Exendin-4, a glucagon-like peptide-1 receptor analogue, accelerates diabetic wound healing

Jun-Neng Roan, MD,a,b Han-Ni Cheng, MSc,c Chao-Chung Young, MD, PhD,d Chi-Ju Lee, MSc,e Ming-Long Yeh, PhD,e,f Chwan-Yau Luo, MD, MSc,b Yau-Sheng Tsai, PhD,a and Chen-Fuh Lam, MD, PhDg,h,*

a Institute of Clinical Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan
b Division of Cardiovascular Surgery, Department of Surgery, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan
c Department of Anesthesiology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan
d Department of Dermatology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan
e Department of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan
f Medical Device Innovation Center, National Cheng Kung University, Tainan, Taiwan
g Department of Anesthesiology, Buddhist Tzu-Chi General Hospital and Tzu-Chi University School of Medicine, Hualien, Taiwan
h Department of Anesthesiology, Taipei Medical University Hospital and School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

A B S T R A C T

Background: Diabetes disregulates inflammatory responses and impairs vascular function in wounds. Glucagon-like peptide-1 receptor (Glp-1R) agonists are hypoglycemic agents with pleiotropic vascular protective and anti-inflammatory effects. The therapeutic potential of a Glp-1 analogue in a diabetic rat model of excisional wound injury was investigated.

Materials and methods: Excisional wounds were created on the dorsum of streptozotocin-induced diabetic rats, which received placebo or Glp-1 analogue exendin-4 (Ex4; 0.5 μg/kg/d, i.p.) for 2 wk. The final-to-initial wound area ratio was measured for 14 d. Levels of superoxide anions and proinflammatory cytokines in wounds were determined.

Angiogenesis was assessed using the Matrigel assay. Expression levels of proangiogenic factors and extracellular matrix proteins were measured.

Results: Ex4 restored wound closure in diabetic rats and significantly suppressed the generation of superoxide anions and interleukin-6 in wounds. The number of circulating endothelial progenitor (CD34+/KDR+) cells increased significantly in Ex4-treated diabetic
rats, which also showed increased capillary tube formation. Protein levels of vascular endothelial growth factor receptor-2, phosphorylated endothelial nitric oxide synthase, matrix metalloproteinase-2, and transforming growth factor-β were increased in diabetic rats receiving Ex4 therapy. Ex4-enhanced vascularity, dermal regeneration, and epidermal regeneration, while it decreased hemorrhaging and increased the number of proliferative cells in the dermis.

Conclusions: Ex4 accelerated excisional wound healing in subjects with diabetes. Glp-1R activation attenuates inflammatory response and enhances angiogenesis during the early proliferation phase of wound healing in diabetic subjects, while it enhances transforming growth factor-β/matrix metalloproteinase-mediated regeneration during the maturation phase. These results suggest that Ex4 could be used as a standard hypoglycemic agent in diabetic patients with wound injury.

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Introduction

Wound healing involves the orchestration of four complex dimensional processes which include inflammation, angiogenesis, and regenerative reactions. These processes were commonly categorized into inflammation, proliferation, and remodeling phases. Diabetes mellitus (DM) is the most common systemic disease that impairs wound healing, which leads to nonhealing and chronic ulcers. More than 20% of patients with diabetes develop foot ulcers during their lifetime. A nationwide survey in the United States showed a nearly 20% increase of hospital admission for diabetic foot ulcers. Diabetes also significantly increases medical expense in the management of chronic wounds, including revascularization and osteomyelitis, by additional US $9-13 billions to the costs associated with diabetes itself.

There are multiple factors that impair the recovery of a diabetic wound, including focal vascular insufficiency, dysregulation of inflammatory, and angiogenic responses, formation of advanced glycation products, and the presence of peripheral neuropathy. Persistence of inflammation and neutrophil infiltration are characterized by the chronic upregulation of proinflammatory cytokines and superoxide anions in the diabetic wounds. Hyperglycemia has also been known to impair proangiogenic effect by the downregulation of endothelial nitric oxide synthase (eNOS) activity and the attenuation of mobilization of endothelial progenitor cells (EPCs). Hence, endothelial dysfunction associated with suppression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF) plays a major role in the delayed wound healing during the proliferative phase in diabetes. In the remodeling or mature phase of wound healing, diabetes affects the biosynthesis of extracellular matrix growth factors and matrix metalloproteinases (MMPs), thereby attenuating wound tensile strength.

Glucagon-like peptide-1 (Glp-1) is a gut-derived hormone secreted by the small intestine under a fine-tuned regulation of blood glucose concentration. Glp-1 stimulates the release of insulin through binding to the Glp-1 receptor (Glp-1R) on pancreatic beta cells. In addition to hypoglycemic effect, Glp-1 and its receptor agonists have recently been reported to exert cardioprotective effects in the experimental and clinical studies. The protective effect of Glp-1 is attributed to the suppression of programmed cell death and amelioration of oxidative stress-induced vascular endothelial dysfunction. This study tested the hypothesis that parenteral administration of a Glp-1 analogue, exendin-4 (Ex4), might enhance wound healing in an experimental model of diabetes due to the systemic anti-inflammatory and proangiogenic responses that were independent from its glycemic control effect.

Materials and methods

Induction of hyperglycemia in rats

The animal studies were conducted in compliance with the Animal Center of the National Cheng Kung University and approved by the Institutional of Animal Care and Use Committee (IACUC approval no: 103303; The National Cheng Kung University, Tainan, Taiwan). Male Sprague–Dawley rats at the age of 6-8 wk (200-300 g) were used in this study. Diabetes was induced by a single dose of streptozotocin (65 mg/kg, i.p.). Hyperglycemia, defined as a random blood glucose level over 250 mg/dL, was confirmed 72 h later using a glucose meter sensor (Accu-Chek Active, Roche). Physiological variables including body weight, water, and chow consumption were recorded.

Rat model of wound healing

A full-thickness circular excisional wound (approximately 2 cm in diameter) was created on the dorsum of rat along the midline. The wound was covered by a transparent dressing (3M Health Care, St Paul, MN). Animals were then randomly assigned to control or Ex4 treatment group and received intraperitoneal injection of normal saline or Ex4 (0.5 mg/kg/d, Sigma Aldrich) for 14 d after operation. Glp-1R analogues have been widely used in the control of blood glucose levels for the past decade, and they are most commonly administered via subcutaneous injection in type II diabetic patients. Therefore, our study design followed the clinical scenario that the application of parenteral injection of Ex4 as a supplemental hypoglycemic agent in diabetic patients with wounds could reduce hospitalization costs associated with diabetes itself.
patients may provide additional effect in the promotion of wound healing. Excisional wounds were photographed under a fixed magnification (8×), and areas of the wound were measured using the TwinCAT software (version 3.2, TCAM Development Inc, Bellevue, WA) at different time points after wound injury.21 Wound recovery rates were evaluated using the formula as22:

\[
\% \text{Wound recovery on Day } X (Dx) = \frac{(\text{Wound area on } D_0) - (\text{Wound area on } Dx)}{\text{Wound area on } D_0} \times 100\%
\]

**Blood sampling and analyses**

Whole blood was collected immediately following euthanasia by direct cardiac aspiration under deep anesthesia (pentobarbital 250 mg/kg). Mononuclear cells were isolated and stained with fluorescent conjugated antibodies for CD34 (Biovendor, bs-2038R) and KDR (Novus Biologicals, NB100-2382SS) using a flow cytometry, as the cell markers for circulating EPCs.16,21 Serum samples were analyzed for superoxide anions and other biochemical concentrations.

**In vivo angiogenesis assay**

Matrigel (1 mL; BD Bioscience, San Jose, CA) containing fibroblast growth factor (10 ng/mL, R&D System, Minneapolis, MN) and heparin (60 U/mL) was injected subcutaneously into the rat abdominal wall 11 d after Ex4 treatment. Following euthanasia at day 14, the Matrigel plug was excised, and the contents of hemoglobin in the matrigel were measured.21 The degrees of angiogenesis were also determined by the immunofluorescence expression of specific cell markers for EPCs (CD133+/KDR+; CD133, Miltenyi Biotec, Germany; KDR, Abcam, Cambridge, MA, USA) in the matrigel.

**Measurements of tissue inflammation reaction**

Concentrations of superoxide anions and proinflammatory cytokines in the wound tissues were measured, respectively, determined by the chemiluminescence assay and an ELISA kit (R&D systems), respectively. Tissue activity of myeloperoxidase was assayed as the previously described methods.21

**Western bloting**

Soluble proteins extracted from wound tissues (50 μg) were loaded into polyacrylamide gels and transferred onto nitrocellulose membranes. Mouse monoclonal anti-eNOS (1:2000; BD Transduction Labs, 610296), anti-phosphorylated (p)-eNOS (1:2000; BD Transduction Labs, 612392), anti-Heme oxygenase (HO)-1 (1:5000; Stressgen, OSA-111), anti-MMP-2 (1:2000; Milipore, MAB3308), anti-HIF-1α (1:2000; GeneTex, GTX30105), anti-phosphorylated (phospho)-p47(phox (1:2000; BD Transduction Labs, 610355), anti-VEGF receptor (VEGFR)-2 (1:2000; NOVUS; NB100-2382), anti-transforming growth factor (TGF)-β1 (1:2000; R&D system; MAB240), and anti-β-actin (1:5000; Genetex, GTX109639) primary antibodies were used. Appropriate primary antibodies were immunoblotted, and the horseradish peroxidase-linked secondary antibody-enhanced bands were visualized using chemiluminescence. Protein levels were quantified by scanning densitometry (Scion Image, Frederick, MD).

**Histology examinations**

Biopsies of 4% paraformaldehyde solution-fixed wound tissues were embedded in paraffin wax and sectioned at 5-μm intervals. Sectioned tissues were then stained with Hema-toxyn & eosin and Masson’s trichrome stains. The histologic sections were independently reviewed by a dermatologist and a pathologist, who were blinded to the treatment groups. A modified histologic grading scale was used by assessing vascularity, granulation formation, and periepidermal regeneration.23 Three-point scales were applied to vascularity (1, severely altered angiogenesis with one or two vessels per site and endothelial edema, thrombosis, and/or hemorrhage; 2, moderately altered angiogenesis with three to four vessels per site, moderate edema, and hemorrhage; and 3, normal angiogenesis with more than seven vessels per site with only mild edema), granulation tissue formation (1, thin granulation layer; 2, moderate granulation layer; and 3, thick granulation layer), and periepidermal regeneration (1, little regeneration; 2, moderate regeneration; and 3, complete regeneration).

**Immunohistochemically analysis**

Immunohistochemically analysis was performed on the 4-μm-thick formalin-fixed paraffin-embedded tissue sections using the Bond-Max Automated IHC stainer (Leica Biosystems Newcastle Ltd, Australia). Rabbit primary anti-VEGFR-2 (1:400 dilution, Dako, M727329), anti-Ki-67 (1:200 dilution, Dako, M724801), and anti-TGF-β1 (1:20 dilution, Dako, M727329) were used.

**Statistical analysis**

Unless otherwise specified, the results are presented as mean ± standard deviation. Data were compared by using one-way or two-way repeated measures analysis of variances followed by Dunnett’s post hoc test. Statistical significance was accepted at a level of P < 0.05.
Ex4 accelerated wound recovery rate in diabetic rats

Compared with controls, wound recovery rates were significantly reduced in hyperglycemic rats (Fig. 1). Treatment with Ex4 significantly potentiated the closure rates of wound healing in diabetic animals that were similar to the control group (Fig. 1). The mean recovery rate on POD 14 was 64% in the diabetic group, which was significantly lower than the control and DM+Ex4 groups (83% and 80%, respectively; \( P < 0.001 \) compared with DM group, \( n = 6-8 \); Fig. 1).

Ex4 modulated inflammation responses in the wound

At POD 3, the expressions of phospho-p47phox and HO-1 in the wound tissue were not different among the three treatment groups (Fig. 2A), but these proteins were reduced to the levels of controls in diabetic rats treated with Ex4 at POD 14, while the expressions were remained at significantly higher levels in the DM group without Ex4 treatment (Fig. 2A). The activity of polymorphonuclear leukocytes and oxidative reaction were quantified by the tissue levels of myeloperoxidase and superoxide anions, respectively. Activities of myeloperoxidase were not different at the early (POD 3) and late (POD 14) stages among the three treatment groups (data not shown), whereas Ex4 significantly suppressed generation of superoxide anions in the diabetic rats at POD 14 (Fig. 2B). In addition, systemic administration of Ex4 significantly suppressed serum concentrations of interleukin (IL)-6 at POD 3 in hyperglycemic rats (Fig. 2C).

Ex4 promoted systemic proangiogenic effects in vivo

Ex4 significantly increased capillary tube formation and content of hemoglobin in the Matrigel plugs implanted in

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**Table – Blood glucose, lipid profile, and body weight on the day of sacrifice.**

<table>
<thead>
<tr>
<th>Blood and physiological parameters</th>
<th>Control</th>
<th>DM</th>
<th>DM + Ex4</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>208.1 ± 19.9*</td>
<td>455.2 ± 33.9</td>
<td>438.5 ± 43.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>56.3 ± 8.0</td>
<td>114.8 ± 49.4</td>
<td>116.5 ± 30.7</td>
<td>0.580</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>52.4 ± 6.1</td>
<td>54.2 ± 5.9</td>
<td>54.0 ± 3.8</td>
<td>0.297</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>360.8 ± 26.4</td>
<td>242.5 ± 16.2</td>
<td>264.0 ± 19.0</td>
<td>0.029</td>
</tr>
<tr>
<td>Oral intake (g/d)</td>
<td>21.2 ± 2.9</td>
<td>40.1 ± 2.7</td>
<td>42.6 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water intake (mL/d)</td>
<td>57.0 ± 7.1</td>
<td>171.3 ± 16.9</td>
<td>178.0 ± 18.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DM = diabetes mellitus; Ex4 = exendin-4; HDL = high-density lipoprotein.
The 14-d average intake after wound creation was also recorded.
*, †, ‡: Control group compared with DM and DM + Ex4.
Data were analyzed using one-way ANOVA. Mean ± SE.

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Fig. 1 – The recovery of excisional wound. (A) Representative photographs of wound closure at postoperative days (POD) 1, 3, 7, and 14. Bar scale represents 1 cm in length. (B) Quantification of wound area recovery from POD 1 to 14. * \( P < 0.001 \), DM versus control and DM + Ex4. \( n = 6-8 \) for each group. (Color version of figure is available online.)
the subcutaneous layer of diabetic rats (Fig. 3A and B, respectively). Numbers of CD34+/KDR+ EPCs were significantly enhanced in circulation of diabetic rats after 14 d of Ex4 treatment (Fig. 3C). In vivo proangiogenic assay also confirmed that these CD133+/KDR+ EPCs were present in the matrigel and were significantly attenuated in the diabetic animals (Fig. 3D). Daily injection of Ex4 in rats with diabetes increased seeding of EPCs in the matrigel (Fig. 3D).

Ex4 enhanced regional angiogenesis in the wound

Levels of angiogenesis in the regenerating wound tissues were measured by the protein expression of HIF-1α, VEGFR-2, and eNOS. Hypoxia-inducible factor is a critical regulator for angiogenesis during acute phase of tissue injury. Hyperglycemia suppressed the expression of HIF-1α at the early phase (POD 3) of wound regeneration, but Ex4 enhanced the protein levels of HIF-1α at POD 14, while the level remained low in the diabetic rats without Ex4 treatment (Fig. 4). On the other hand, the expressions of eNOS and its phosphorylated form, and the VEGF receptor tyrosine kinase (VEGFR-2) were clearly sustained in the DM+Ex4 group at POD 14 (Fig. 4).

Ex4 restores regeneration activity in the wound

In comparison to controls, tissue expressions of MMP-2 and TGF-β1 were significantly reduced in hyperglycemic rats during the late tissue remodeling phase (POD 14; Fig. 5). Administration of Ex4 restored the MMP-2 and TGF-β1 abundances in the healing wound of diabetic rats (Fig. 5).
Histologic examinations

Compared to controls, the wound tissue obtained from DM rats showed decreased vascularity, thickened granulation layer with impaired regeneration in the epidermis and upper dermis (Fig. 6A). Treatment with Ex4 enhanced vascularity, dermal and epidermal regeneration, and decreased hemorrhage. Immunostaining of wound tissue confirmed the increased expression of VEGFR-2 in the papillary epidermal layer of the diabetic wounds treated with Ex4 (Fig. 6B). In addition, expressions of cell proliferative and tissue regenerative markers, namely Ki-67 and TGF-β1, were augmented in subepidermal layer after Ex4 treatment in diabetic rats, suggesting the augmented proliferation activity during the regenerative phase (Fig. 6B).

Discussion

Wound repair involves the interactions among different cell populations including platelets, macrophages, neutrophils, fibroblasts, keratinocytes, endothelial cells, and proangiogenic cells. Harmonization of the repair process depends on an intimate cell-to-cell communication through direct contact or cytokines and growth factors, such as TGF-β, VEGF, HIF-1α, TNF-α, and cytokines such as IL-1 and IL-6. In addition, activity of MMPs contributes important tissue proteinases to control closure of epithelium and cultivate a favorable microenvironment for the proliferation and maturation of wound healing. Previous experimental and clinical studies clearly indicated that diabetes impairs wound healing by prolongation of inflammatory reaction, attenuation of regional angiogenesis, and dysregulation of remodeling process. Although manipulations of individual growth factors or cytokines have been tested in the treatment of diabetic wounds, currently, there is currently still a lack of pharmaceutics that are therapeutic throughout entire phases of wound healing which can also potentially serve as supplementary agents to the standard antihyperglycemic therapy in diabetic subjects with wound injury. In this regard, we tested the therapeutic effect of Ex4, a novel parenteral hyperglycemic agent that mediates pleiotropic anti-inflammatory and proangiogenic effects, in a diabetic rat model of excisional wound injury.

During the inflammatory phase of wound healing, wound size enlarges due to the amplification of aggregation responses through initiation of coagulation cascade and chemoattractant reaction in the infiltrating inflammatory cells. In rats with diabetes, we found that the excisional wound bulked significantly in size at POD 3, suggesting a more profound inflammatory reaction at POD 3 suggesting a more profound inflammatory reaction in diabetes. The wound became contracted and gradually reduced in size from POD 3 to POD 14 during the proliferative and remodeling phases. Our results clearly demonstrated that wound closure was significantly delayed in diabetic rats during these regenerative phases of wound healing, which is consistent with the clinical and experimental reports. The most important finding of this study was that administration of Ex4 from the first day after wound injury significantly restored the healing process of
excisional wound in diabetes that was similar as the controls with normoglycemia. Since these findings support that the therapeutic effects of Ex4 can potentially affect all phases of wound healing, we analyzed the molecular changes in the different phases of regenerating wounds in diabetic animals after Ex4 treatment.

Acute inflammation reaction is the beginning process of wound healing after hemostasis, involving the regulation of nervous system, capillary vasodilation, granule release of cytokines, and growth factors to stimulate the wound repair process and angiogenesis. Consistent with previous reports, creation of a full-thickness excisional wound in diabetic rats induced significantly higher tissue expression of nicotinamide adenine dinucleotide phosphate oxidase (phospho-p47phox) and the generation of superoxide anions at POD 3, suggesting the proinflammatory status in subjects with diabetes during the acute phase of wound repair. In diabetic rats without Ex4 treatment, the proinflammatory response in the wound was prolonged up to POD 14 with significantly higher tissue expression of phospho-p47phox and generation of superoxide anions. Following treatment with Ex4 for 2 wk, protein level of nicotinamide adenine dinucleotide phosphate oxidase and tissue concentration of superoxide anions were significantly suppressed in diabetic rats.

Fig. 4 – Ex4 enhanced the expression of proangiogenic proteins in the wound tissue. Diabetes suppressed the abundance of hypoxia-inducible factor (HIF) 1-α and eNOS at postoperative (POD) 3; \( P < 0.001 \), control versus DM and DM + Ex4; \( **P = 0.011 \). Treatment with Ex4 marginally upregulated the expression of HIF 1-α at POD 14 \( (P = 0.125) \), but significantly enhanced the protein levels of VEGFR-2, eNOS, and p-eNOS on D14 in Ex4-treated diabetic rats, \( *P = 0.009, **P = 0.029, #P < 0.001; n = 6 \) different animals in each group.
rats. Attenuation of HO-1, a reactive antioxidant gene also reinforced that Ex4 reduced the overall proinflammatory status in the diabetic wound. In addition, intraperitoneal injection of Ex4 significantly attenuated the plasma concentrations of the proinflammatory cytokine IL-6 at POD 3 in diabetic animals. IL-6 is an important mediator during acute inflammatory reaction via the activation of IL-6/gp 130 trans-signaling pathway. Previous studies have demonstrated that significant elevation of IL-6 is a characteristic biomarker for inflammation in metabolic syndrome. Administration of Glp attenuated the systemic levels of IL-6 in patients with type 1 diabetes and improved vascular endothelial function that is consistent with our findings in experimental model of diabetes.

Proliferative phase of wound healing involves profound angiogenesis and formation of granulation tissue. The prolonged inflammatory reaction results in delayed wound proliferation in subjects with diabetes. We used several assays to determine the degrees of angiogenesis in this model of wound healing. Circulating EPCs exert important regenerative function as an endogenous repair mechanism in forming new vessels by direct incorporation and paracrine-mediated effects in wound healing. Clinical investigations showed that circulating CD133+/CD34+ cells were significantly increased after acute wound injury, and these cells were reduced in diabetic patients. Transplantation of autologous G-CSF-mobilized peripheral CD34+ cells provided potential therapeutic effects in diabetic patients. Therefore, we measured the number of EPCs in rats after creation of an excisional wound to assess degree of angiogenesis. EPCs were reduced in diabetic rats and treatment with Ex4 significantly enhanced numbers of EPCs in the circulation, indicating that Ex4 mobilized these bone marrow-derived progenitors in diabetes. We also measured the homing of EPCs using an in vivo assay and found that more CD133+/KDR+ expressed cells were identified on the matrigel isolated from rats with diabetes. Consistently, capillary tube formation and content of hemoglobin were significantly increased in the implanted matrigel following treatment with Ex4 in diabetic animals. Furthermore, the expressions of eNOS and its phosphorylated form were significantly suppressed in diabetes and were upregulated by Ex4 treatment up to 14 d after wound injury. Similarly, the important receptor in VEGF-mediated mitogenesis VEGFR-2 and the synergistic proangiogenic factor HIF-1α were enhanced in the wound tissue of Ex4-treated diabetic rats, underscoring that activity of angiogenesis in diabetes was potentiated by Ex4.

In accordance with improvement in inflammation and angiogenesis, administration of Ex4 restored the expressions of TGF-β1 and MMP-2 in the wound at the remodeling phase, which were significantly reduced in diabetes. Isoforms of TGF-β family are important mediators in wound healing, involving regulation of inflammation, stimulation of angiogenesis, proliferation of fibroblasts, synthesis of collagen, and remodeling of the extracellular matrix. Lower activity of TGF-β is associated with impaired wound healing and implantation of exogenous TGF-β1 reversed wound healing in rats with experimental hyperglycemia. Previous studies showed that TGF-β1 promotes activity of MMP-2 and facilitates wound healing process. Consistent with these molecular changes, histologic sections of the regenerated wounds demonstrated that Ex4 increased vascular density, formation of granulation tissue and cell proliferative index (Ki-67) in the epidermal and dermal layers in diabetic rats.

To our knowledge, two previous studies had investigated the applications of Glp-1 analogues or dipeptidyl-peptidase-IV inhibitors (DDP-IV) in wound regeneration in normoglycemic or animals with metabolic syndrome. Topical injection of high dose Ex4 (5 μg/kg) accelerated wound healing rates through activation of myofibroblasts in nondiabetic mice. Schürmann et al. showed that oral administration of linagliptin (a dipeptidyl-peptidase-IV inhibitor, 3 mg/kg/d) in diabetic obese mice enhanced re-epithelialization of excisional wounds at 10 d

**Fig. 5 – Ex4 treatment restored the tissue expression of MMP-2 and TGF-β1 in diabetic rats at postoperative day (POD) 14, *P = 0.025 and **P = 0.003 for DM versus DM + Ex4. n = 6 for each group.**
after surgery by attenuation of infiltration of neutrophils and proinflammatory reaction. In addition to the anti-inflammatory and proliferative properties, our study provides more integrated and additional mechanistic investigations into the therapeutic effects of Ex4 in the wound regeneration of diabetes by modulation of regional or systemic inflammation, promotion of angiogenesis, and potentiation of wound maturation.
Previous studies indicated that treatment with Ex4 alone does not significantly affect the blood glucose levels, particularly with low therapeutic doses (0.5 μg/kg/d). However, blood glucose could better be controlled by parenteral injection of higher doses of Ex4 (up to 20 μg/kg/d) over a 2-wk treatment period in diabetic rats. Therefore, we believe that the hypoglycemic effect of Ex4 is treatment dose and duration dependent in experimental animals. In this study, we showed that low dose Ex4 mediated anti-inflammatory and proangiogenic effects, in turn improving excisional wound healing, which were consistent with the characteristic responses reported by other study groups. In addition, our study also supports the concept that the pleiotropic protective effect of Ex4 in wound repair is independent from its hypoglycemic reaction.

There were several limitations in this study. First, streptozotocin-induced diabetes is not clinically compatible with the majority of type II diabetes in humans. We admit that the immune diabetic models are more compatible with type II diabetes, but streptozotocin-induced insulin-dependent chronic hyperglycemia is also a valid experimental model for wound healing, particularly when the extraglycemic effect of a drug or compound is tested. Second, the closure of excisional wounds in this study might be affected by wound contracture rather than the true effect of re-epithelialization. Nevertheless, our results clearly showed that administration of Ex4 accelerated wound closure in diabetic rats that was comparable with controls, while the diabetic wounds remained poorly healed up to 14 d after injury.

Conclusions

Our study signifies the beneficial pleiotropic effects of Ex4 in the healing process of diabetic wounds. Intraperitoneal administration of Ex4 modulates inflammatory responses by suppressing regional generation of superoxide anions and systemic levels of proinflammatory cytokine IL-6 during the early phases of wound healing. Treatment of Ex4 enhances angiogenesis via mobilization of EPCs and induction of proangiogenic microenvironment for wound proliferation. The synergistic anti-inflammatory and proangiogenic effects of Ex4 are associated with upregulation of TGF-β1/MMP-2 pathway in the formation of a more mature dermal granulation. Collectively, we suggest that apart from the glycemic controlling effect, Ex4 might potentially be a hypoglycemic agent in diabetes with wound injury or subjected to skin excision during surgical intervention.

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Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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