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Role of Nitric Oxide in Extracellular Matrix Metabolism and Inflammation in Diabetic Wound Healing

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1. Introduction

Diabetes and its subsequent complications present a significant challenge to our healthcare system. In particular, chronic diabetic wounds are a major cause of morbidity and use of healthcare resources in the U.S. Recent statistics reveal that 15% of diabetic patients develop foot and ankle chronic ulcers (Singh et al., 2005). Of these chronic wounds, 1 in 5 results in amputation. Further statistics reveal that 60% of nontraumatic lower-limb amputations occur in people with diabetes (Reiber, 2001). Chronic diabetic wounds result in a state of chronic inflammation about the wound site with decreased collagen and nitric oxide (NO) levels. The aims of these studies were to examine whether NO donor compounds will decrease inflammation and extracellular matrix destruction and simultaneously improve collagen production and wound healing. Specifically, we examined the effects of NO donors NOR-3 and SNOG on expression of matrix metalloproteinases (MMP)-1, -2. -8, -9 and -13, the inflammatory mediator interleukin-6 and collagen types I and type III by normal and diabetic fibroblasts under both normoxic and hypoxic states.

2. Methods

Prior studies by others have resulted in the development of a catalog of factors (altered MMP/TIMP ratio, decreased cell proliferation and migration, changes in growth factors [TGF-β, VEGF, PDGF-BB] and cytokines [IL-1β, IL-6, TNF-α], extracellular matrix, oxygenation, and nitric oxide) which are important in normal wound healing and demonstrate the dysregulation that exists in chronic wounds (Chen et al., 1999; Cook et al., 2000; Lobmann et al., 2002; Efron and Moldawer, 2004;Vandeberg et al., 2005). What was intriguing to us was the observation that a number of MMPs were known to be elevated in chronic wounds and that nitric oxide levels were quite low, but no one had drawn the connection that nitric oxide may regulate MMPs and promote wound healing by altering the MMP/TIMP ratio. By returning the MMP/TIMP ratio to near normal levels, matrix remodelling and growth factor regulation of the healing process could begin. The experiments described in this chapter were designed to examine these questions by testing

short-acting and long-acting NO donor compounds in fibroblast culture and in an *in vivo* skin wound repair model. We chose to evaluate enhancement of matrix production by measuring type I and III collagen expression and amelioration of inflammation by measuring interleukin-6 (IL-6) production. We have previously demonstrated that long-acting NO donors significantly raise NO levels and reduce MMP gene expression in human diabetic skin fibroblast cultures (Burrow et al., 2007).

2.1 Diabetic ulcer model

Genetically diabetic, male C57BL/KsJ-*m*+/+*Lepr*^{db} mice and control parental strain C57BL/KsJ mice were obtained from Jackson Laboratories (Bar Harbor, ME). According to an IACUC-approved protocol, mice were anesthetized and a 10mm full thickness wound was created on the dorsum. Treated mice received a single topical application of vehicle or 500 nM (±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamide (NOR-3) or S-nitrosoglutathione (SNOG) applied to the wound. The rate of wound healing was compared throughout the experimental period and wound area was determined by tracing the edge of the wound onto a glass microscope slide and determining changes in wound area by use of Adobe Photoshop.

2.2 Cell culture and treatment

Primary dermal fibroblasts were isolated from normal and diabetic mice by mincing excised skin samples and digesting for 2 hours in 0.20% collagenase (type I, Worthington, Freehold, NJ) in serum-free Dulbecco's modified Eagle's medium (Mediatech Inc., Manassas, VA) at 37°C. The dissociated cells were cultured in Dulbecco's modified Eagle's medium with 20% FBS (Atlanta Biologicals, Norcross, GA) and 1% antibiotic/antimycotic supplement at 37°C with 100% humidity in 5% CO2 in air. Third passage fibroblasts were treated with control media, short-acting NOR-3, or long-acting SNOG NO donor compounds. Cells were incubated at 37ºC in Dulbecco's Modified Eagle's Medium + 20% FBS + antibiotics for 1, 3 or 7 days. Similar experiments were carried out using human skin fibroblasts from age- and gender-matched non-diabetic and diabetic human subjects. Human diabetic and control fibroblasts (catalog no. GM00043, normal fibroblasts, and catalog no. GM01486, maturity onset diabetes fibroblasts) were obtained from the Coriell Institute for Medical Research (Camden, NJ). GM00043 cells were obtained at passage 11 and used for experiments at passages 14–16. GM01486 cells were obtained at passage 3 and used at passages 4–6. The cells were grown in T75 tissue culture flasks (Falcon BD, Bedford, MA) in minimal essential medium containing 15% fetal bovine serum (catalog no. 100-602; Gemini Bio-Products, West Sacramento, CA) and 1% penicillin-streptomycin at 37°C, following the recommended split ratios of 1:4 for the normal GM00043 cells and 1:3 for the diabetic GM01486 cells. For experiments, cells were plated in 24-well tissue culture plates (BD Biosciences, San Jose, CA) at a seeding density of 40,000 cells/well and cultured to confluence. The experiments were conducted under normoxic conditions (20% oxygen) and hypoxic conditions (2 % oxygen) using a Model NU-4950 CO_2 incubator with the ability to accurately regulate the oxygen level (Nuaire Corp., Plymouth, MN).

2.3 MMP/gelatin zymography

MMP-2 and MMP-9 activity in control and 500 nM SNOG-treated fibroblasts were assessed by gelatin zymography. Samples were separated under non-reducing conditions on 10%

SDS-PAGE mini-slab gels co-polymerized with 150 µg/ml type I gelatin, washed in 5% Triton X-100, and equilibrated with collagenase assay buffer (50 mM Tris, 200 mM NaCl,

5 mM $CaCl_2$, pH 7.2) at 37°C. Counterstaining with Coomassie brilliant blue revealed MMP-2 and -9 as cleared bands.

2.4 MMP and collagen gene expression

Total RNA was isolated from the non-diabetic and diabetic skin fibroblast cultures using TRIzol and then reverse transcribed using specific primers for MMP-2, -9 and -13, and Types I and III collagen. To determine the effects of NO donor on mRNA levels for each MMP, we performed real-time PCR analyses using 18S rRNA as the index gene, specific primers/probes for each MMP, and a Prism 7000 detection system (Applied Biosystems, Foster City, CA).

2.5 Determination of growth factor and cytokine levels

Release of cytokines and growth factors into the media of the cultures was quantified by use of Quantikine ELISA kits from R and D Systems. Colorimetric determination of interleukin-6 and PDGF-BB levels were done following manufacturer's specifications.

3. Results

3.1 Effects of NO donors on MMP expression

Expression of mouse MMP-2, MMP-9, and MMP-13 was measured using real time PCR. MMP-2 and MMP-9 were expressed in both normal and diabetic fibroblast cultures after 1 day of culture, but expression in the diabetic cells was 5-8 times higher than that found in normal (Figure 1). In contrast, MMP-13 was expressed by both cells at equivalent levels. When S-nitroso-N-acetylpenicillamine (SNAP), a NO donor with a half-life of 5 hours, was added to the cultures, a dose-dependent decrease in MMP-9 expression was observed (Figure 2). These results validate our previous findings in human skin fibroblasts (Burrow et al., 2007) and confirm that it is reasonable to expect mouse fibroblast MMP expression to be similar to that found with the human fibroblasts.

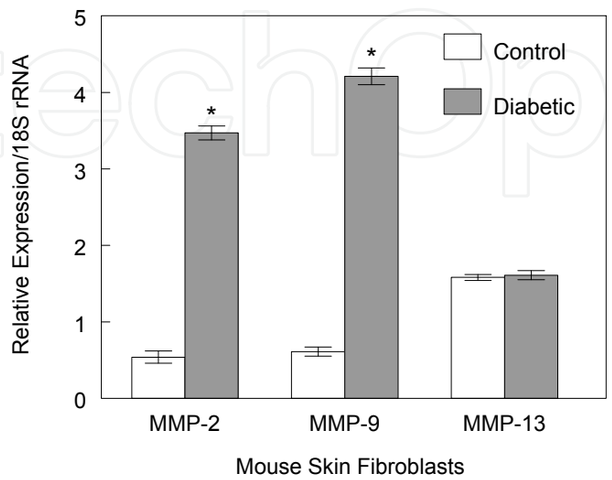

Fig. 1. Matrix metalloproteinase gene expression in control and diabetic mouse fibroblasts.

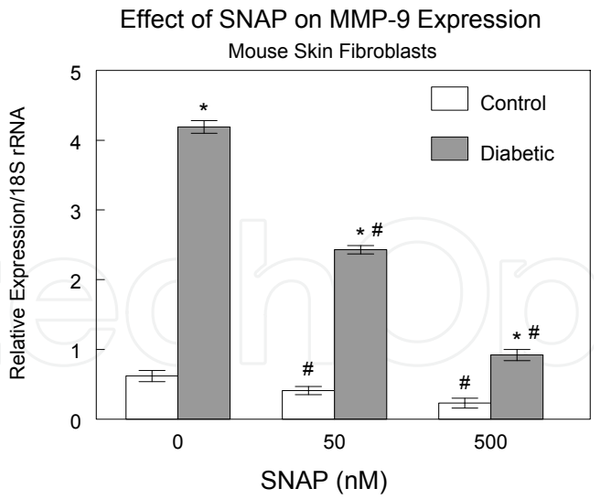

Fig. 2. Effect of NO donor SNAP on MMP-9 expression by control and diabetic mice fibroblast.

3.2 Effects of NO donors on skin wound healing

In the diabetic ulcer model, it can be clearly seen that wound area in the diabetic animals does not appreciably change over time. In contrast, normal mice mount a significant healing response and the wounds are virtually healed by 4 weeks. Treatment with 500 nM NO donor compound SNOG (Figure 3) or SNAP (data not shown) elicited a healing response in the diabetic animals that temporally approximated the normal healing process. Interestingly, normal animals treated with NO donors also displayed accelerated healing compared with normal controls. When 500 nM NOR-3, the fast acting donor was used, there was no effect on wound healing (Figure 4).

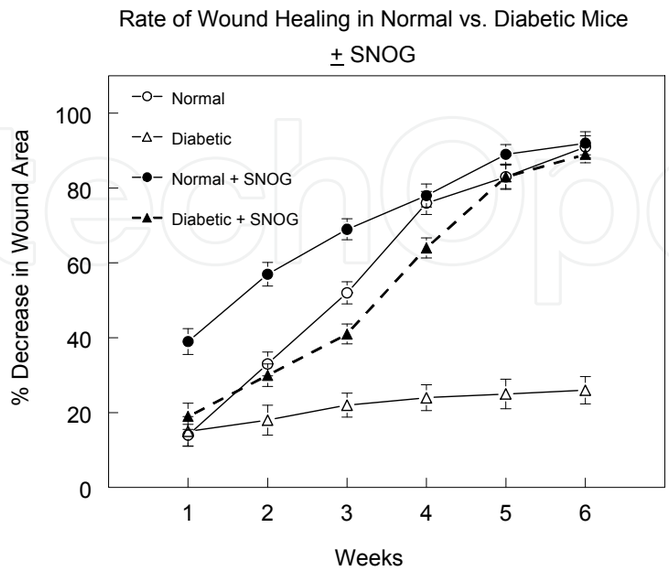

Fig. 3. Effect of NO donor SNOG on skin wound healing in normal and diabetic mice

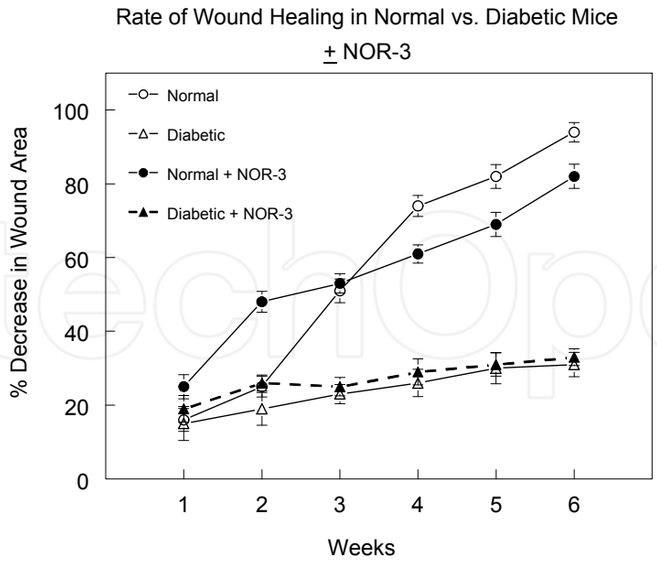

Fig. 4. Effect of NO donor NOR-3 on skin wound healing in normal and diabetic mice

3.3 Effects of NO donors on MMP activity determined by gelatin zymography

Cultured fibroblasts from the skin tissue of the normal and genetically-diabetic mice were examined for differences in MMP activity using gelatin zymography. Clear zones indicate MMP activity and the results demonstrate that equivalent cell numbers of diabetic fibroblasts show higher MMP-9 enzyme activity as compared to normal fibroblasts (Figure 5, compare MMP-9 signal in lane 3 versus lane 7). When treated for 24 hours with 500 nM SNOG, a significant reduction in MMP-9 activity was evident for both the non-diabetic and diabetic fibroblast cultures (see lanes 4 and 8). There was no such effect observed for MMP-2.

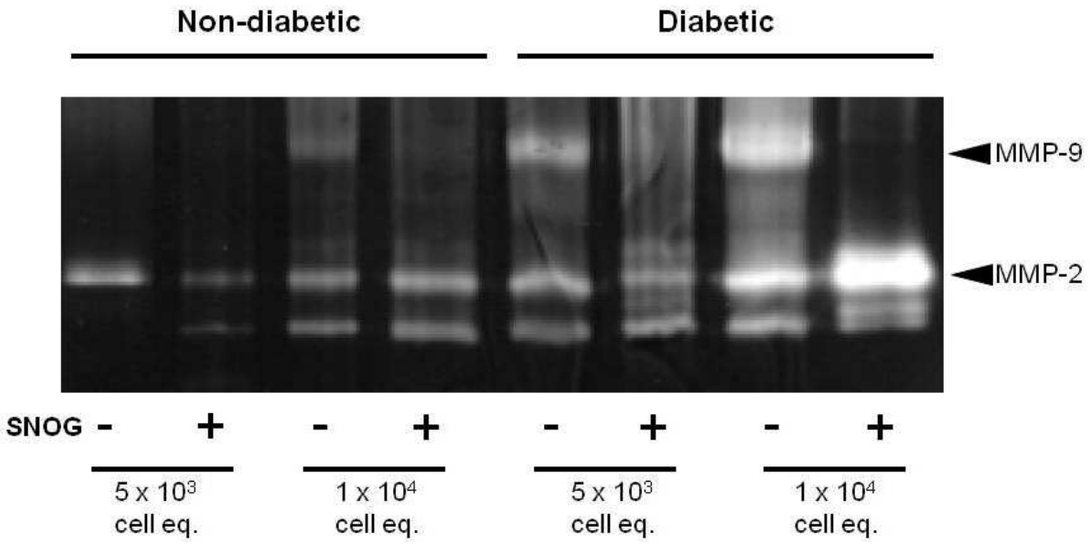

Fig. 5. Zymographic analysis of MMP enzyme activity before and after SNOG treatment of mouse fibroblast cultures.

3.4 Effects of NO donors on collagen gene expression

Type I and III collagen gene expression was significantly lower under hypoxic conditions and in diabetic fibroblasts. Significant differences were noted in regards to collagen gene expression in diabetic fibroblasts and under hypoxic conditions, mimicking the *in vivo* situation of chronic wounds. Collagen type I and III gene expression in our control samples was decreased in diabetic fibroblasts when compared to normal fibroblasts. Also, when comparing normoxic to hypoxic states, expression of type I and III collagen genes was less in the hypoxic group.

Type III collagen gene expression was significantly increased under normoxic conditions with the addition of the nitric oxide donor compound SNOG. Treatment with 1 nM SNOG, the long-acting NO donor, was noted to increase the levels of type III collagen expression by diabetic fibroblasts after three days of culture (Figure 6). In addition, the effect was dose-dependent for the diabetic cultures. No significant effect was noted on day 1 of culture at any concentration tested (data not shown). In contrast to the diabetic fibroblasts, normal fibroblasts only responded with increased expression at the 100 nM dose.

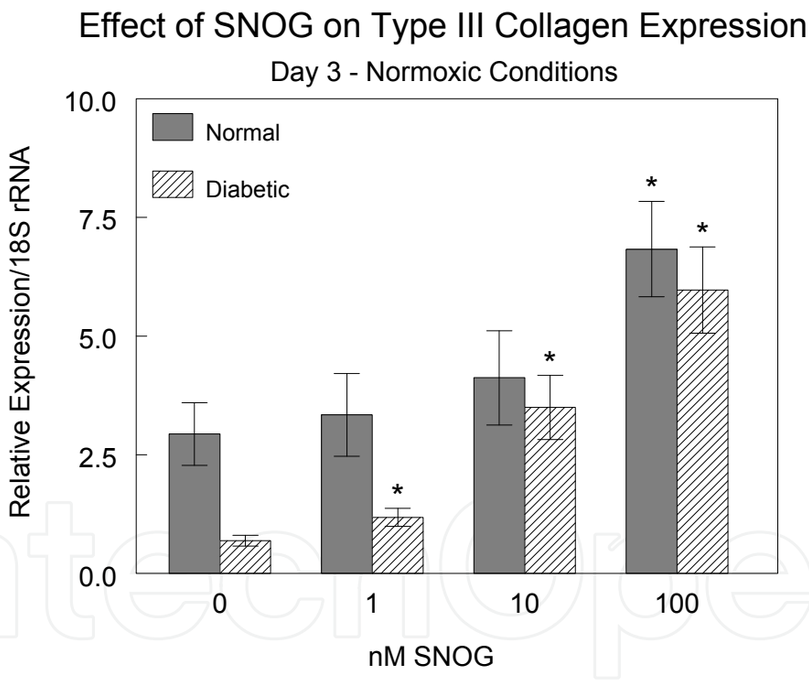

Fig. 6. Effect of NO donor SNOG on Type III collagen expression by normal and diabetic human fibroblasts cultured for three days under normoxic conditions.

Type III collagen gene expression was increased under hypoxic conditions with the addition of SNOG. Treatment with 1nM SNOG was noted to increase the levels of type III collagen expression by the diabetic fibroblasts on day 3 of culture (Figure 7). In addition, the effect was dose-dependent. However, the effect was only significant for the normal fibroblasts at 10 nM and 100 nM SNOG-treated cultures. No significant effect was noted on day 1 of culture at any concentration tested (data not shown).

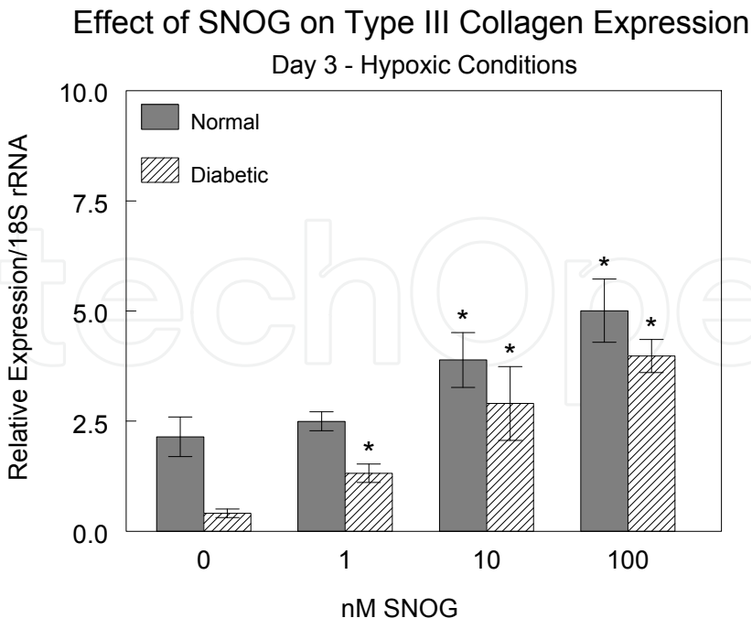

Fig. 7. Effect of NO donor SNOG on Type III collagen expression by normal and diabetic human fibroblasts cultured for three days under hypoxic conditions.

Type I collagen gene expression by normal fibroblasts at day 3 was not affected by SNOG treatment under normoxic conditions (Figure 8). A similar trend was noted with diabetic cells under normoxic conditions for type I collagen expression, except that a significant increase was observed with 100 nM SNOG treatment. No effect on type I collagen expression was noted for either normal or diabetic fibroblasts cultured for 3 days under hypoxic conditions (Figure 9).

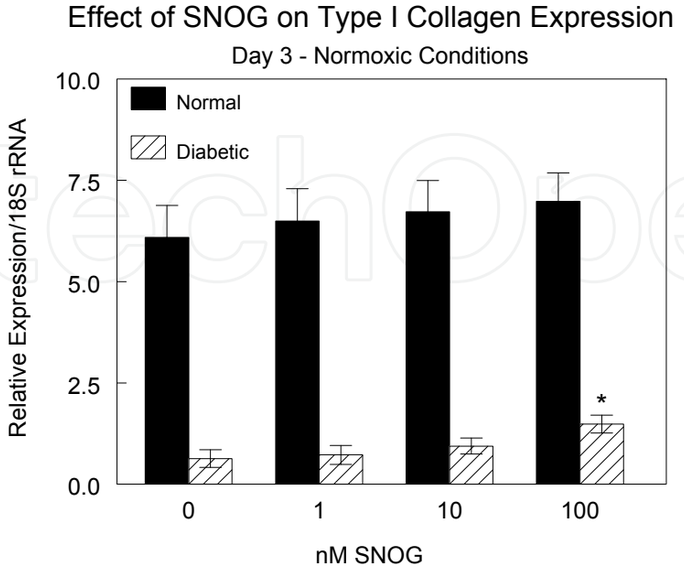

Fig. 8. Effect of NO donor SNOG on Type I collagen expression by normal and diabetic human fibroblasts cultured for three days under normoxic conditions.

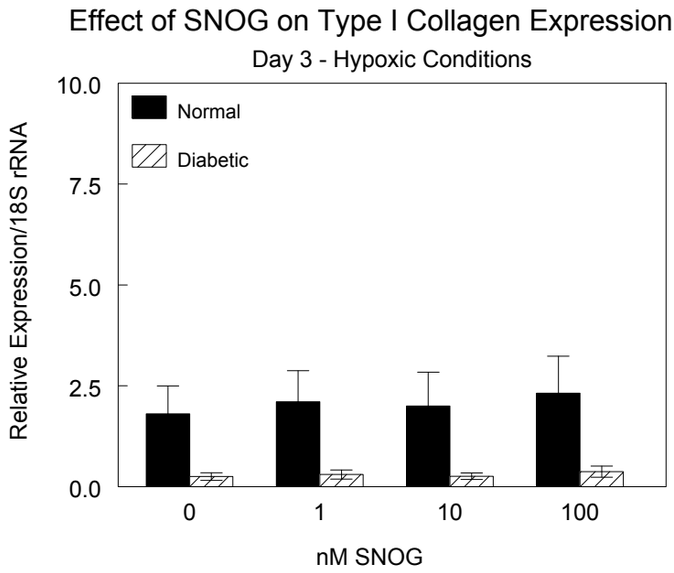

Fig. 9. Effect of NO donor SNOG on Type I collagen expression by normal and diabetic human fibroblasts cultured for three days under hypoxic conditions.

However, after seven days of culture under normoxic conditions following a single addition of SNOG, type I collagen expression by normal and diabetic fibroblasts was found to be increased (Figure 10). A significant increase was observed at 1nM SNOG for the normal fibroblasts and the effect was dose-dependent. Significant increases were also noted for the diabetic cultures, however only at the 10 and 100nM doses. Dose-dependent increases in type I collagen expression for normal and diabetic fibroblasts were also seen under hypoxic conditions (Figure 11).

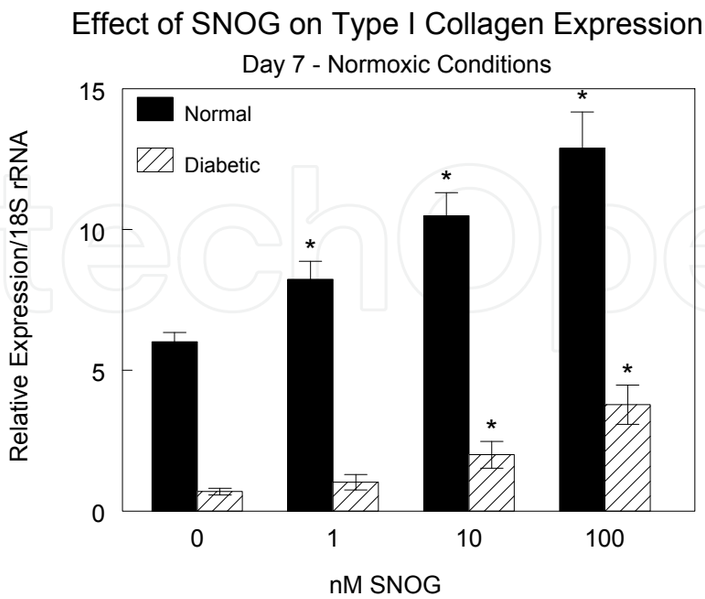

Fig. 10. Effect of NO donor SNOG on Type I collagen expression by normal and diabetic human fibroblasts cultured for seven days under normoxic conditions

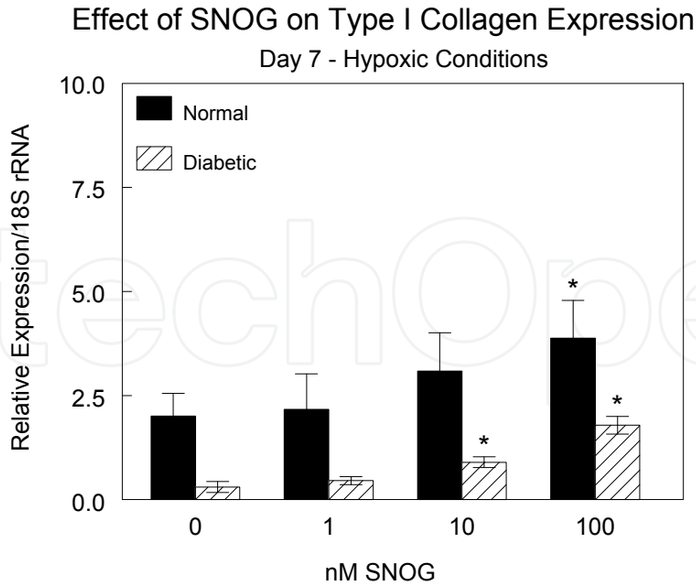

Fig. 11. Effect of NO donor SNOG on Type I collagen expression by normal and diabetic human fibroblasts cultured for seven days under hypoxic conditions.

In contrast, the short-acting NOR-3 NO donor had no significant effect upon collagen gene expression by either normal or diabetic fibroblasts cultured under either normoxic or hypoxic conditions. No effect was observed under normoxic (data not shown) or hypoxic conditions for type III collagen expression by normal or diabetic fibroblasts cultured for three days in the presence of NOR-3 (Figure 12). Similarly, there was no effect on type I collagen expression for cultures grown under hypoxic conditions for three days (Figure 13). Additonally, no effects were noted on days 1 or 7 of culture (data not shown).

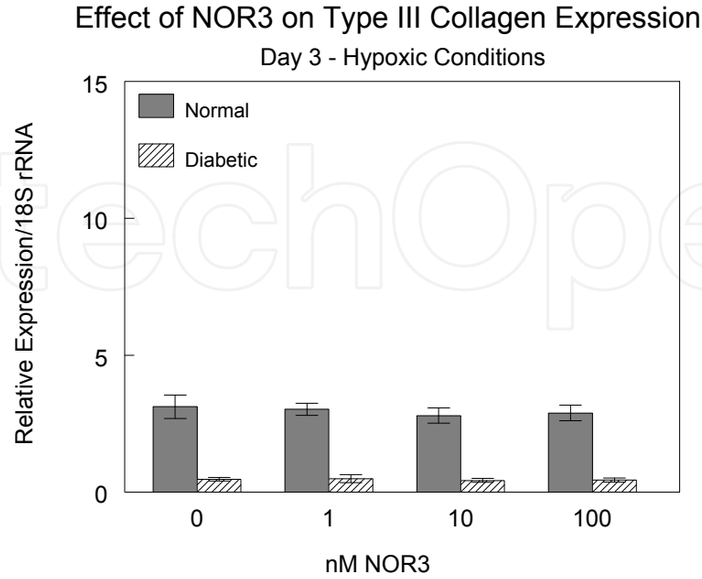

Fig. 12. Effect of NO donor NOR-3 on Type III collagen expression by normal and diabetic human fibroblasts cultured under hypoxic conditions.

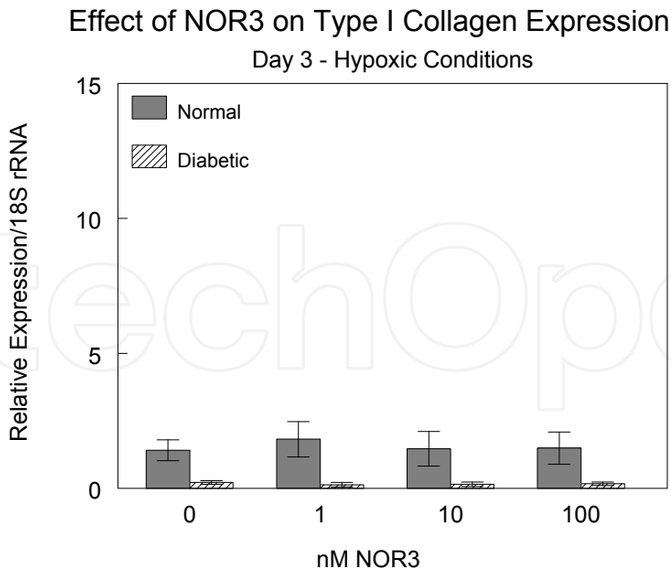

Fig. 13. Effect of NO donor NOR-3 on Type I collagen expression by normal and diabetic human fibroblasts cultured under hypoxic conditions.

3.5 Effects of NO donors on interleukin-6 production

The nitric oxide donor SNOG reduced IL-6 production by normal and diabetic skin fibroblasts grown under normoxic conditions and hypoxic conditions. At the control (0 nM) concentration, diabetic cells show significantly greater production of IL-6 than normal fibroblast cultures. The long-acting nitric oxide donor SNOG dose-dependently reduced IL-6 production for diabetic fibroblasts cultured either under normoxic conditions (Figure 14) or hypoxic conditions (Figure 15). In contrast, normal fibroblasts only demonstrated an effect at the highest dose of NO donor under either normoxic or hypoxic conditions.

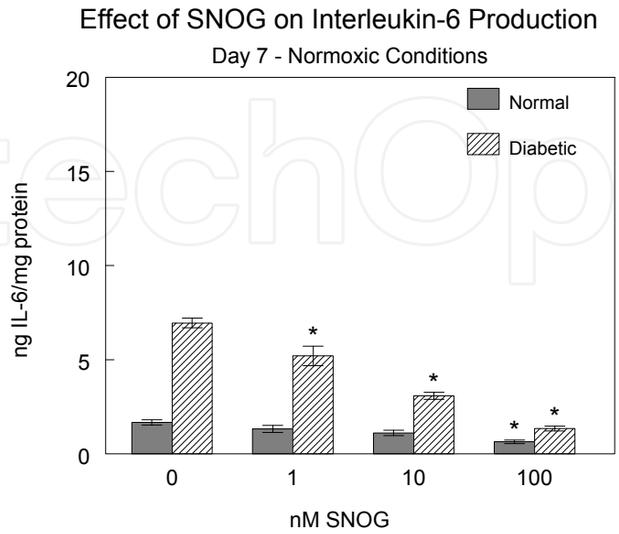

Fig. 14. Effect of NO donor SNOG on IL-6 production by normal and diabetic human fibroblasts cultured under normoxic conditions.

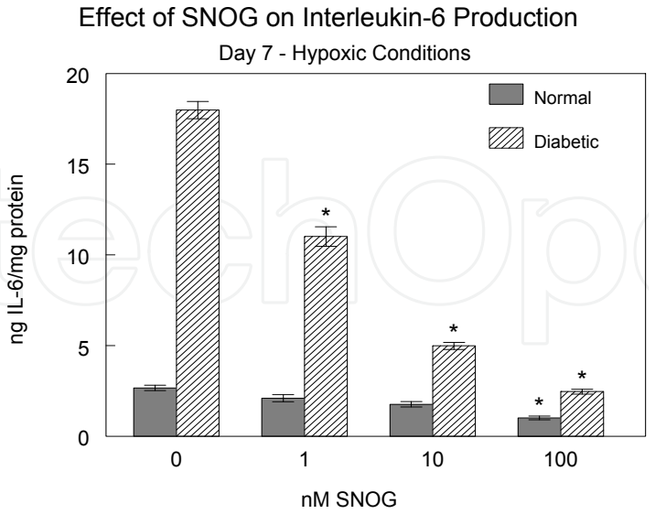

Fig. 15. Effect of NO donor SNOG on IL-6 production by normal and diabetic human fibroblasts cultured under hypoxic conditions.

The short-acting NO donor NOR-3 had no significant effect upon IL-6 production in either normal or diabetic fibroblasts cultures grown under either normoxic conditions (Figure 16) or hypoxic conditions (Figure 17).

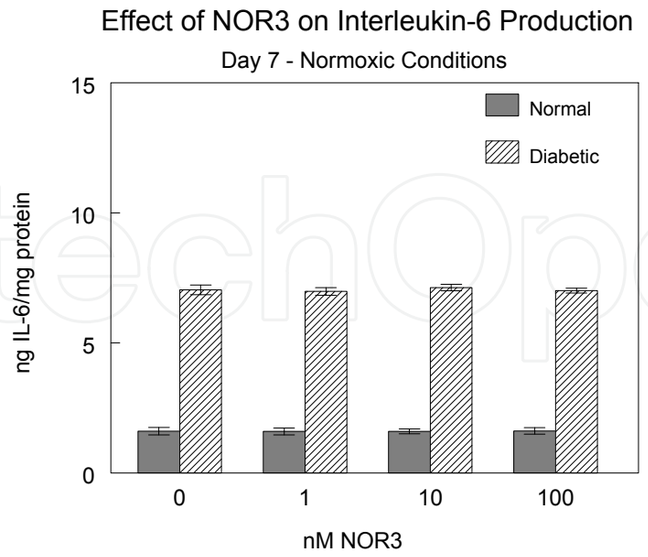

Fig. 16. Effect of NO donor NOR-3 on IL-6 production by normal and diabetic human fibroblasts cultured under normoxic conditions.

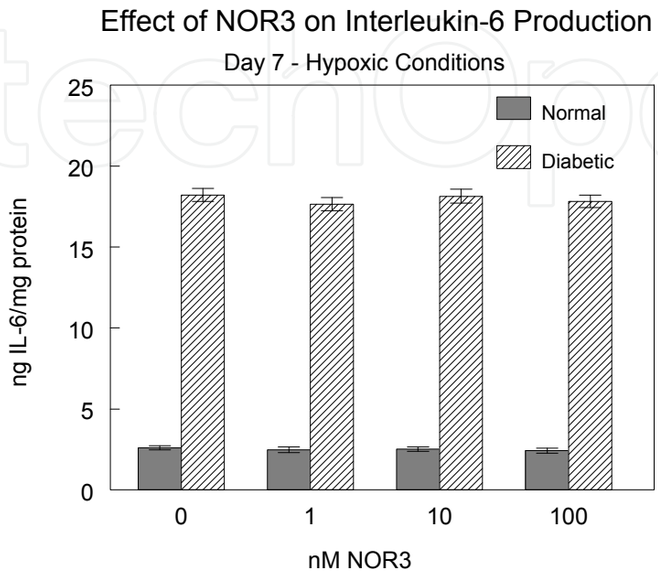

Fig. 17. Effect of NO donor NOR-3 on IL-6 production by normal and diabetic human fibroblasts cultured under hypoxic conditions.

3.6 Effects of NO donors on PDGF-BB production

Nitric oxide donor treatment had no effect on PDGF-BB production by normal fibroblasts grown under normoxic or hypoxic conditions. Under normoxic conditions, the normal cells produced twice as much PDGF as the diabetic cells, whereas in the hypoxic environment the normal cells produced five times as much PDGF (data not shown). Neither of the nitric oxide donors tested had any significant effect on PDGF production in either of the environmental conditions that were statistically significant.

3.7 Summary

MMP-9 and interleukin-6 expression is higher, and type I and type III collagen expression is lower, in diabetic fibroblasts in both normoxic and hypoxic states as compared to normal fibroblasts. With the addition of the NO donor with the relatively long half-life (SNOG), MMP-9 gene expression and enzyme activity were decreased. Additionally, production of the inflammatory cytokine interleukin-6 was decreased by increasing NO levels with SNOG. Concurrently, type I and type III collagen expression were increased. Treatment with SNOG

resulted in a significant effect upon collagen type III production in a dose-dependent fashion in both normal and diabetic fibroblasts under both normoxic and hypoxic states. In diabetic fibroblasts, under a normoxic state, the effect did not become significant until the 1nM SNOG-treated cells on day 3, and continued at the 10nM and 100nM concentrations on days 3 and 7. The effect upon collagen type III production in diabetic fibroblasts, under a hypoxic state, was found to be significant at the 1nM, 10nM, and 100nM in SNOG-treated cells on days 3 and 7. The effect of the addition of SNOG to both normal and diabetic fibroblasts under both normoxic and hypoxic states also results in a significant increase in collagen type I production in a dose dependent fashion. The effect in diabetic fibroblasts, under a normoxic state, was noted to be significant at the 100nM concentration of the SNOG donor compound on day 3. The effect in diabetic fibroblasts, under a hypoxic state, was found to be significant at the 10nM and 100nM level on day 7. The addition of NOR-3 had no significant effect upon the production of type I or III collagen in normal or diabetic fibroblasts.

4. Conclusion

Chronic diabetic wounds present a significant challenge to healthcare providers and resources. Significant amounts of time, money and resources have gone into prevention and treatment of these wounds. However, despite these efforts, chronic wounds continue to pose a serious problem. This has led researchers to focus more on understanding the molecular organization and function of normal wound healing and how this process is altered in chronic diabetic wounds. This is a very complex process that occurs through a series of highly integrated interactions between a multitude of varying cell types, growth factors, and cytokines. In diabetics, these wound healing interactions are disrupted and the process of wound healing seems to be locked in a perpetual state of chronic inflammation.

In diabetic wound healing, a physiologic state consistent with decreased nitric oxide (NO) levels results, leading to an impaired inflammatory response, decreased collagen production, and decreased wound-breaking strength (Witte et al., 2002). Prior studies have also revealed the MMP levels are significantly elevated in chronic diabetic wounds, thus creating an imbalance in matrix breakdown and release of growth factors that are important for normal wound healing (Wysocki et al., 1993; Trengove et al., 1999; Lobmann et al., 2002). Chronic diabetic wounds appear to be physiologically trapped in the inflammatory stage of wound healing and nitric oxide may promote normal healing by selectively reversing dysregulated factors (e.g.: localized ischemia, cell proliferation, MMP/TIMP ratio, growth factors/ cytokines, matrix synthesis) which prevent progression to the reepithelialization phase of wound healing. Additionally, nitric oxide may play a larger role in diabetic foot problems as low levels of endothelial nitric oxide synthase, an enzyme responsible for nitric oxide production, is associated with bone fractures (Loveridge et al., 2002) and Charcot neuroarthropathy (La Fontaine et al., 2008).

Prior studies have shown that introduction of a NO donor can improve wound healing (Shabani et al., 1996; Bohl-Masters et al., 2002). The mechanism(s) responsible for NO-induced healing remains unclear. Our studies reveal significant differences in the levels of MMP-9, NO synthetases, PDGF-BB, interleukin-6 and types I and III collagen in normal

and diabetic fibroblasts. With the addition of NO donor compounds, the levels of extracellular matrix-degrading MMP-9 and inflammatory mediator interleukin-6 were decrease in skin fibroblast cultures. NO donors stimulated type I and III collagen expression in both normal and diabetic fibroblasts and under both normoxic and hypoxic states. Thus, the results of this study combined with previous studies involving chronic diabetic wounds, the potential exists to develop a topical media to apply to chronic wounds that would introduce these NO donors to the wound site to improving the healing potential of these wounds.

Although it is known that diabetic patients display impaired wound healing, the mechanism for this impairment is not fully understood. Because fibroblasts are essential for dermal wound repair, the current study compared the *in vitro* behavior of human dermal fibroblasts. It has been reported that fibroblasts from diabetic wounds continue to display impaired proliferation even when taken out of their *in vivo* diabetic environment and cultured *in vitro* (Burrow et al., 2007; Loot et al., 1999; Hehenberger et al., 1998). Therefore, identical tissue culture conditions should not obscure differences between our experimental groups.

Diabetic cells when compared to normal cells demonstrated a decreased gene expression in types I & III collagen, eNOS and iNOS, and PDGF-BB, with an increased production of IL-6. Within the hypoxic condition the expression of types I & III collagen, eNOS and iNOS and PDGF-BB were further reduced in both the diabetic and normal cells, whereas the IL-6 production was only significantly increased within the diabetic fibroblasts. With the treatments of the NO donors, it was demonstrated that when evaluating the types I & III collagen and eNOS and iNOS only SNOG was seen to have an effect on either type, with its most profound effect on type III collagen in both environmental conditions. The IL-6 production was only decreased by the long-acting NO donor SNOG. Neither NO donor demonstrated a significant effect upon PDGF-BB production.

In 2005, we demonstrated that the addition of the NO donor SNAP to skin wounds in a diabetic mouse model significantly increased the rate and extent of wound healing (Stehly et al., 2005). The wound healing rates in the diabetic treatment groups approximated the normal mice. In a study by Bohl-Masters et al., 2002, a polyvinyl alcohol hydrogel impregnated with NO donor was used to increase the local NO concentration in a wound healing model in diabetic and normal mice. They found that wound healing in the NO donor-treated diabetic mice was either the same or slightly less than in the control group. However, the quality of granulation tissue was much improved in the presence of nitric oxide donor.

NO has been shown to be significantly reduced in chronic ulcers. Impaired healing of diabetic wounds is thought to be related to diminished NO production (Witte et al., 2002; Schaffer et al., 1997). Because the level of NO in chronic wounds is low, and elevation of NO enhances wound healing, a number of other approaches have been tried to deliver NO therapeutically. Studies using compounds such as L-arginine (Shi et al., 2003), vitamin B_{12} (Bauer et al., 1998), and multivitamin therapy using a combination of folic acid, vitamin B_6, and vitamin B_{12} (Boykin et al., 2005) have reported concomitant increases in wound tissue NO level and healing. The latter study demonstrated that serum

homocysteine, an inhibitor of NOS, is elevated in patients with chronic wounds; with multivitamin therapy, this inhibitor is decreased and associated with enhanced healing. Both of these approaches employ systemic delivery of the compounds to raise NO, but do not involve a local targeted delivery of NO directly into the wound site. Very few investigations have sought to deliver NO directly into wounds. For example, one study using a NO-modified polyvinyl alcohol hydrogel effectively healed cutaneous wounds in diabetic mice (Bohl-Masters et al., 2002). Increased production of extracellular matrix molecules was noted in this study, but no attempt was made to measure changes in MMP activity. Other approaches used to deliver NO to the wound have used linear polyethyleneimine/NO adducts (Bauer et al., 1998) and delivery of iNOS naked DNA via a collagen sponge (Thornton et al., 1998). Both of these approaches reported toxic effects related to the delivery of excessive levels of NO to the wound, suggesting the need for a more finely controlled transient release of NO. The longer-acting NO donor compounds, SNAP and SNOG, used in the current study, and similar cell-permeable compounds which produce transient increases in tissue NO concentration, should provide a means of optimizing the therapeutic effect. NO donor compounds may ultimately provide a new therapeutic tool for the treatment of diabetic foot wounds.

The purpose of this study was to assess the effect that NO donor compounds would have upon the production of type I and type III collagen production in diabetic fibroblasts both in normoxic and hypoxic (representative of chronic diabetic wounds) states. Prior studies have mainly focused upon the effect in normal fibroblasts under normoxic conditions. This study demonstrated that collagen type I and type III production was decreased in diabetic fibroblasts under both normoxic and hypoxic states when compared to normal fibroblasts without the addition of NO donor compounds. Thus, providing a catalyst to increase the amount of type I and type III collagen could potentially improve wound healing in diabetic chronic wounds.

To examine the time dependence of NO action in wound healing, this study utilized a shorter acting NO donor compound (NOR-3) and a relatively long-acting NO donor, SNOG, to assess their effects upon collagen production. Through the introduction of these agents in this study, significant increases in collagen type I and type III production were observed. In fact, the effect of the SNOG NO donor compound upon type III collagen production in diabetic fibroblasts demonstrated a significantly greater dose dependent effect than that observed in normal fibroblasts. Type I collagen levels were also significantly increased with the addition of the SNOG NO donor compound in both diabetic and normal fibroblasts. However, the dose dependent relationship for collagen type I production was essentially the same for both normal and diabetic fibroblasts.

The short acting NO donor compound (NOR-3), was not found to have any significant effect upon collagen type I or III production in diabetic fibroblasts. This finding, along with previous findings with the use of NOR-3 potentially indicate that relatively longer-acting NO donor compounds are more effective at NO and collagen production in diabetic fibroblasts. Thus, using a relatively longer-acting NO donor compound could potentially expose the chronic wound to longer duration of NO further increasing the wound healing potential and being present throughout more of the wound healing stages.

The results observed in this study are very encouraging in regards to possible future treatment of chronic diabetic wounds. By studying the effects of these NO donor compounds in diabetic fibroblasts under normoxic and hypoxic states we have been able to create an environment more closely resembling a chronic wound state in human diabetic patients. From the results of this and other studies in our laboratory we now have the potential to develop a topical treatment for chronic diabetic wounds based upon optimal delivery of NO to assist with wound healing. Preliminary studies are currently being performed examining SNOG attachment and release from both collagen-based and hydroxyapatite-based scaffolds. The goals of these studies are to develop new types of synthetic graft materials for the treatment of diabetic skin and bone wounds respectively. Future research will be directed toward development and optimization of these and other NO delivery systems for the treatment of wounds and other diabetic complications.

5. Acknowledgments

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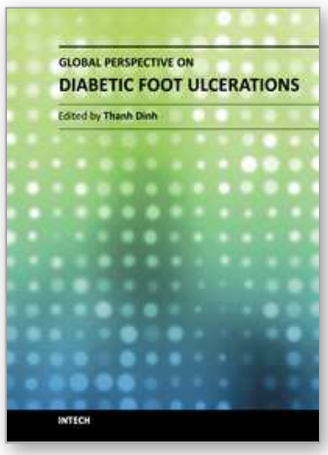

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Over the last decade, it is becoming increasingly clear that diabetes mellitus is a global epidemic. The influence of diabetes is most readily apparent in its manifestation in foot complications across cultures and continents. In this unique collaboration of global specialists, we examine the explosion of foot disease in locations that must quickly grapple with both mobilizing medical expertise and shaping public policy to best prevent and treat these serious complications. In other areas of the world where diabetic foot complications have unfortunately been all too common, diagnostic testing and advanced treatments have been developed in response. The bulk of this book is devoted to examining the newest developments in basic and clinical research on the diabetic foot. It is hoped that as our understanding of the pathophysiologic process expands, the devastating impact of diabetic foot complications can be minimized on a global scale.

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