PLASMINOGEN APPLICATION IMPROVES PLASTIC CLOSURE OF WOUND DEFECTS IN PATIENTS WITH CHRONIC DIABETIC WOUNDS

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ABSTRACT

Background. Chronic diabetic foot ulcers and wounds are significant complications associated with diabetes, comprising approximately 85% of purulent-necrotic lesions affecting the lower extremities. The development of these wounds is influenced by pathogenetic factors such as hyperglycemia, neuropathy, and existing infections, which contribute to metabolic disturbances, including tissue hypoxia and the activation of proteolytic enzymes known as matrix metalloproteinases (MMPs).

Aim. To explore the therapeutic potential of autologous plasminogen in facilitating the healing process of diabetic wounds through the modulation of MMP activity.

Materials and Methods. The study enrolled 45 patients diagnosed with chronic diabetic wounds, who were assigned to two distinct groups. The control group (n=25) received conventional treatment approaches, while the intervention group consisted of 20 patients treated with autologous plasminogen applications.

Results. After 18 days of treatment, a substantial reduction of 3.5-fold in MMP-2 and MMP-9 activity was observed within the intervention group, accompanied by complete wound closure in 16 patients. Additionally, four patients underwent autodermoplasty, successfully achieving wound defect closure through effective graft integration. In contrast, the control group exhibited consistently elevated MMP activity levels throughout the entire observation period.

Conclusions. The activity of matrix metalloproteinases (MMPs) in chronic diabetic wounds reaches dramatic levels, making spontaneous wound healing impossible. The application of autologous Pg allows modulation of this activity and creates favorable conditions for wound healing by reducing excessive MMP activity, improving blood supply, and resolving inflammatory processes.

Keywords: *chronic wounds, diabetes mellitus, matrix metalloproteinases, plasminogen, autodermoplasty.*

Introduction

Patients with diabetes mellitus are quite sensitive to any trauma due to the neuropathy, as well as a predisposition to slow or non-healing wounds. The primary presentations of tissue injury in the lower limbs among individuals with diabetes mellitus encompass chronic wounds and trophic ulcers, constituting approximately 85% of cases. The residual fraction comprises abscesses, phlegmons, osteomyelitis, and purulent arthritis, which manifest either due to trophic ulcers or as a consequence of traumatic events [1]. Consequently, the issue of effectively addressing wound defects through plastic surgical techniques becomes notably pertinent. Hyperglycemia serves as the principal pathogenetic mechanism contributing to the development of chronic diabetic wounds, as it exerts a toxic effect on tissues, causing metabolic disorders that lead to neuropathy, angiopathy, and immunosuppression [2].

These metabolic disturbances promote tissue hypoxia and subsequent activation of proteolytic enzymes. In physiological conditions, matrix metalloproteinases (MMPs) play a vital role in the degradation of the extracellular matrix (ECM), tissue remodeling, cell migration, and inflammation,

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thereby contributing to each phase of wound healing [3]. Proteases actively participate in the processes of wound healing, making the assessment of proteolytic activity a useful biomarker for wound healing status. Among all Ps, MMP-2 and MMP-9 are vitally important proteases, which are typically involved in the normal tissue remodeling during wound healing [4]. However, it has been reported that a significant increase in MMP activity is a key factor contributing to impaired wound healing in diabetic skin ulcers and other vascular complications. Moreover, excessive MMP activity has been shown as one of the most important risk factors for dermal graft failure during autodermaplastic procedure [5]. Therefore, the exploration of strategies aimed at modulating proteolytic activity is anticipated to yield advantageous outcomes in the management of recalcitrant wound healing cases.

Recent studies have identified plasminogen (Pg) as a crucial participant in the wound healing process that was designated as a "master regulator" of wound healing [6]. Currently, over 12 different cellular receptors for Pg have been identified, which are potentially associated with wound healing. This suggests that apart from fibrinolysis, Pg may act as a signaling molecule that regulates and coordinates the activity of monocytes/macrophages, keratinocytes, platelets, and other cells involved in wound healing [7]. Although many studies performed on animal models are accumulated to report beneficial effects of Pg application on healing process of both acute and chronic wounds, including in diabetic mammals so far there were no attempts made in clinical studies to explore Pg as a possible remedy to improve closure rates and quality of tissue reparation.

Therefore, the **aim** of this study was to investigate the effects of autologous Pg application on MMP activity, closure of chronic wounds, and success of autodermoplasty in patients with diabetes mellitus.

Materials and Methods

The study comprised a cohort of 45 individuals aged 45 to 81 years, diagnosed with diabetes mellitus, who received outpatient care at the Central Polyclinic of the Ministry of Internal Affairs of Ukraine between 2021 and 2023. These patients presented with neuropathic diabetic wounds of prolonged duration, exceeding 6 weeks, and were classified as wounds that are not at risk of amputation within the next year according SVS WIFI classification [8]. Written informed consent was obtained from all participants, who also provided explicit authorization for the dissemination of research findings. Ethical considerations were rigorously observed, with all research procedures and protocols scrutinized and endorsed by the local ethics committee. Adherence to the ethical guidelines prescribed by the most recent iteration of the Helsinki Declaration (2013) was meticulously ensured throughout the course of the investigation.

The patients were randomly divided into two groups. The groups were representative in terms of age, complications, and comorbidities. The control group (25 patients) received treatment for diabetic wounds according to standard protocols, which included local application of antiseptics and wound dressings. All patients were administered glucose-lowering medications. In the intervention group (20 patients), in addition to standard wound care, autologous plasminogen was topically applied to the wound surface at a dose of 1.0 mg/mL of sterile buffered physiological solution every 2 days for a total of 20 days (10 applications in total). Biopsies from the wound bed in patients with diabetes (100±3) mg were collected before the start of treatment (day 0) and on the 18th day of the current treatment period.

Native form of Pg (Glu-Pg) was obtained from fresh citrated plasma of donors (patients with diabetes from the study group) using affinity chromatography on Lys-Sepharose (GE Healthcare, Amersham, UK) in the presence of the serine protease inhibitor aprotinin (10 mg/mL) following the method described by Lijnen [9] with minor modifications. The purity of the obtained Pg preparations was evaluated by denaturing gel electrophoresis on a 10% polyacrylamide gel (SDS-PAGE). The gel electrophoresis results indicated that the Glu-Pg preparations isolated from donor plasma were electrophoretically homogeneous (purity level 99%). Prior to use, the Pg preparations were tested for spontaneous amidolytic activity using a photometric method with the specific chromogenic substrate plasmin S2251, and only those preparations that did not show spontaneous activity were included in the study. The protein preparations were concentrated, sterilized by ultrafiltration, frozen, and stored at – 20°C until use.

The activity of MMP-9 in skin wound biopsies was evaluated by gelatin zymography and compared to the MMP-9 activity in biopsies taken from acute wounds. Gelatinolytic activity was assessed by separating proteins (50 μg per lane) on 8% polyacrylamide gel co-polymerized with gelatin (5 mg/ml). Following denaturing gel electrophoresis, the gels were subjected to two 30-minute

washes with cold 2.5% (i/v) Triton X-100 to eliminate SDS. Subsequently, they underwent five 5-minute washes with cold deionized water. The gels were then incubated for 16 hours at 37°C in a developing buffer (50 mM Tris-HCl, pH 7.6, containing 0.15 M NaCl, 5 mM $CaCl_2$, 1 mM $ZnCl_2$, and 0.02% Tween-20). Zymograms were stained using a 0.15% ethanol solution of Coomassie R-250. Destaining was carried out using a dye-free solution consisting of 30% methanol and 10% acetic acid. After destaining, the gel exhibited a uniform blue background, except for regions where MMPs migrated and digested the substrate. Gelatinolytic activity was identified as transparent bands against the stained gelatin background. The resulting MMP bands were visualized and subjected to quantitative densitometric analysis.

The statistical analysis of the gelatin zymography data was performed using the Mann-Whitney U test to assess differences between mean parameters. All variables were expressed as mean ± standard error of the mean (SEM). A significance level of $p<0.05$ was considered statistically significant for all tests. The statistical calculations were conducted using the "OriginPro" software (version 9.0 SR2 Pro English).

Results and Discussion

Conservative therapy often plays an independent role in the treatment of neuropathic diabetic wounds. According to some authors, the healing of foot ulcers reaches 80–90%, with approximately two-thirds of patients who do not require surgical intervention [10]. To achieve a positive outcome in such cases, adherence to treatment protocols, aseptic and antiseptic practices, and an unrestricted duration of treatment (up to 8 months) are necessary. However, chronic wounds pose a potential risk of further infection and the development of new purulent foci in the extremity. Therefore, the primary focus of treating these wounds is early closure of the wound defects to prevent complications and tissue destruction progression [11]. The primary requirements for undertaking plastic surgery involve achieving the patient's overall well-being, ensuring sufficient blood circulation to the soft tissues of the limb, and resolving any purulent inflammation present. Optimal conditions for plastic surgery include a wound with minimal bacterial colonization (below the critical level of 105 microbial bodies per 1 gram of tissue) and the presence of vibrant, moist granulation tissue with minimal exudation. The objective of the surgical procedure is to achieve full closure of the wound defect and prevent deformities.

The following types of plastic surgeries are commonly performed: autodermoplasty (ADP) for extensive defects on the dorsal foot; tissue expansion or local tissue flaps for medium-sized defects on the shin; local tissue flaps in the ankle joint area and minor linear wounds on the dorsal foot; local tissue flaps for defects in "pressure points"; autodermoplasty for defects in the medial arch of the foot; rotation of dorsal flaps to close dorsal and plantar defects; rotation of plantar flaps to close lateral and dorsal defects of the foot.

The application of dressings in patients from the main group resulted in complete healing in 16 patients with wounds measured up to 50 cm^2 within 16–18 days of treatment. However, in 4 patients with larger wounds exceeding 50 cm^2 on the posterior surface of the shin and foot, the main objective was wound debridement and preparation for closure. Prior to the start of treatment, the wounds exhibited characteristic features: the wound surface was covered with fibrin, it was swollen and protruding above the skin surface, the granulation tissue was coarse-grained and had an unhealthy pale gray color, and there was moderate exudate with an unpleasant odor (*Fig. 1*).

After the application of autologous Pg dressings using the authors' method, complete debridement of the wounds from fibrin and necrotic tissue was observed, and a reduction in edema was noted starting from day 10 of treatment. The granulation tissue appeared fine-grained and had a "healthy" appearance, with minimal exudate. Pathogenic microorganisms were not detected in the bacteriological examination, and histological analysis showed the absence of a "biofilm." From day 14 onwards, progressive reduction in the wound area was observed, indicating favorable conditions for performing plastic surgery (*Fig. 2*).

The gelatinase activity analyzed by zymography assay in bioptates from chronic wounds before the Pg treatment was high, that is generally in agreement with earlier published data [12]. Levation of MMP-9 levels, which is a typical characteristic of chronic wound processes, was observed on the zymograms (*Fig. 3*). However, a significant decrease in MMP activity in the wound tissues by 3.5 times was observed on day 18 of treatment ($p<0.01$). Meanwhile, the MMP activity in acute wounds remained minimal throughout the entire treatment period, and by day 18, it exhibited a trace level (*Fig. 4*). Then, the wound defects were closed using full-thickness perforated skin grafts

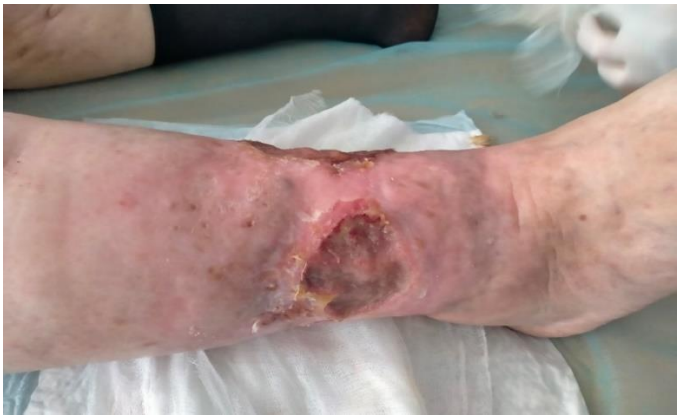

Fig. 1. Diabetic trophic ulcer prior to treatment with autologous plasminogen.

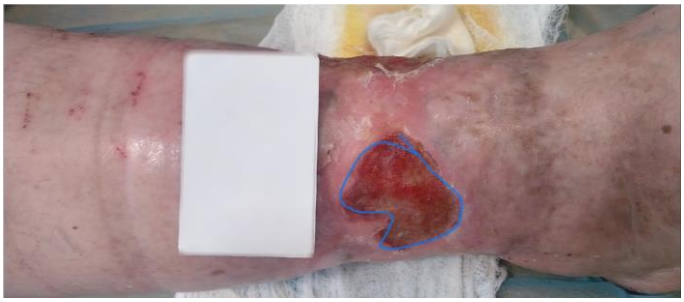

Fig. 2. Diabetic trophic ulcer on the 12th day of treatment with autologous plasminogen.

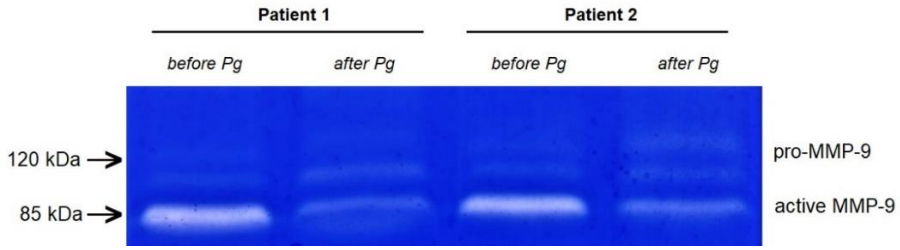

Fig. 3. Decrease activities of matrix metalloproteinases due to treatment by autologous plasminogen.

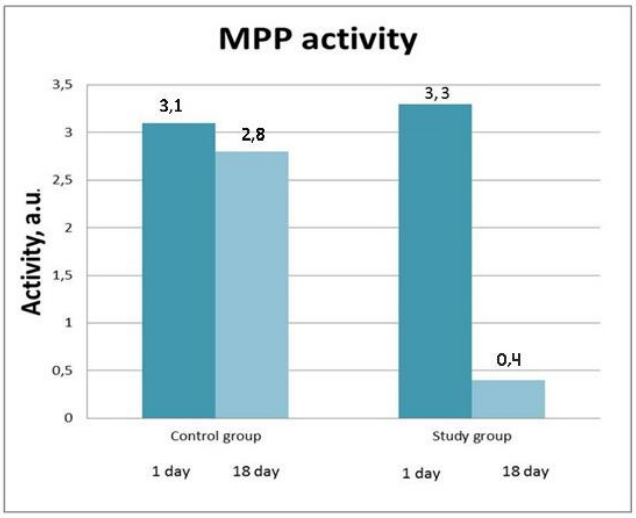

Fig. 4. Effect of plasminogen application on the levels of MMP-9
in chronic wound tissue of diabetic patients (typical gelatin zymography).

with the assistance of autodermoplasty (*Fig. 5*). All grafts successfully engrafted.

In the control group patients with traditional treatment, a decrease in signs of perifocal inflammation and cleansing of the wounds from fibrin and necrotic tissues was observed starting from day 20 of treatment. The level of matrix metalloproteinase (MMP) activity remained consistently high. Subsequently, the cleansing of the wound surface and reduction in wound area occurred gradually with the involvement of new forms of wound coverings and instrumental methods. Wound contraction occurred starting from day 30 of treatment. The prolonged and often unsuccessful healing of chronic diabetic wounds is attributed to the excessive activity of MMPs. Their aggressive action completely negates all treatment efforts for wound healing. Even complete cleansing of the wound from microbial components does not guarantee successful healing. Performing plastic surgery to close the wound surface will not be successful in this case, and will result in complete graft lysis, until the protease activity is neutralized (*Fig. 6*). As demonstrated in our previous studies, despite the use of vacuum therapy in the treatment of diabetic wounds, the level of MMP activity in the wound exudate remains relatively high, even after vacuum therapy had been applied [13].

One of the mechanisms of Pg action in chronic wound processes primarily involves the elimination of tissue hypoxia by dissolving microthrombi in blood vessels through its fibrinolytic properties [14]. In addition, the conversion of plasminogen to plasmin is accompanied by increased activity of pro-inflammatory cytokines present in the wound tissues. The expression of these factors initiates inflammation and stimulates angiogenesis, which is a crucial component in overcoming the "vicious cycle" of chronic inflammation and transitioning the wound process to the proliferation stage thus pushing wound to heal.

Conclusion

The activity of matrix metalloproteinases (MMPs) in chronic diabetic wounds reaches dramatic levels,

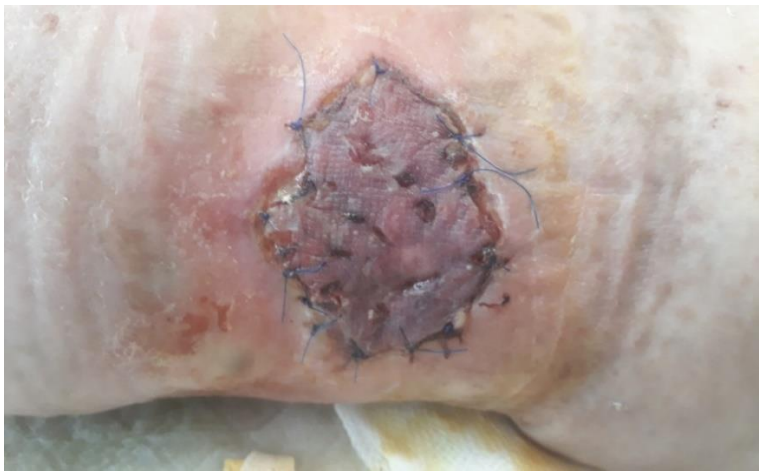

Fig. 5. Graft survival on the 10th day after plastic closure of the wound.

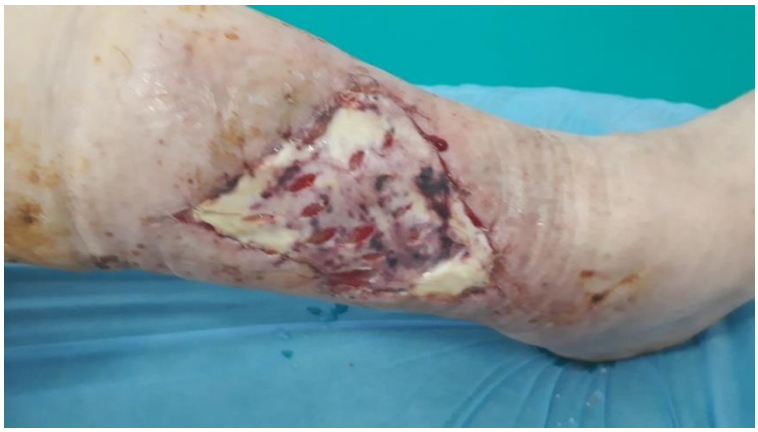

Fig. 6. Graft necrosis on the 10th day after ADP in patient of the control group.

making spontaneous wound healing impossible. The application of autologous Pg allows modulation of this activity and creates favorable conditions for wound healing by reducing excessive MMP activity, improving blood supply, and resolving inflammatory processes. Local administration of autologous Pg represents a promising strategy for developing new therapeutic approaches that improve wound healing in patients with diabetes mellitus.

DECLARATIONS:

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

Data Transparency

The data can be requested from the authors.

Statement of Ethics

The authors have no ethical conflicts to disclosure.

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Consent for publication

All authors give their consent to publication.

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