NITRIC OXIDE DONOR (DETA NONOATE) ENHANCE EXPERIMENTAL WOUND HEALING IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Background: Diabetes is characterized by a nitric oxide deficiency at the wound site. Diabetes is a factor that influences all stages of wound healing. In animals with acute experimental diabetes induced by streptozotocin (STZ), the early inflammatory responses after wounding is impaired, fibroblast and endothelial cell proliferation is reduced as well as accumulation of reparative collagen and gain in wound breaking strength. In this study we investigated whether exogenous nitric oxide supplementation with nitric oxide donor DETA NONOate could reverse impaired healing in diabetes.

Method: In this study male Sprague Dawley Rats were rendered diabetic by intraperitoneal streptozotocin administration. Nine days after induction of diabetes (blood glucose greater than 250 mg/dl), were given full thickness dermal wounds (1×1cm). The test group (n=6) was treated with 100µ mole DETA NONOate in phosphate buffer while control wounds in the control subjects (n=6) received sterile phosphate buffer on the same day and subsequently every three days. Urinary nitrate output was quantitated daily prior to wounding, during wound healing (21 days) and following external wound closure. The rate of wound healing was determined by video image analysis on the day of wounding and every 3 days during wound healing (21days).

Result: The results suggest nitric oxide donor DETA NONOate can reverse impaired healing associated with diabetes (P<0.001) and urinary nitrate (NO³⁻) output may reflect the extent of repair in this wound model (P<0.001).

Conclusion: Site specific delivery of nitric oxide via NO-donor DETA NONOate could be an effective therpeutic strategy to impaired diabetic wound healing.

KEY WORDS: DETA NONOate, Diabetes, Wound healing

INTRODUCTION

Nitric oxide (NO) is a unique, gaseous free radical that is an important physiologic mediator for autonomic functions such as vasodilation, neurotransmission, and intestinal peristalsis. Recent wound healing studies of NO-mediated enhanced tissue repair¹,². In diabetes, an endogenous deficiency in NOS enzyme leads to decreased wound NO production and a spectrum of related pathologies,³,⁴ such as impaired cutaneous vasodilation, decreased neurogenic vascular response, diabetic neuropathy, endothelial cell function that inhibit the processes necessary for granulation tissue
formation and reduction in the accumulation of reparative collagen. As a mediator of tissue repair, NO has shown to promote angiogenesis and cellular migration, increase wound collagen deposition and collagen cross linking, regulate vasodilation, inhibit platelet aggregation, inhibit endothelial-leukocyte cell adhesions, modulate endothelial proliferation and apoptosis, increase the viability of random cutaneous flaps, and enhance cellular immunomodulation & bacterial cytotoxicity.

Based on previous findings that diabetes is characterized by reduced nitric oxide levels in the wound environment. In this study we investigated whether exogenous nitric oxide supplementation with nitric oxide donor DETA NONOate could reverse impaired healing in diabetes. The 24 hr urine samples were collected throughout the healing period (21 days). Wound closure profiles were examined by video image every 3 days and urinary nitrate NO$_3$ output was measured by Griess reagent.

**MATERIALS AND METHOD**

DETA NONOate [(z) - 1 - [2 - Amino ethyl] - N - (2 - ammonioethyl) amino - diazen - 1 - ium - 1, 2 - diolate] was purchased from Alexis Co. (Switzerland). Low nitrate diet (2% L-arginine) was obtained from Pasture Institute, Tehran, Iran and Potassium Nitrate (99.9%) from Merck chemical Company (Germany). Vanadium (III) Chloride (99%) was purchased from Johnson Mattley GmbH. Blood glucose levels were measured with glucose oxidase kit. (Zist Chimi Chemical Co. Tehran, Iran) Griess Reagent was purchased from Alexis Biochemicals (Switzerland). All procedures used for animal experimentation were approved by the animal care committee of Tehran University of Medical Sciences.

Male Sprague-Dawley Rats (Tehran University of Medical Sciences animal house, Tehran, Iran) were acclimatized for one week they were given water and libitum, and fed a low nitrate containing diet (2% L-arginine). Animals were transferred to separate metabolic cages. Nine days before wounding, 12 rats were injected intraperitoneally (IP) with streptozotocin (STZ) (55 mg/kg body weight in citrate buffer 0.1 mol/L, pH 4.5) to induce diabetes. Evidence of diabetes was confirmed by blood glucose levels greater than 250 mg/dl and excessive urination. Daily urine samples were collected at every 24 hr intervals. To inhibit bacterial growth, 5ml of 3 M HCl was added to each urine collection (pH=1) and urine samples were kept frozen until analysed (-70°C). Before wounding, the rats were anesthetized with Nembutal (40 mg/Kg i.p.). The dorsal surface of each rat was properly shaved and given full thickness dermal wounds approximately 1 cm x 1 cm. The test group (n=6) was treated with 100µmole DETA NONOate in phosphate buffer while control wounds (n=6) received sterile phosphate buffer on the same day and every 3 days. Urinary nitrate (NO$_3$) was quantitated daily prior to wounding, during wound healing (21 days) and following external wound closure. The rate of wound healing was determined by video image analysis. 48hr following wounding and every 3 days, wounds were video taped using Nikon Colpix 5000. The urinary nitrate level was determined using Griess reagent. SPSS computer software was used for data management and analysis.

**RESULTS**

Promising results have been obtained from studies using non-soluble, polymeric DETA NONOate as NO donating agent during cutaneous healing in rats. The urinary nitrate (NO$_3$) profiles for diabetic rats with DETA NONOate and controls is shown in Figure 1. Each point represents the mean daily urinary nitrate output for each group. Topical application of DETA NONOate was made on days 0, 3, 6, 9, 12, 15, 18 and 21. The mean pre wound urinary nitrate output for NONOate group was 4.9 ± 0.21 µmol/day. The largest post wound urinary nitrate increase for NONOate group occurred from days zero to eleven. Mean urinary nitrate output from days zero to eleven for NONOate group was 9.59 ± 0.67 µmol/day which was significantly
higher than pre wound levels (P<0.001). Mean Urinary nitrate output for control group was 8.8 ± 0.7 µ mol/day which was significantly higher than pre wound levels (P<0.001). Mean urinary output between days twelve to twenty one for NONOate and control groups was 10.18 ± 0.86 and 9.3 ± 0.89 µ mol/day respectively. Control diabetic rats had significantly less urinary nitrate (NO\textsubscript{3}) output than the test group (P<0.001), where there is a significant difference (P<0.001) between the test and the control group. A significant peak in nitrate (NO\textsubscript{3}) output occurred between days 12-13 when the external wound was approximately 65% closed. Diabetic rats whether treated with DETA NONOate or not, exhibited a significant increase in urinary nitrate (NO\textsubscript{3}) output within 24 to 48 hr post wounding period. During a 3 day period, all the rats were removed from their cages and video imaged. Wound closure rates for both DETA NONOate treated and control rats are shown in Figure 2. Based on percent wound open (relative to initial wound area), the healing of wounds with topical DETA NONOate was significantly enhanced (P<0.001) relative to controls. Wounds of DETA NONOate treated rats were 90 ± 0.72 percent open 3 days post wounding compared to 95 ± 0.201 percent for controls. By day 12,
deficient in nitric oxide. The Nitric Oxide donor DETA NONOate represents a potential treatment for acutely impaired healing in diabetes. NO donors can partially reverse the impaired healing in diabetes and in parallel restore wound NO Levels towards more normal values. Urinary nitrate (NO$_3^-$) output reflects the extent of wound repair, and is important throughout the wound healing. The results obtained indicate an increase in urinary NO$_3^-$ output between days 12-13 though the external wound closure is almost complete. The results of this study shows that DETA NONOate applications could raise the urinary NO$_3^-$ levels and the subsequent elevated levels compared to control rats indicates that significant amounts of NO were delivered to the wound site. This can be related to an increased biochemical activity and revascularization at the wound site and it is possible that urinary NO$_3^-$ output had dropped to essentially pre-wounding levels.

In summary, wound healing is a complex biological process and site specific delivery of nitric oxide via NO-donor DETA NONOate could be an effective therapeutic strategy to impaired wounds. Nitric Oxide is vital to healing process. Novel therapies in wound care management rely heavily on our current knowledge of wound healing process. Recently investigators have implicated Nitric Oxide (NO) in the exertion of regulatory forces on various cellular activities of inflammatory and proliferative phases of wound healing. However a better understanding of the regulation and functions of Nitric Oxide supplementation as means of elevating Nitric Oxide at wound site is clearly required.

REFERENCES