Peptides 78 (2016) 1–10

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Nesfatin-1 improves oxidative skin injury in normoglycemic or hyperglycemic rats

Ali Solmaz[a,1], Elif Bahadır[b,1], Osman B. Gülçiçek[a], Hakan Yiğitbaş[a], Atilla Çelik[a], Ayça Karagöz[c], Derya Özsavcı[d], Serap Şirvancı[c], Berrak Ç. Yeğen[b,*]

[a] General Surgery Clinic, Bağcılar Training and Research Hospital, Istanbul, Turkey
[b] Department of Physiology, Marmara University Faculty of Medicine, Istanbul, Turkey
[c] Department of Histology & Embryology, Marmara University Faculty of Medicine, Istanbul, Turkey
[d] Department of Biochemistry, Marmara University Faculty of Pharmacy, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form 9 December 2015
Accepted 23 December 2015
Available online 30 January 2016

Keywords:
Nesfatin-1
Vascular endothelial growth factor (VEGF)
Hyperglycaemia
Surgical wound
Neutrophil infiltration
Oxidative injury

ABSTRACT

Hyperglycemia is one of the major causes of suppressed angiogenesis and impaired wound healing leading to chronic wounds. Nesfatin-1 a novel peptide was reported to have antioxidant and anti-apoptotic properties. This study is aimed to investigate the potential healing-promoting effects of nesfatin-1 in non-diabetic or diabetic rats with surgical wounds. In male Sprague-Dawley rats, hyperglycemia was induced by intraperitoneal (ip) injection of streptozotocin (55 mg/kg). Under anesthesia, dorsum skin tissues of normoglycemic ($n = 16$) and hyperglycemic rats were excised (2×2 cm, full-thickness), while control rats ($n = 16$) had neither hyperglycemia nor wounds. Half of the rats in each group were treated ip with saline, while the others were treated with nesfatin-1 (2 μg/kg/day) for 3 days until they were decapitated. Plasma interleukin-1-beta (IL-1β), transforming growth factor-beta (TGF-β-1), IL–6 levels, and dermal tissue malondialdehyde (MDA), glutathione (GSH) levels, myeloperoxidase (MPO) and caspase-3 activity were measured. For histological examination, paraffin sections were stained with hematoxylin-eosin or Masson's trichrome and immunohistochemistry for vascular endothelial growth factor (VEGF) was applied. ANOVA and Student's *t*-tests were used for statistical analysis. Compared to control rats, skin MPO activity, MDA and caspase-3 levels were increased similarly in saline-treated normo- and hyperglycemic rats. Nesfatin-1 depressed MDA, caspase-3, MPO activity and IL-1β with concomitant elevations in dermal GSH and plasma TGF-β-1 levels. Histopathological examination revealed regeneration of epidermis, regular arrangement of collagen fibers in the dermis and a decrease in VEGF immunoreactivity in the epidermal keratinocytes of nesfatin-1-treated groups. Nesfatin-1 improved surgical wound healing in both normo- and hyperglycemic rats via the suppression of neutrophil recruitment, apoptosis and VEGF activation.

1. Introduction

Almost all acute wounds created by surgical procedures heal by an orderly and timely process, resulting mostly in structurally and functionally satisfactory outcomes [1]. During the healing process of a wound, cell proliferation and migration, collagen deposition and remodeling, wound contraction, and angiogenesis take place, which involve the activities of various inflammatory cells like neutrophils, macrophages, fibroblasts/myofibroblasts, keratinocytes, and endothelial cells [2,3]. The wound tissue releases pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), initiating a healing process accompanied with inflammatory events [4,5]. In the course of wound healing, the cells that participate in wound healing produce $\cdot O_2^-$ and the resultant reactive oxygen species (ROS) possess deleterious effects on wound healing by inhibiting cell migration and angiogenesis [6]. Thus, if the produced oxidants cannot be eliminated by anti-oxidative systems, a delay in wound healing takes place [7].

Delayed skin wound healing and impaired soft tissue regeneration are common causes of morbidity and mortality in patients with diabetes mellitus, but the underlying pathophysiological mechanisms are not well defined yet. It has been suggested that

* Corresponding author at: Marmara University, School of Medicine, Temel Tip Bilimleri Binasi, Basibuyuk Yerleskesi, Maltepe, 34854 Istanbul, Turkey.
E-mail addresses: byegen@marmara.edu.tr, berrakyegen@gmail.com (B.Ç. Yeğen).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.peptides.2015.12.006
0196-9781/

hyperglycemia delays wound healing and particularly alters angiogenesis and extracellular matrix remodeling [8] via the generation of advanced glycation end-products, hyperosmolarity and oxidant formation, which activate inflammatory cytokines and alter collagen synthesis [9–11]. Spravchikov et al. [12] have shown that chronic skin complications of diabetes occur directly due to high glucose exposure and impaired insulin signaling, which impair glucose utilization of skin keratinocytes, and the subsequent skin proliferation and differentiation. Diabetic leg and foot ulcers heal more rapidly in patients with lower HbA1c, verifying the correlation between hyperglycaemia and impaired wound healing [13]. An altered immune function with decreased chemotaxis, phagocytosis, bacterial killing, and reduced heat shock protein expression was proposed to be responsible for the early phase, while altered leukocyte infiltration and elevated IL–6 in wound fluid were observed in the late inflammatory phase of impaired wound healing in diabetes [14–16]. Moreover, hyperglycemia is one of the patient-related risk factors predisposing for surgical site infections in several surgical disciplines [17]. Based on the oxidative nature of impaired healing in diabetes, many compounds with anti-oxidant actions were used in attempts to treat diabetic wounds [18–21]. Accordingly, α-lipoic acid, ascorbic acid, carnosine and various plant extracts have been screened for their therapeutic potentials in wound healing [22–24].

Nesfatin-1, a recently identified satiety-inducing adipokine derived from nucleobindin-2 (NUCB2) in hypothalamic nuclei, was recently shown to have anti-inflammatory effects via the maintenance of the intracellular antioxidant pools [25–27]. Previous studies in murine pancreatic beta cells have demonstrated the co-expression of precursor pronesfatin and insulin immunoreactivity, suggesting a potential role for pronesfatin-encoded peptides in regulating islet biology [28,29]. When the pancreatic cell lines or specifically β-cells were incubated in high glucose, nesfatin-1 levels [30] and nesfatin-1-induced glucose-dependent insulin secretion were stimulated [31]. On the other hand, both NUCB2 and preproinsulin mRNA expressions were down-regulated in streptozotocin (STZ)-induced type 1 diabetic mice [31]. However, the possible healing-promoting effect of nesfatin-1 on surgical wounds complicated with hyperglycemia was not elucidated before. The current study is aimed to investigate the potential effects of nesfatin-1 on the oxidative stress that occurs during the healing of surgical wounds under normoglycemic or hyperglycemic conditions.

2. Materials and methods

2.1. Animals

Forty-eight male Sprague-Dawley rats (300–400 g), obtained from Bağcılar Training and Research Hospital Animal Center (BADABEM), were housed in cages (one per cage) under controlled room temperature (21 ± 2° C), humidity (60–70%) with 12-h light-dark schedule and were fed with standard pellet, ad libitum. All experimental procedures were approved by the Bağcılar Training and Research Hospital Animal Care and Use Committee (2013-40).

2.2. Induction of hyperglycemia

Rats were randomly assigned to normoglycemic ($n = 16$) and hyperglycemic ($n = 16$) groups. On the first day of the experiment, a group of rats was injected intraperitoneally with a single dose of streptozotocin (STZ, 55 mg/kg; Sigma–Aldrich, St. Louis, MO, USA), freshly prepared in cold 0.1 M citrate buffer (pH 4.5). On the third day of injections, blood was withdrawn from the tail veins and fasting blood glucose levels were measured by a glucometer (IME-DC GmbH, Germany). STZ-injected animals with a blood glucose level of ≥200 mg/dL were considered as hyperglycemic; those below that limit were not included in the further steps of the experiment. Fifteen days later, skin incisions were made, and the rats were treated for 3 days with either saline or nesfatin-1, while the control groups with no incisions ($n = 16$) were anesthetized and treated with either saline or nesfatin-1 for 3 days.

2.3. Excision wound model and experimental design

Hyperglycemic rats ($n = 16$; on the 16th day of STZ injection) and non-STZ-injected normoglycemic ($n = 16$) rats were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Under aseptic conditions, the dorsal skin of the animals was shaved and cleaned with povidone-iodine solution, and an open excision-type square-shaped full-thickness wound (approximately 2×2 cm^2, ≈400 mm^2) was created to the depth of loose subcutaneous tissue as reported previously [32]. Following the operation, intraperitoneal acetaminophen (Fentanyl Citrate®, Abbott, IL, USA; 0.1 mg/kg) was injected for analgesia. After recovery from anesthesia, rats were housed individually in disinfected cages and were wet dressed with saline twice a day to avoid exposure and prevent infection.

Normoglycemic non-wounded control rats, as well as normoglycemic and hyperglycemic rats with skin incisions were treated for 3 consecutive days with either saline (1 ml/kg/day) or nesfatin-1 (2 μg/kg/day). The rationale for the dose of nesfatin-1 depends upon our previous studies in which we have investigated the effective dose between 0.1–10 μg/kg [25,26]. On the 19th day of the experiment, all rats were euthanized under anesthesia, and blood was collected by cardiac puncture, centrifuged and stored at −80 °C for the measurement of transforming growth factor-(TGF)-β, interleukin (IL)–6, IL-1β and cortisol in the plasma. Skin tissue samples were taken from the wound sites, stored at −80 °C for the measurement of malondialdehyde (MDA), glutathione (GSH) levels, myeloperoxidase (MPO) and caspase-3 activities. For histological examination, dermal tissue samples were processed by routine techniques and fixed in 10% (v/v) buffered p-formaldehyde before embedding in paraffin.

2.4. Measurement of plasma cortisol, TGF-β, IL–6, IL-1β

Plasma levels of cortisol, TGF-**β** and pro-inflammatory cytokines IL–6 and IL-1β were quantified using enzyme-linked immunosorbent assay (ELISA) kits specific for the rat, according to the manufacturers' instructions and guide-lines (Platinum Elisa, eBioscience). These particular assay kits were chosen because of their high degree of selectivity, sensitivity and inter- and intra-assay precision, and the small amount of plasma sample required to conduct the assay.

2.5. Measurement of MDA and GSH levels

As signs of lipid peroxidation products, malondialdehyde (MDA) levels were assessed in the skin samples obtained from the wound sites. Samples were homogenized in 10 volumes of 10% trichloracetic acid and centrifuged at 3000 rpm for 15 min at 4 °C. Supernatant was taken and re-centrifuged at 15,000 rpm at 4 °C for 8 min. The lipid peroxide levels were expressed in terms of MDA equivalents as nmol MDA/g tissue [33]. GSH was determined by a modified Ellman procedure based on spectrophotometric measurement [34]. The results were given in milimoles GSH per gram tissue.

2.6. Measurement of MPO activity

Myeloperoxidase (MPO), mainly released by activated neutrophils, is accepted as an indicator of neutrophil accumulation in the inflamed tissues. Using a procedure similar to that documented