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Chinese medicine ulcer oil promotes the healing of diabetic foot ulcers

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Abstract

Objective: This study aimed to investigate the mechanism by which Chinese herbal medicine ulcer oil (UO) accelerates ulcer healing in a diabetic ulcer rat model.

Methods: Sprague Dawley rats were allocated at random into four groups: a control group, a positive control group (PC), a UO treatment group and an ethacridine lactate solution treatment group. Subcutaneous tissue was surgically removed from the rats on days 3, 7 and 14. The levels of protein phosphotyrosine phosphatase 1B (PTP1B), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and advanced glycation end products (AGEs) were detected using western blot analysis.

Results: PTP1B protein expression was significantly lower in the UO group compared with the PC group. VEGF protein expression was significantly higher in the UO group than in the control group on day 3. PDGF protein expression in the UO group was significantly higher than in the PC group on day 3. AGE expression was significantly lower in the UO group than in the PC group.

Conclusions: UO may downregulate PTP1B and AGEs and upregulate VEGF and PDGF, which may contribute to the inhibition of the inflammatory response and promote the healing of diabetic foot ulcers.

Keywords

Traditional Chinese medicine, ulcer oil, diabetic foot ulcer, vascular endothelial growth factor, diabetic rat model, inflammatory response

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Introduction

Diabetic foot ulcers (DFUs) are a chronic severe complication of diabetes and the leading cause of long-term diabetes peripheral vascular occlusive lesions, which lead to ischemia, peripheral neuropathy, abnormal sensations and frequent infections. DFUs are a serious health issue;[1] epidemiological studies have shown that the prevalence of diabetic lower limb distal nerve lesions is 30%–50%.[2]

Patients with DFU experience greatly reduced quality of life and many cannot work, placing substantial economic pressure on their families and on society.[3,4] Recent studies suggest that plant extracts can improve the healing of ulcers in different organs.[5–9] Ulcer oil (UO) is a Chinese herbal medical treatment mainly composed of Cortex Phellodendri and and *Angelica dahurica* that is used externally to remove toxins and promote wound healing. In this study, we investigated the mechanism by which UO accelerated ulcer healing in a diabetic ulcer rat model.

Materials and methods

Animals

This study was approved by the Animal Care and Use Committee of Beijing University of Chinese Medicine. Specific pathogen free (SPF) male Sprague Dawley (SD) rats (80–100 g) were maintained at 40%–70% humidity and 20–26°C with circulating air under SPF conditions. Streptozocin (STZ) was purchased from Sigma-Aldrich (St Louis, MO, USA). UO was prepared at the Beijing University of Chinese Medicine Dongzhimen Hospital. Ethacridine lactate solution (ELS) was provided by Hebei Wuluo Pharmaceutical Co. Ltd. (Hengshui, Hebei, China).

The 48 SPF male SD rats were allocated at random to four groups: (1) a control group, (2) a positive control group (PC), (3) a UO group and (4) an ELS group. The UO and ELS groups were treated with UO and ELS, respectively, at a dose of 1 mL/cm^2 once a day at 08:00. The control and PC groups were treated with iodine to disinfect the wounds. All rats were examined on follow-up days 3, 7 and 14.

All rats except those in the control group were treated with STZ (50 mg/kg) via intravenous tail injection. Six to ten hours after the STZ injection, rats were injected with 0.5% glucose. Blood sugar was monitored for each group on days 3 and 7. To establish the ulcer model, the rats were anaesthetised via an intraperitoneal injection of 10% chloral hydrate anaesthesia (0.004 mL/g). An approximately $4 \times 4\,cm^2$ partial full-thickness skin incision was made on the back of the rats to form wounds. The wounds were painted with glacial acetic acid daily for a week. A week later, diabetic ulcers had formed.

Western blot analysis

The wound tissues were dissected and lysed in 0.5 mL radioimmunoprecipitation assay buffer on ice. The homogenate was centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatant was collected and protein concentration was determined using a bicinchoninic acid assay. Equal amounts of protein (50 μg) were analysed using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred to polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were incubated with 5% skimmed milk for 1 hour, then incubated with rabbit polyclonal antibodies for protein phosphotyrosine phosphatase 1B (PTP1B), vascular endothelial growth factor (VEGF), advanced glycation end products (AGEs) and monoclonal antibody for platelet-derived growth factor (PDGF) (Abcam, USA) overnight at 4°C. The membranes

were washed three times, and then incubated with peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG (APPLYGEN) for 1 hour at room temperature. The membranes were washed, ECL detection was performed and the images were scanned for densitometry analysis.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA). All statistical data were expressed as the mean ± standard deviation (mean ± SD). The data were analysed using one-way analysis of variance followed by the least significant difference test. A P value <0.05 was considered statistically significant.

Results

AGE expression in the four groups

First we compared AGE levels in each group on days 3, 7 and 14. Compared with the control group, AGE levels were significantly higher in the PC group on day 3 ($P < 0.05$), but significantly lower in the UO and ELS groups than in the PC group (both $P < 0.05$). On day 14, AGE levels showed no significant differences among all four groups (Figure 1).

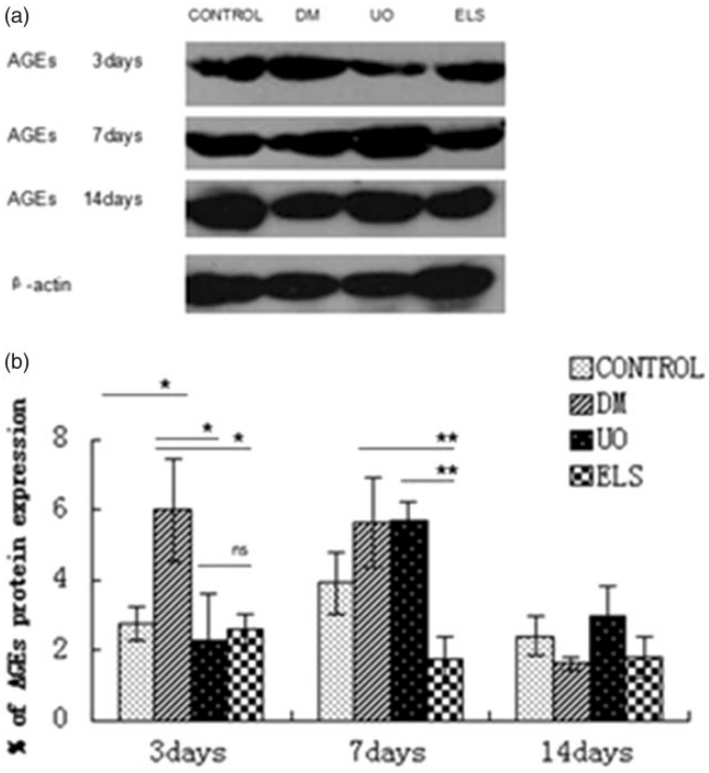

Figure 1. Levels of advanced glycation end products (AGEs) in diabetic ulcer subcutaneous tissues of each group of rats. A. Representative blots. β-actin was the loading control. B. Densitometry analysis of AGE levels. Means ± SD, n = 3, *$P < 0.05$, **$P < 0.01$. PC, positive control; UO, ulcer oil; ELS, ethacridine lactate solution.

PDGF expression in the four groups

We compared PDGF levels in each group on days 3, 7 and 14. On day 3, PDGF levels were significantly lower in the PC group than in the control group ($P < 0.05$), but were significantly higher in the UO group than in the PC group ($P < 0.05$). On days 7 and 14, PDGF levels showed no significant differences among all four groups (Figure 2).

VEGF expression in the four groups

We also compared VEGF levels in each group on days 3, 7 and 14. On day 3, VEGF levels were significantly higher in the UO group than in the other groups ($P < 0.05$). On days 7 and 14, VEGF levels showed no significant differences among all four groups (Figure 3).

PTP1B expression in the four groups

Finally, we compared PTP1B levels in each group on days 3, 7 and 14. On days 3 and 7, PTP1B levels showed no significant differences among all four groups. On day 14, PTPB1 levels were significantly higher in the PC group than in the other groups ($P < 0.05$) (Figure 4).

Discussion

The wound healing process comprises three overlapping phases: (1) the inflammatory stage, (2) the proliferation stage and (3) the remodelling stage. The acute wound

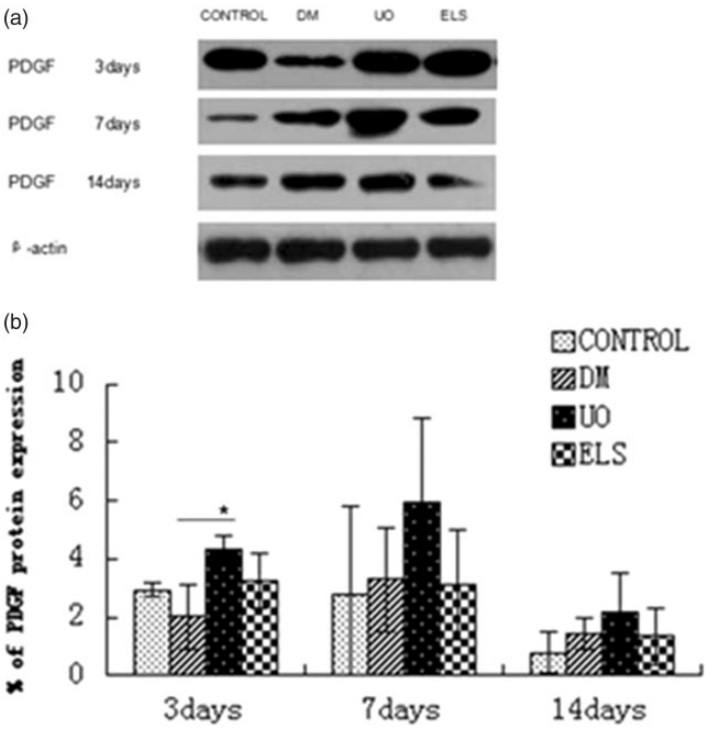

Figure 2. Levels of platelet-derived growth factor (PDGF) in diabetic ulcer subcutaneous tissues of each group of rats. A. Representative blots. β-actin was the loading control. B. Densitometry analysis of PDGF levels. Means ± SD, n = 3, *$P < 0.05$. PC, positive control; UO, ulcer oil; ELS, ethacridine lactate solution.

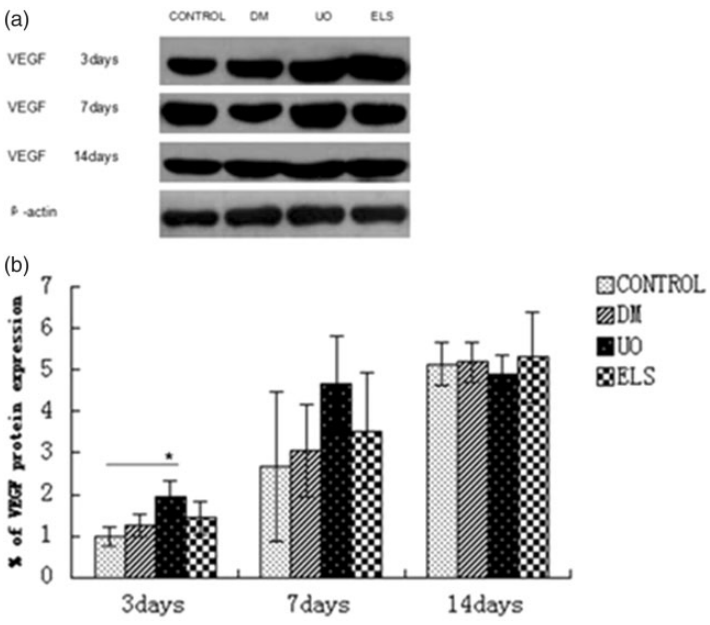

Figure 3. Levels of vascular endothelial growth factor (VEGF) in diabetic ulcer subcutaneous tissues of each group of rats. A. Representative blots. β-actin was the loading control. B. Densitometry analysis of VEGF levels. Means ± SD, n = 3, *$P < 0.05$. PC, positive control; UO, ulcer oil; ELS, ethacridine lactate solution.

healing process gradually occurs after these three phases. However, healing of diabetic ulcers is slow, because long-term sustained high blood sugar results in the accumulation of AGEs and damage to vascular endothelial cell function and structure.[10]

A variety of growth factors have been used to accelerate wound healing, with relatively stable effects. However, some treatments are inappropriate, as they increase the risk of complications.[11,12] Traditional Chinese medicine has advantages for the clinical treatment of diabetic ulcers at different stages. UO is a traditional Chinese medical topical treatment that contains Cortex Phellodendri and *Angelica dahurica*. It is used to clear heat, remove toxins, promote wound healing and moisturise the skin. Pharmacological studies have shown that *Angelica dahurica* can inhibit bacterial growth and has an anti-inflammatory effect on tissue inflammation and swelling.[13]

AGE levels are very low under normal conditions. Diabetic and inflammatory stress increase AGE levels and the binding of AGEs to receptors causes oxidative stress, forming a positive feedback loop.[14] In this study, we found that AGE levels were significantly higher in the PC group and lower in the UO and ELS groups. These data indicate that UO may alleviate oxidative stress and reduce damage.

PTP1B negatively regulates insulin signal transduction pathways through phosphorylation of the substrates. Overexpression of PTP1B reduces insulin signal transduction and inhibits glucose uptake and glycogen synthesis, which lead to insulin resistance. Recent study findings suggest that PTP1B inhibits wound healing by inactivating growth factors in diabetic wounds.[15] In this study, we compared PTP1B expression level in wound tissues of each group on days 3, 7 and 14, and found that PTP1B

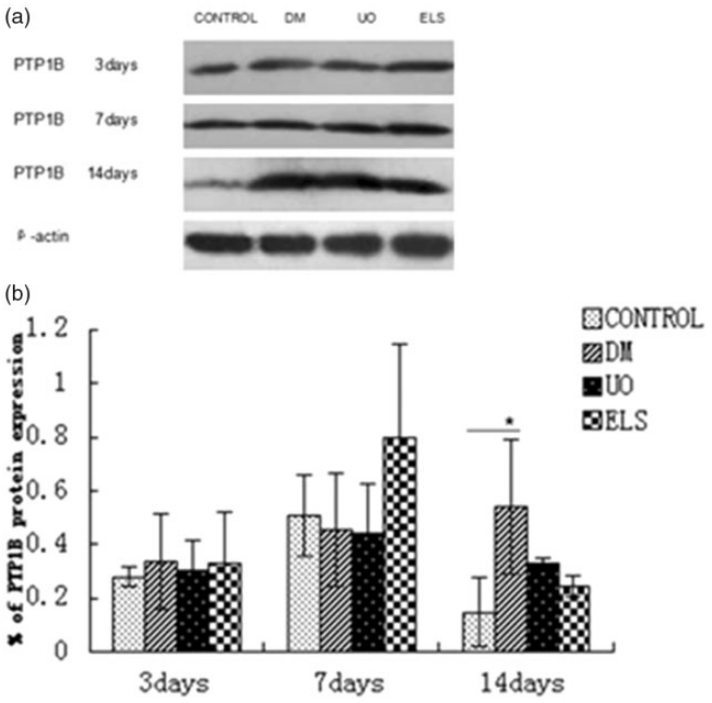

Figure 4. Levels of protein phosphotyrosine phosphatase (PTP1B) in diabetic ulcer subcutaneous tissues of each group of rats. A. Representative blots. β-actin was the loading control. B. Densitometry analysis of PTP1B levels. Means ± SD, n = 3, $^*P < 0.05$. PC, positive control; UO, ulcer oil; ELS, ethacridine lactate solution.

expression in the PC group was higher than in the control group. Additionally, PTP1B expression was lower in the UO group than in the PC group. These data indicate the inhibitory role of PTP1B in wound healing. UO may downregulate PTPB1 expression to promote healing of DFUs.

Insufficient action of wound growth factors is considered a main cause of diabetic ulcers. PDGF can accelerate cell proliferation and promote wound healing in normal tissues.[16] In this study, we found that PDGF levels in diabetic ulcer wounds were lower in the PC group than in the control group on day 3, but that PDGF and VEGF levels were higher in the UO group than in the PC group on day 3. DFUs could be treated and even prevented by controlling the expression of VEGF, which controls new blood vessel formation.[17] One study showed that VEGF expression in diabetic skin samples was lower than in control samples, and reduced VEGF expression in patients with vascular endothelial dysfunction was significantly correlated with increased DFUs.[18] In addition, upregulation of VEGF expression can reduce the incidence of DFUs.[19] Therefore, exogenous VEGF has a curative effect on diabetic foot disease.

In summary, using a diabetic ulcer rat model we demonstrated that UO can downregulate PTP1B and AGEs, and upregulate VEGF and PDGF expression, which in

combination contribute to wound healing. These effects were most pronounced during the inflammation period, suggesting that UO may inhibit the inflammatory response. UO shows promise as a possible treatment for DFUs.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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