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ORIGINAL RESEARCH

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Infliximab accelerates wound healing in diabetic mice by aggravating macrophage polarization

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Abstract

Delayed wound healing is one vital complication of diabetes mellitus (DM) that adversely impacts patient quality of life. Infliximab (INF), a monoclonal tumor necrosis factor α (TNF-α) antibody, has been investigated for its therapeutic potential across various diseases through displaying anti-inflammation ability. However, the regulatory mechanisms by which INF influences and correlates with delayed wound healing in DM remain unclear. In this study, we first induced diabetes in mice using streptozotocin (STZ) to establish a DM model, and then excision wounds were generated. We observed that wound closure was significantly retarded in DM mice, but this effect was reversed following INF treatment (5 mg/kg). Moreover, INF treatment attenuated the heightened inflammation observed in DM mice. Furthermore, we found that INF expedited the M2 phenotype polarization in DM mice. Mechanistically, INF was shown to delay activation of the nuclear factor kappa-B (NF-κB) pathway. To sum up, our findings demonstrate that in diabetic mice, INF facilitates wound healing by modulating macrophage polarization, and refrained the NF-κB pathway, supporting INF as a promising treatment approach for improving wound healing in diabetics.

Keywords

Infliximab; Wound healing; Macrophage polarization; Diabetes mellitus; NF-κB pathway

1. Introduction

Wound healing refers to the sequential process of hemostasis, inflammation, proliferation and remodeling aimed at restoring the skin to its original state [1]. Delayed wound healing is a significant global health challenge, particularly prevalent among patients with diabetes mellitus (DM) [2, 3]. Approximately 15% of DM patients suffer from nonhealing ulcers [4]. In DM, chronic inflammation often prolongs the inflammatory phase of wounds, leading to delayed or nonhealing outcomes [5]. Thus, there is an urgent need to explore novel targeted therapies for improving delayed wound healing in DM.

Several drugs have been investigated to ameliorate wound healing in DM. For instance, carnosine has been manifested to promote wound healing in a type 2 DM mouse model [6], while 11,12 epoxyeicosatrienoic acid improves wound healing outcomes in DM [7]. Additionally, adenosine diphosphate activates the P2Y12 receptor to facilitate wound healing in DM mice [8], and a triazolothiadiazine derivative stimulates chemokine (C-X-C motif) receptor 4 (CXCR4) signaling to accelerate wound healing [9]. Infliximab (INF), one monoclonal antibody for tumor necrosis factor-alpha (TNF-α), displays anti-inflammatory influences [10]. In addition, in arthritis rat model, INF has been demonstrated to alleviate bone loss and inflammation [11]. Moreover, INF modulates the NF-κB pathway to suppress oxidative stress and inflammation in H_2O_2-stimulated H9c2 cells [12], and it attenuates TNF-α-mediated pathways to improve outcomes in traumatic brain injury [13]. Moreover, INF can reduce peripheral inflammation and neuroinflammation in hepatic encephalopathy, thereby preserving cognitive and motor functions [14]. However, the specific regulatory mechanisms of INF and its associated pathways in promoting wound healing in DM remain poorly understood.

Macrophages own pivotal roles in wound healing by exhibiting phenotypic plasticity, can change from one pro-inflammatory "M1" phenotype to one pro-healing "M2" phenotype [15]. Thus, macrophage responses are closely interrelated with the impaired wound healing in diabetes, making macrophages a promising therapeutic target [16]. However, the precise impact of INF on macrophages and its modulation of wound healing in DM require further investigation.

This study goaled to probe the regulatory influences of INF in DM mice. Our results demonstrate that INF facilitated wound healing in DM mice by modulating macrophage polarization, and refrained the NF-κB pathway. These data may supply new insights of INF in ameliorating wound healing in DM.

2. Materials and methods

2.1 Animal model

Male C57BL/6 mice (9 weeks old, n = 18), purchased from Vital River (Beijing, China), were housed under standard conditions (22 ± 1 °C, 12/12 light/dark cycle) with ad libitum access to water and food [17, 18]. DM was induced by intraperitoneal injection of streptozotocin (STZ, S0130, Sigma; St. Louis, MO, USA) at a dose of 100 mg/kg dissolved in citrate buffer (0.01 M), administered twice weekly. Successful establishment of the DM model was confirmed when blood glucose levels reached 250 mg/dL or higher. Full-thickness excisional wounds (1 cm diameter) were then created on the dorsal skin of the mice under pentobarbital sodium anesthesia using sterile ophthalmic scissors [19]. In the DM + 5 mg/kg INF group, DM mice received intraperitoneal injections of INF (5 mg/kg in 200 μL saline, ©Remicade, Janssen Biotech, Horshamn, PA, USA) once every 7 days [20]. Wound healing progress was monitored and photographed at 3, 7 and 14 days post-wounding [21]. By day 14, wounds in the Control group were typically healed. The mice were then separated into three groups: Control, DM and DM + 5 mg/kg INF (n = 6 mice per group).

The formula: Closure rate (%) = [1 − (wound area on day 3, 7 or 14/wound area on day 0)] × 100%.

2.2 Western blot

Proteins extracted from wound tissues were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and migrated to polyvinylidene fluoride (PVDF) membranes. After sealing, the membranes were mixed with primary antibodies (all purchased from Abcam (Shanghai, China)), such as Collagen I (1/1000; ab260043), alpha smooth muscle actin (α-SMA, 1/1000; ab5831), Arg-1 (1/1000; ab315110), cluster of differentiation 206 (CD206, 1 μg/mL; ab64693), inducible nitric oxide synthase (iNOS, 1/1000; ab178945), p-p65 (1/1000; ab76302), p65 (1/2000; ab32536), p-inhibitory subunit of nuclear factor kappa B alpha (p-IκBα, 1/1000; ab92700), IκBα (1/1000; ab32518) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1/1000; ab8245) overnight. The membranes were then mixed with a secondary antibody (1/1000; ab7090) and visualized using a chemiluminescence detection kit (89880, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.3 ELISA

ELISA kits of interleukin-6 (IL-6, ab222503), TNF-α (ab208348) and interleukin-1 beta (IL-1β, ab197742) were all purchased from Abcam (Shanghai, China), and employed in line with the manufacturer's instructions.

2.4 Statistical analysis

Data were analyzed using GraphPad Prism software 9 (GraphPad Software, San Diego, CA, USA) and presented as mean ± standard deviation (SD). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A p-value less than 0.05 was deemed as statistically significant.

3. Results

3.1 Infliximab accelerated wound healing in DM mice

The wound healing closure rate was found to be decreased in DM mice compared to controls, and this effect could be reversed by using INF (5 mg/kg) treatment (Fig. 1A,B). Additionally, the protein expressions of Collagen I and α-SMA, markers of wound healing, were reduced in DM mice and were restored after INF treatment (Fig. 1C). Overall, INF demonstrated promising effects in accelerating wound healing in DM mice.

3.2 Infliximab reduced inflammation in DM mice

As shown in Fig. 2, the levels of IL-6, TNF-α and IL-1β (pro-inflammatory cytokines) were elevated in DM mice, but these effects were attenuated following INF treatment. These findings indicate that INF reduced inflammation in DM mice.

3.3 Infliximab facilitated macrophage polarization

Further experiments showed that the protein expressions of Arg-1 and CD206 (markers of M2-type macrophages) were decreased, while iNOS (a marker of M1-type macrophages) was increased in DM mice. However, these changes were reversed after INF treatment (Fig. 3). Thus, INF facilitated M2 macrophage polarization.

3.4 Infliximab delayed NF-κB pathway activation

Lastly, we found that the protein expressions of p-p65/p65 and p-IκBα were increased, whereas IκBα was decreased in DM mice. Moreover, these alterations could be mitigated following INF treatment (Fig. 4). Collectively, INF refrained the NF-κB pathway.

4. Discussion

INF is recognized for its anti-inflammatory properties and therapeutic potential in various diseases [11–14]. However, the specific regulatory effects of INF and its associated pathways on delayed wound healing in DM remain unclear. In this study, we observed that the wound healing closure rate was delayed in DM mice and that this effect was reversed following INF treatment at a dose of 5 mg/kg. Inflammation plays a crucial role in the context of wound healing in DM. For instance, sustained oxygenation promotes angiogenesis and reduces inflammation, thereby enhancing diabetic wound healing [22]. Additionally, substance P influences inflammation and macrophage phenotype to accelerate wound healing in DM [23], while chick early amniotic fluids (ceAF) can mitigate inflammation, thereby improving diabetic wound healing [24]. Moreover, miR-155 enhances fibroblast growth factor 7 (FGF7) expression, exacerbating wound inflammation in DM

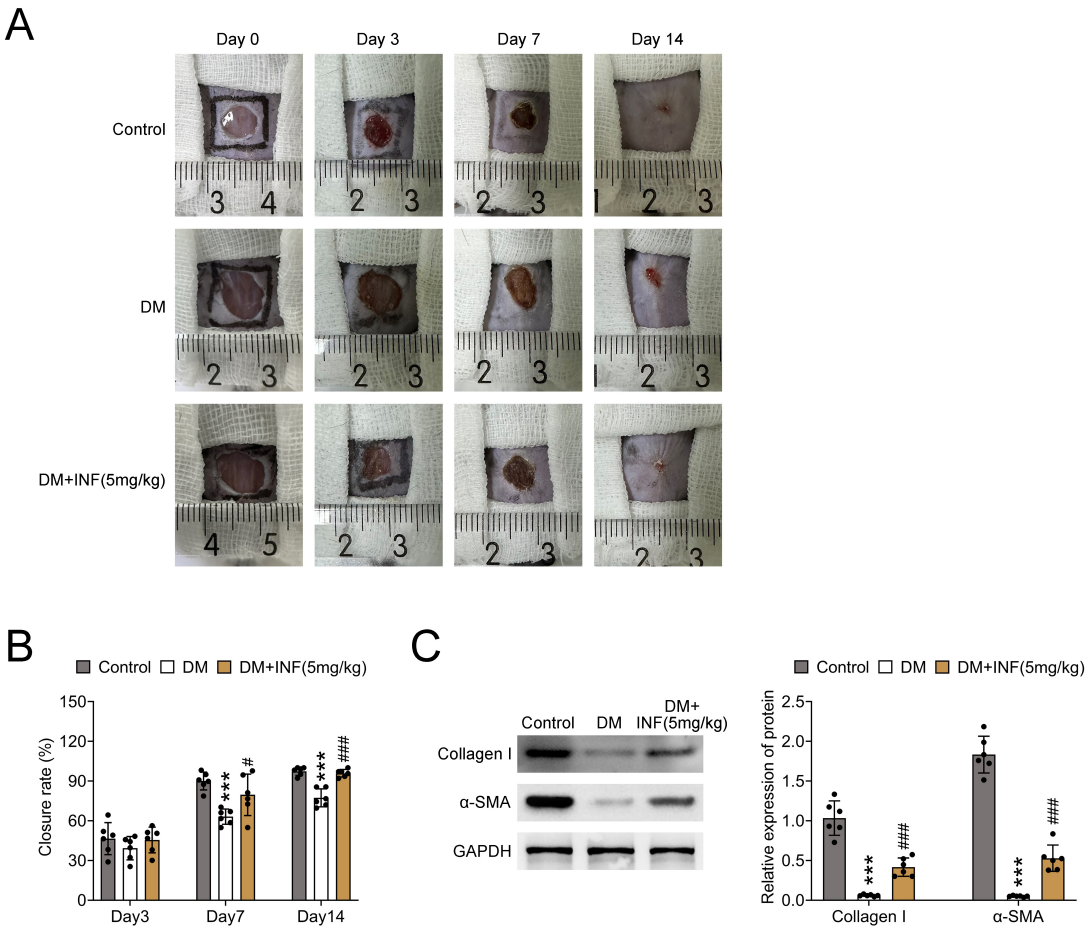

FIGURE 1. Infliximab accelerated wound healing in DM mice. The mice were divided into Control, DM and DM + 5 mg/kg INF groups. (A,B) Measurement of wound healing closure rates. (C) Protein expressions of Collagen I and α-SMA were determined by western blot. N = 6 mice per group. ***$p < 0.001$ *vs.* Control; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ *vs.* DM. DM: diabetes mellitus; INF: Infliximab; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; α-SMA: alpha smooth muscle actin.

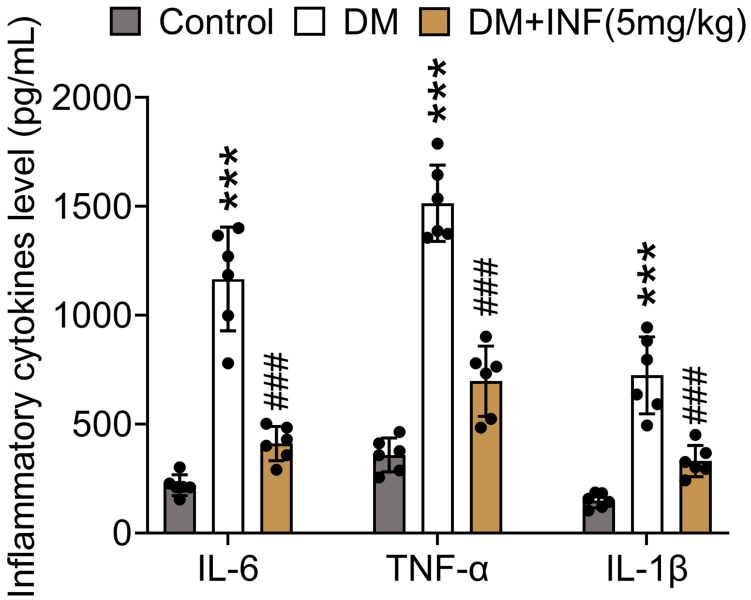

FIGURE 2. Infliximab reduced inflammation in DM mice. The mice were divided into Control, DM and DM + 5 mg/kg INF groups. Levels of IL-6, TNF-α and IL-1β were assessed using ELISA. N = 6 mice per group. ***$p < 0.001$ *vs.* Control; $^{\#\#\#}p < 0.001$ *vs.* DM. DM: diabetes mellitus; INF: Infliximab; IL-6: interleukin-6; TNF-α: tumor necrosis factor α; IL-1β: interleukin-1 beta.

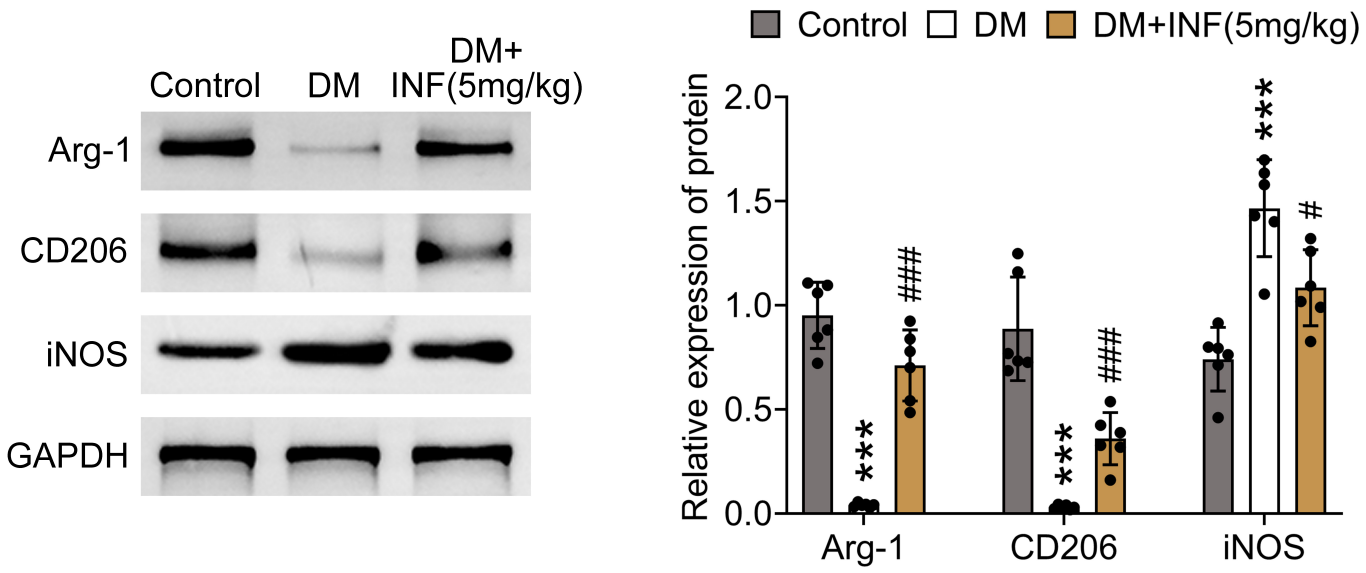

FIGURE 3. Infliximab facilitated macrophage polarization in DM mice. The mice were divided into Control, DM and DM + 5 mg/kg INF groups, and their protein expressions of Arg-1, CD206 and iNOS were determined by western blot. N = 6 mice per group. ***$p < 0.001$ *vs.* Control; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ *vs.* DM. DM: diabetes mellitus; INF: Infliximab; CD206: cluster of differentiation 206; iNOS: inducible nitric oxide synthase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

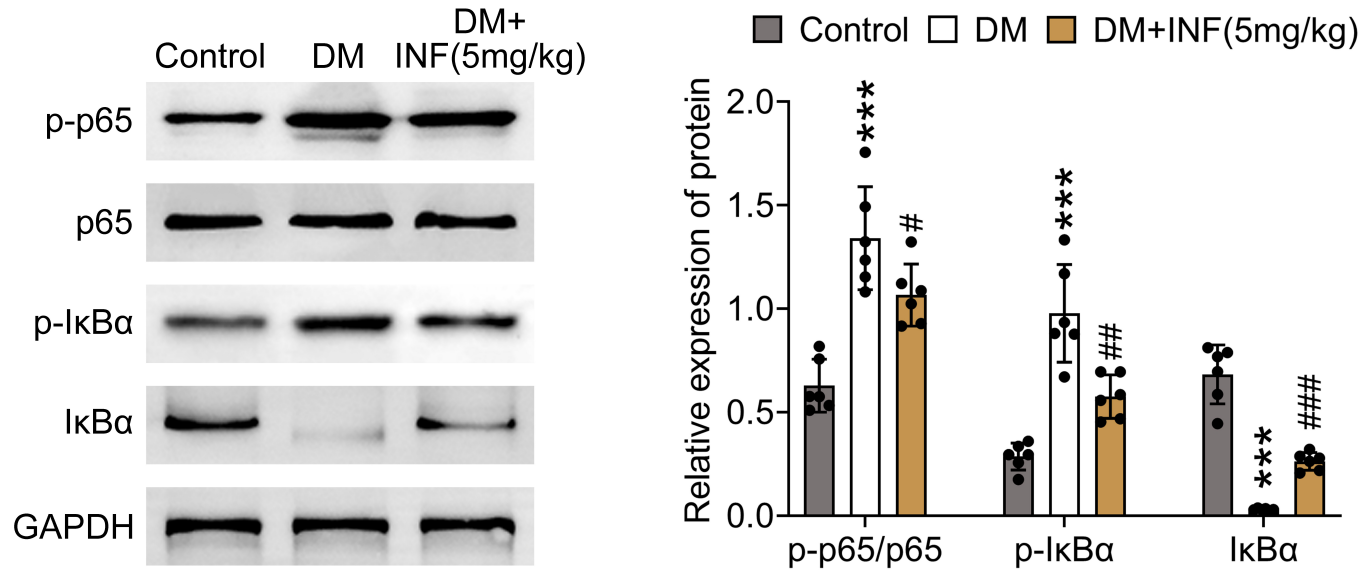

FIGURE 4. Infliximab refrained the NF-κB pathway in DM mice. The mice were divided into Control, DM and DM + 5 mg/kg INF groups. The protein expressions of p-p65, p65, p-IκBα and IκBα were analyzed by western blot. N = 6 mice per group. ***$p < 0.001$ *vs.* Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs.* DM. DM: diabetes mellitus; INF: Infliximab; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; IκBα: inhibitory subunit of nuclear factor kappa B alpha.

[25]. INF has been demonstrated to mitigate inflammation [26], but its specific effects on wound inflammation in the context of DM progression remain poorly understood. In our study, we found that heightened inflammation in DM mice was attenuated following INF treatment.

M1 macrophages in diabetic wounds exhibit impaired polarization towards M2 macrophages, contributing to a prolonged inflammatory state that hinders angiogenesis and collagen deposition, thereby delaying or preventing wound healing [3]. Numerous studies have probed the impact of M1/M2 polarization on wound healing in DM. For instance, exosomal miR-146a-5p has been revealed to influence macrophage M1/M2 polarization and accelerate diabetic wound healing [27]. Additionally, IL-25 induces M2 polarization to enhance diabetic wound healing [28], and resveratrol accelerates M2 polarization to improve wound healing outcomes in DM [29]. However, the specific regulatory role of INF in M1/M2 polarization during DM progression remains unclear. Consistent with these previous studies, our findings also demonstrate that INF facilitates M2 macrophage polarization in DM mice.

The retardation of the NF-κB pathway has been identified as beneficial for wound healing in DM. For instance, stro-

mal vascular fraction gel modulates the NF-κB pathway to ameliorate wound healing and peripheral nerve restoration in DM [30]. Additionally, astragalus polysaccharide regulates the β-catenin/NF-κB axis to influence macrophage M2 polarization and inflammation, thereby alleviating diabetic ulcers [31]. Furthermore, camel milk peptide suppresses the NF-κB pathway, affecting redox status and immune response and promoting wound healing in DM [32]. In previous studies, INF has been shown to alleviate inflammation in H_2O_2-evoked H9c2 cells by retarding the NF-κB pathway [33]. Moreover, INF can suppress NF-κB activation, thereby mitigating lung metastasis in breast cancer [12]. Therefore, the specific regulatory impacts of INF on the NF-κB pathway in DM require further investigation. In our study, we also confirmed that INF refrained the NF-κB pathway.

5. Conclusions

This study manifested that in diabetic mice, INF facilitated wound healing by modulating macrophage polarization, and refrained the NF-κB pathway. Despite these findings, several limitations should be acknowledged. INF shows promise as one hopeful agent for ameliorating wound healing, suggesting new research avenues and potential clinical applications, particularly when compared with cell therapy approaches [34, 35]. Future studies could further explore INF's comparative effectiveness with other treatments for diabetic wound healing and investigate its long-term effects.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

MC and FW—designed the study and carried them out; prepare the manuscript for publication and reviewed the draft of the manuscript. MC, MHD and QY—supervised the data collection, analyzed the data, interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Animal Experimental Ethical Inspection Form of Southeast University (Approval no. 20230308015).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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