman assay primer sets (Applied Biosciences, Foster City, California), per the manufacturer's specifications. cDNA was loaded onto the 48.48 Dynamic Array (Fluidigm, South San Francisco, California) for quantitative polymerase chain reaction amplification using Universal PCR Master Mix (Applied Biosystems) with Taqman assay primer sets, as previously described.²⁹

Statistical analysis.

All experiments were conducted in triplicates unless stated otherwise. Results are presented as mean $\pm$ standard error of the mean. Data analysis was performed using either Student's *t* test (migration assay) or one-way ANOVA (SDF-1 ELISA). Results were considered significant for **P* < 0.05. Unless otherwise stated, all measurements were obtained from independent experiments performed at least in triplicate.

RESULTS

MK0626 enhances diabetic wound healing.

A standardized excisional wound model was used to examine the effects of MK0626 on murine diabetic wound healing.³⁰ Mice treated with MK0626 had a significantly faster healing rate than glipizide-treated and untreated mice (Fig 2A–C). This effect became evident by day 6, with MK0626 wounds showing significantly reduced mean wound area relative to original wound size ($63.15 \pm 2.41\%$) as compared to glipizide-treated ($74.30 \pm 1.92\%$, $P = 0.04$) and untreated groups ($75.01 \pm 0.78\%$, $P = 0.02$). Furthermore, MK0626 wounds closed significantly faster (15.33 ± 0.42 days) than glipizide-treated (17.33 ± 0.42 days, $P = 0.007$) and untreated groups (19.00 ± 0.45 days, $P = 0.005$).

MK0626 treatment increases vascularization and CXCL12/SDF-1 expression.

To evaluate the mechanism by which MK0626 treatment improves wound healing in diabetic wounds, immunohistochemistry was performed on tissue samples harvested at post-injury day 7 (Fig 3A–D). Wounds of MK0626-treated mice showed significantly increased vascular density (1.05 ± 0.11 AU) as compared to glipizide-treated (0.75 ± 0.04 AU, $P = 0.04$) and untreated groups (0.63 ± 0.07 AU, $P = 0.004$; Fig 3A, B). Further, wounds of MK0626-treated mice showed significantly increased SDF-1 expression (1.05 ± 0.14 AU) as compared to glipizide treated (0.291 ± 0.197 AU, $P = 0.04$) and untreated groups (0.179 ± 0.13 AU, $P = 0.04$; Fig 3C, D). There was no significant difference between glipizide-treated and untreated groups.

MK0626 treatment improves wound quality.

H&E was performed to assess tissue quality in healed wounds. Dermal thickness was significantly increased in wounds from MK0626-treated mice (66.56 ± 5.04 μm), as compared to the glipizide-treated (49.57 ± 4.01 μm , $P = 0.03$) and untreated groups (44.87 ± 2.91 μm , $P = 0.03$; Fig 3E, F). These results suggest that increased neovascularization underlies improved wound healing in MK0626-treated mice with subsequent improvement in the dermal thickness of the healed wounds.

MK0626 expands BM-MPC population in bone marrow.

As diabetes has been shown to disrupt BM-MPC population dynamics and function,¹⁴ and SDF-1 is known to regulate the bone marrow stem cell niche,^{31,32} we sought to investigate the effect of MK0626 on BM-MPC population dynamics. Bone marrow was harvested from MK0626, glipizide, or untreated mice and evaluated using flow cytometry (Fig 4A, B). FACS analysis showed that the proportion of BM-MPCs with the surface marker profile Lin-/CD45-/Sca+1 cells was significantly increased in MK0626-treated (0.011 ± 0.0004), as compared to glipizide-treated (0.007 ± 0.0005 , $P < 0.01$) and untreated groups (0.007 ± 0.011 , $P < 0.01$).

Single cell analysis demonstrates symmetrical depletion of MPC subpopulations.

Our lab previously found that specific pro-vasculogenic BM-MPC subpopulations become depleted in the setting of diabetes.³³ In order to determine whether MK0626-induced

improvements in BM-MPC function were related to changes in population dynamics, we examined the effect of MK0626 on the BM-MPC transcriptome at a single cell level. Using microfluidic based single cell analysis, we found similar gene expression profiles in MK0626-treated, glipizide-treated, or untreated mice (Fig 5A, B, S2, S3). These results indicate that the therapeutic benefit of MK0626 is likely not due to alterations in BM-MPC gene expression, but rather through the increased total number and recruitment.

MK0626 enhances recruitment of BM-MPCs to the wound site.

After identifying that MK0626 treatment increased the number of BM-MPCs in the bone marrow, we explored the effect of MK0626 treatment on BM-MPC recruitment potential. FACS analysis showed that MK0626 treatment led to a significant increase in the proportion of BM-MPCs present at the wound site ($0.08 \pm 0.01\%$), when compared with both glipizide-treated ($0.05 \pm 0.003\%$, $P < 0.05$) and untreated groups ($0.04 \pm 0.005\%$, $P < 0.05$; Fig 4C).

MK0626 increases SDF-1 expression in fibroblasts and improves BM-MPC responsiveness to SDF-1.

Having established that MK0626 improves wound healing and BM-MPC recruitment, we investigated potential mechanisms for this effect. First, we explored the effect of MK0626 treatment on SDF-1 expression in fibroblasts cultured under high-glucose conditions. MK0626-treated fibroblasts demonstrated significantly increased SDF-1 expression, as compared to untreated fibroblasts (0.44 ± 0.03 ng/mL vs 0.29 ± 0.08 ng/mL, $P = 0.04$; Fig 6A). These results suggest that MK0626 treatment enhances fibroblast SDF-1 expression in the healing wound.

Next, we isolated BM-MPCs from control, glipizide, or MK0626 treated mice using FACS. BM-MPCs were expanded in culture, and their migratory function was measured using an SDF-1 migration assay (Fig 6B, C). BM-MPCs harvested from MK0626-treated mice demonstrated significantly increased migration (8.4 ± 0.94 cells/hpf) compared to BM-MPCs harvested from both Glipizide-treated (6.4 ± 0.73 cells/hpf, $P < 0.05$) and untreated mice (5.4 ± 0.60 cells/hpf, $P = 0.009$).

These results suggest that enhanced recruitment of BM-MPCs following MK0626 treatment results not only from increased SDF-1 expression at the wound site, but also an enhanced capacity of BM-MPCs to respond to SDF-1 signaling.

MK0626 modifies gene expression in murine wounds.

Quantitative RTPCR analysis demonstrated that MK0626 treated wounds had significantly lower mRNA expression of *tgfb1* (0.969 ± 0.12 vs 2.98 ± 0.14), *tgfb3* (0.794 ± 0.12 vs 1.53 ± 0.10), *mcp1* (1.19 ± 0.19 vs 2.41 ± 0.16), and *mmp9* (1.58 ± 0.16 vs 2.50 ± 0.25) than untreated wounds ($P < 0.05$). Alternatively, *vegfa* expression showed no difference between groups ($P > 0.05$; Fig 6D).

DISCUSSION

Chronic wounds reduce quality of life for patients and are an increasing burden on the health care system.^{2,34} The high incidence of chronic wounds in diabetic patients, which can necessitate limb amputation, underlines a vital need for new, effective therapies.

Diabetic wound healing is characterized by defects in hypoxia-induced neovascularization in the skin.³ Although diabetic wound healing has been studied extensively, effective therapeutic options for patients remain elusive. Diabetes medications, such as the sulfonylurea agent glipizide, have shown little to no effect on the sequelae of diabetes such as cardiovascular disease, stroke, and chronic wound progression. The ability of endogenous stem cells, such as BM-MPCs, to support neovascularization suggests a potential therapeutic approach for diabetic wounds.³⁵ In this study, we sought to test a novel small molecule inhibitor of DPP-4 (MK0626) for its effect on endogenous progenitor cells and diabetic wound healing. As a relatively new class of oral antidiabetic agents, DPP-4 inhibitors are typically prescribed to patients whose response to sulfonylureas and/or metformin is unsatisfactory. Along with glucagon-like peptide-1, the prescription of DPP-4 inhibitors in the type II diabetes market has increased substantially worldwide.^{16,17} In addition to its primary action on glycemic control, its additional effects associated with acceleration of wound healing offers further benefit for diabetic patients with chronic ulcers.

Using a murine excisional wound model, we found that DPP-4 inhibition with MK0626 significantly accelerates diabetic wound healing, as compared to glipizide-treated and untreated mice. A significant increase in vascularity and in dermal thickness of the MK0626-treated mice was also noted. We then explored the potential mechanisms underlying these findings. Given the established trophic effects of SDF-1 on progenitor cells,³⁶ we investigated the effects of MK0626 treatment on progenitor cell presence in the bone marrow and at the wound site. Our data indicate that MK0626 increases both the number of BM-MPCs available for recruitment, as well as the number of BM-MPCs recruited to the wound. This was an interesting finding, as our laboratory previously demonstrated that diabetes negatively influences the function and number of circulating BM-MPCs,³³ and Gallagher et al previously established that diabetes impairs progenitor cell recruitment.¹⁰ These results complement those of Li et al, which demonstrated that treatment with MK0626 increases the number of circulating endothelial progenitor cells and improves blood flow in a murine model of hind-limb ischemia.³⁷ MK0626 appears to at least partially reverse the impairments in progenitor cell number and recruitment seen in diabetes.

Our laboratory has previously found that diabetes not only influences availability and recruitment of BM-MPCs, but influences their function and causes subpopulation gene expression deficits.³³ To assess the effects of DPP-4 inhibition on BM-MPC function, we tested the migratory capacity of MPCs cultured in high-glucose conditions and exposed to MK0626. Our data show that MPC migratory capacity was increased in the MK0626 group, reflecting improved MPC function. Furthermore, fibroblasts cultured under high-glucose conditions that were exposed to MK0626 expressed significantly more SDF-1 protein than their untreated counterparts. In order to test whether MK0626 treatment could restore MPC transcriptional heterogeneity, we then analyzed the gene expression of BM-MPCs harvested

from diabetic bone marrow and exposed to MK0626 on a single cell level. Our data indicate that MK0626 treatment had little effect on the BM-MPC transcriptome. It should be noted, however, that genes associated with chemotaxis or migration, were not inspected at a single cell level. Thus, the observed enhancement in BM-MPC recruitment is likely due to increased chemotactic signaling by SDF-1 as a result of its protection from DPP-4-mediated degradation.

Inflammation and fibrosis are an integral part of normal healing.^{38,39} However, excessive inflammation is associated with the progression to nonhealing wounds,⁴⁰ and excessive fibrosis associated with the formation of hypertrophic scars.⁴¹ Therefore, therapeutic modulation of these pathways may help prevent chronic wound progression and improve scarring for patients. Interestingly, DPP-4 pathway activation has been shown to correlate with inflammation through T cell and macrophage activation.⁴² Additionally, Bostick et al recently showed that DPP-4 inhibition reduces myocardial fibrosis in mice.⁴³ To test whether DPP-4 had any effect on fibrosis or inflammation, we performed qRT-PCR on several pro-fibrotic and pro-inflammatory genes implicated in wound healing. We found that wounds of MK0626-treated mice exhibited reduced expression of the pro-fibrotic genes *tgfb1*, *tgfb3*, and *mmp9*, as well as the pro-inflammatory gene *mcp1*, compared to untreated wounds. We previously identified a subpopulation of fibroblasts, lineage-traced by the embryonic expression of Engrailed-1 that was responsible for scarring and fibrosis. Flow cytometry revealed DPP-4 labeled this subpopulation of fibroblasts, and inhibition of DPP-4 via topical application of diprotin A resulted in decreased scarring and fibrosis.⁴⁴ Taken together, these findings suggest that DPP-4 inhibition can improve wound healing through a variety of mechanisms that include neovascular, inflammatory, and fibrotic pathway components.

In summary, this study demonstrates that treatment with a DPP-4 inhibitor significantly improves wound healing, angiogenic capacity, and endogenous progenitor cell recruitment in the setting of diabetes. Improvements in MPC number, function, and trafficking in both the bone marrow and wound site in response to SDF-1 appear to play an important role in this process. As MK0626 has shown promise in treating a variety of other clinical problems including hepatic steatosis,⁴⁵ metabolic cardiomyopathy,⁴³ and obesity-induced renal injury,⁴⁶ testing the safety and efficacy of MK0626 in more advanced animal models will be needed to assess this drug's suitability for clinical translation.

CONCLUSION

Here we demonstrate that DPP-4 inhibition with MK0626 improves diabetic wound healing through increased angiogenesis, dermal thickness, and recruitment of MPCs from the bone marrow. Furthermore, MK0626 treatment increases SDF-1 expression in fibroblasts cultured under high-glucose conditions, and increases the chemotactic potential of cultured MPCs, representing a potential mechanism for enhanced BM-MPC recruitment. Modulating the SDF-1 pathway through DPP-4 inhibition with MK0626 stimulates angiogenesis, increases progenitor cell recruitment, and represents a promising therapeutic strategy in the SDF-1 deficient diabetic state. Given these encouraging results, further studies are needed to examine the effect of MK0626 treatment for diabetic wounds in the clinical setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BM-MPC	bone marrow-derived mesenchymal progenitor cell
DMEM	Dulbecco's Modified Eagle Medium
DPP-4	dipeptidyl peptidase-4
FBS	fetal bovine serum
MPC	mesenchymal progenitor cell
NIH	National Institute of Health
PBS	phosphate buffered saline
qRT-PCR	quantitative reverse transcription polymerase chain reaction
SDF-1	stromal cell-derived factor-1
VEGF	vascular endothelial growth factor
WT	wild type

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Background

Diabetes is a growing public health concern resulting in an increase in the prevalence of diabetic nonhealing wounds. In these chronic wounds, there is a disruption of the pivotal hypoxia-inducible factor1a (HIF-1a)/stromal cell-derived factor-1 (SDF-1) neovascularization pathway.

Translational Significance

We demonstrate here the efficacy of an oral therapy that modulates the HIF-1a/SDF-1 axis through inhibition of DPP-4. This results in decreased enzymatic cleavage of SDF-1 and increased neovascularization, stem cell recruitment, and ultimately improves diabetic nonhealing wounds.

Fig 1. MK0626 schematics. (A) Effect of MK0626 on DPP-4 and SDF-1. (B) Experimental design. DPP-4, dipeptidyl peptidase-4; SDF-1, stromal cell-derived factor-1.

Fig 2. MK0626 improves cutaneous wound healing in a humanized model of excisional wound healing in mice. (A) Gross photographs of wounds ($n = 12$ per group) during healing in control (top row) vs glipizide-treated (middle row) and MK0626-treated (bottom row) mice. (B) Wound healing curve showing wound size as a percentage of original wound vs time in days ($n = 12$ per group; $*P < 0.05$). (C) Bar graph showing the difference in time to complete healing of wounds in control vs glipizide-treated and MK0626-treated mice ($n = 12$ per group; $*P < 0.05$).

Fig 3.

MK0626 treatment increases vascularization and CXCL12/SDF-1 expression. (A) CD31 immunofluorescence of day 7 wounds in control vs glipizide-treated and MK0626-treated mice. (B) Bar graph of CD31 immunofluorescence from (A) (* $P < 0.05$). (C) SDF-1 immunofluorescence of day 7 wounds in control vs glipizide-treated and MK0626-treated mice. (D) Bar graph of SDF-1 immunofluorescence from (C) (* $P < 0.05$). (E) H&E showing dermal thickness of day 7 wounds in control vs glipizide-treated and MK0626-treated mice. Region of healed wound is identified by black dashed line (F) Bar graph of dermal thickness (microns) from (E) (* $P < 0.05$). HPF, high-powered field; SDF-1, stromal cell-derived factor-1.

Fig 4. MK0626 expands BM-MPC population and enhances recruitment. (A) FACS plots showing Lin-/CD45-/Sca-1+ cells in the bone marrow of control vs glipizide-treated and MK0626-treated mice. (B) Bar graph of FACS plots from (A) (* $P < 0.05$). (C) Bar graphs showing Lin-/CD45-/Sca-1+ cells in wounds of control vs glipizide-treated and MK0626-treated mice (* $P < 0.05$).

Fig 5. Single cell analysis demonstrates symmetrical depletion of MPC subpopulations. Hierarchical clustering of BM-MPCs from control vs glipizide-treated and MK0626-treated mice by (A) treatment group and (B) cell clusters.

Fig 6.

MK06266 increases SDF-1 expression in fibroblasts and modifies gene expression in wounds. (A) SDF-1 expression in fibroblasts cultured in high glucose conditions with or without MK0626 (* $P < 0.05$). (B) Immunofluorescence imaging of BM-MPCs from control vs glipizide-treated and MK0626-treated mice undergoing SDF-1 migration assay. (C) Bar graph of immunofluorescence imaging from (B) (* $P < 0.05$). (D) qRT-PCR of MK0626-treated wounds demonstrate significantly lower mRNA expression of *tgfb1*, *tgfb3*, *mcp1*,

and *mmp9* vs control wounds (* $P < 0.05$). HPF, high-powered field; SDF-1, stromal cell-derived factor-1.

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