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Review

The potential of transdermal nitric oxide treatment for diabetic peripheral neuropathy and diabetic foot ulcers

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ABSTRACT

The Center for Disease Control (CDC) estimates that 29 million Americans have diabetes, and 70% of diabetic patients develop diabetic peripheral neuropathy [1,2]. Up to 27% of the direct medical cost of diabetes may be attributed to DPN [3]. A 2013 article from the American Diabetes Association reported a $176 billion direct medical cost of diabetes in 2012 [4]. DPN patients often suffer from shooting and burning pain in their distal limbs and a severe loss of sensation. Diabetic foot ulcers, infections, and amputations may follow. Currently available treatments: tricyclic antidepressants, anticonvulsants such as gabapentin and pregabalin, serotonin and norepinephrine reuptake inhibitor, duloxetine, topical 5% lidocaine (applied to the most painful area) can manage painful symptoms but do not address the underlying pathologies of DPN and diabetic wound ulcers. A combination of pain-reducing medications can provide relief when individual medications fail, and opioids such as tramadol and oxycodone may be administered with these medications to reduce pain [5]. Due to the prevalence of diabetes, DPN, and diabetic foot ulcers, and because of the lack of available effective treatments to directly address the pathology contributing to these conditions, novel treatments are being sought. Our hypothesis is that a deficiency of nitric oxide synthase in diabetic patients leads to a lack of vascularization of the peripheral nerves, which causes DPN; and this could be treated with vasodilators such as nitric oxide. In this paper, the mechanisms of DPN are reviewed and analyzed to elucidate the potential of a transdermal nitric oxide application for the treatment of DPN and diabetic wound ulcers by increasing vasodilation.

1. Introduction

The diabetic population in America is expanding at an alarming rate. As many as one in three Americans may become diabetic by 2050 [6]. The CDC estimates that 29 million Americans suffer from type 1 or type 2 diabetes, and 70% of diabetic patients develop DPN [1]. The cost of diabetes includes "$176 billion in direct medical costs and $69 billion in reduced productivity." Approximately 65,700 lower-limb amputations are performed annually, and diabetic foot ulcers account for $9 billion to $13 billion of the cost of diabetes [3,4,7]. The incidence will continue to rise as the life-span expectancy continues to increase.

Diabetic peripheral neuropathy (DPN) is a serious and often painful condition with both microvascular and neural components. Common symptoms include decreased somatosensory function and persistent burning pain in the foot. Reduced sensory functions inhibit the patient's perception of foot trauma and can lead to severe injuries and diabetic foot ulcers, which are open sores that are difficult to heal, potentially leading to amputation [2].

Effective treatments are needed to improve the quality of life for those who suffer from the severe complications of diabetes like DPN and diabetic foot ulcers. An effective treatment should address not only painful symptoms but also the underlying pathology. Physicians can currently provide pain management medications but do not currently utilize treatments that directly target the pathology of DPN and diabetic foot ulcers (Table 1) [5,8–17].

An investigation of the literature reveals the potential of nitric oxide, a vasodilator, as a treatment for DPN and diabetic foot ulcers. This paper outlines the pathology of DPN and diabetic foot ulcers and analyzes relevant mechanisms. Finally, the potential of nitric oxide as an improved treatment for these conditions is explored. We propose that a transdermal application of nitric oxide has

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Table 1
Symptomatic therapy for peripheral neuropathy.

Drug Class	Drug	Daily Dose (mg) *unless specified
Analgesic	Capsaicin	0.025–0.1% *3–4 times daily
Anesthetic	Lidocaine	5–8% *up to 3 times daily
Anticonvulsant	Carbamazepine	Up to 800
	Lamotrigine	200–400
	Gabapentin	900–1800
	Pregabalin	160–600
Antioxidant	Alpha-Lipoic-Acid	600
Neurotoxin	Botulinum Toxin	4–10 U *per site
Opioid	Oxycodone CR	10–60
	Tramadol	50–400
	Tapentadol	50–600
SNRI	Duloxetine	20–120
	Venlafaxine	75–225
SSRI	Citalopram	40
	Paroxetine	40
Tricyclic Antidepressant	Amitriptyline	25–150
	Imipramine	25–150
	Desipramine	12.5–150

excellent potential for effectively addressing the pathologies of DPN and diabetic foot ulcers in human patients.

2. Diabetic peripheral neuropathy

DPN is a condition that develops as a complication of diabetes. DPN involves nerve damage throughout the body and especially in the feet. Not all diabetic patients suffer from burning pain, yet many patients report a tingling or burning sensation and the loss of sensory functions. Patients with poorly-controlled diabetes are at a higher risk for developing DPN. The condition is influenced by many factors including elevated blood glucose levels and changes in nerves and microvasculature [2].

The medications and procedures currently available to physicians to treat patients with DPN are limited. Current treatments include non-steroidal anti-inflammatory drugs, anti-depressants, anti-convulsants, and narcotic painkillers [8]. These common clinical treatments for DPN do not directly address the underlying pathology but simply cover painful symptoms. A treatment for DPN is needed that targets the underlying pathology.

2.1. Diabetic foot ulcers

Diabetic foot ulcers are a severe complication of diabetes that can occur along with diabetic neuropathy. These ulcers develop most commonly on the bottom surface of the foot and affect 15% of diabetic patients [18]. DPN patients often experience decreased sensation which may lead to an undetected tissue injury and severe damage. Such an injury is compounded by the decreased vascular function, leading to slower healing and increased risk for infection. Due to compromised injury avoidance and healing systems, even a minor injury can lead to a significant and serious diabetic ulcer. About 50% of patients with diabetic foot ulcers die within five years [19].

Current treatments for diabetic foot ulcers are extremely limited and only prevent additional injury. Dead tissue can be removed surgically to improve the rate of healing, although this rate of healing is still greatly reduced compared to healthy tissue. A physician may also prescribe the use of a cast to prevent further physical trauma to the affected limb [20]. These treatments wholly neglect the underlying tissue pathology.

Due to the poor outcomes for many diabetic wound ulcer patients and the extremely limited treatment options, novel therapies must be investigated until a suitable treatment is found. An effective treatment should directly influence the mechanisms responsible for the development and continuation of the underlying pathology.

3. Pathogenesis of DPN and diabetic foot ulcers

Endothelial cells are dysfunctional in DPN patients, and this appears to permit the continuation of DPN and development of diabetic foot ulcers. DPN patient biopsies were found to have reduced levels of endothelial nitric oxide synthase eNOS, as demonstrated in Fig. 1A [21].

In a mouse model, immunofluorescence studies were performed on mouse nerves, and there was a significant reduction in the presence of eNOS in the control model (which received subcutaneous saline injections) as compared to the non-diabetic animal [22]. Because a quantitative analysis of the fluorescence was not published, the published images have been analyzed using Fiji image analysis software to estimate the difference in fluorescence as indicated in Fig. 1B [23,24].

eNOS produces nitric oxide from L-arginine for vasodilation. Because DPN patients have reduced levels of nitric oxide, impaired vascular function occurs in their distal limbs. A Laser Doppler iontophoresis experiment published by Veves et al. alongside the eNOS immunostaining demonstrated decreased endothelium-mediated vasodilation in DPN patients, confirming the decreased vasodilation in DPN patients which results from low eNOS levels. Poor perfusion produces poor cellular nutrition and impaired immune function, enabling cells to enter a pathogenic state.

Chronic hypoxia is the result of poor perfusion and is detrimental to wound healing. Hypoxia increases the magnitude of the inflammatory response to a wound leading to an increased production of reactive oxygen species which leads to further tissue damage [25]. Hypoxia has been studied thoroughly in the CNS, but

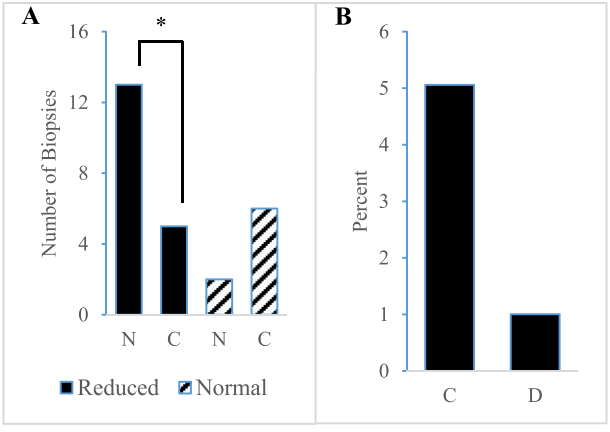

Fig. 1. A: Skin biopsies from the dorsum of the foot were obtained from 36 subjects. Immunostaining for eNOS was performed on the skin samples from both neuropathic (N) and control (C) subjects. Staining revealed that eNOS was significantly reduced or absent as compared to for eNOS in biopsies from control subjects [21]. **B:** Fluorescent immunohistochemistry was performed on the sciatic nerve for eNOS in diabetic saline sham (D) and non-diabetic control (C) mice. The published immunohistochemistry images show a significant reduction in the presence of eNOS for the diabetic animal [22]. To quantify the magnitude of fluorescence reduction, the published images were analyzed using Fiji, an ImageJ distribution. $^{*}P < 0.01$.

few studies have investigated the effects of ischemia in the PNS, "despite the fact" that the PNS is susceptible to impaired perfusion and therefore hypoxia. Hypoxia has also been demonstrated to alter synaptic function and neurotransmitter release [26,27].

Nitric oxide is an endogenous vasodilator synthesized in many tissues including endothelial cells [28]. DPN patients have dysfunctional microvasculature in the peripheral limbs but not necessarily systemic macrovascular dysfunction [21]. This confirms that the distal foot and ankle tissues containing the affected microvasculature could benefit from a transdermal replacement of the deficient signaling molecule. The literature suggests that nitric oxide synthase levels are decreased in DPN patients, and this leads to decreased levels of nitric oxide in the microvasculature. Thus, a poor vasodilatory response is produced, and distal tissues are deprived of essential cellular nutrition. A poor vasodilatory response in the microvasculature may explain why DPN patients are at risk for severe infections and amputations. Nitric oxide shows excellent promise as a treatment for both diabetic foot ulcers and DPN.

One study has found that a nitric oxide-generating spray provided a significant reduction in pain for DPN patients [29]. The spray reduced pain and was associated with improved sleep, mobility, and mood. Although the spray may not be effective for all DPN patients, a transdermal administration of nitric oxide should be attempted before other pharmacological treatments, many of which cause unpleasant side effects and do not directly address the pathology of DPN. The researchers suggested that additional investigation be performed with a larger group of patients to confirm the findings and also determine whether the effects of nitric oxide therapy are sustained during long-term treatment by nitric oxide therapy. Additionally, there may be other physiological improvements from nitric oxide treatment that could be assessed to measure the efficacy of nitric oxide treatment on DPN. Due to the known mechanism of action for nitric oxide, this suggests that vasodilatory improvements of the microvasculature occurred and resulted in improved nerve health. The literature confirms that because neuropathy is a microvascular condition, microvascular dysfunction can lead to nerve dysfunction.

The resultant nerve dysfunction is central to the development of neuropathy [30]. Thus, decreased levels of nitric oxide lead to impaired microvascular function, and microvascular dysfunction leads to neuropathy. Further, an impaired microvasculature provides poor blood supply for distal tissues, explaining the decreased healing as found with wounds that become diabetic foot ulcers. In summary, DPN patients have reduced levels of the nitric oxide synthase enzyme which leads to reduced levels of the vasodilator nitric oxide. Without nitric oxide, a local, endogenous vasodilator, the peripheral vasculature has decreased vasodilatory function which leads to neuropathy.

Impaired microvasculature can also lead to the development of diabetic foot ulcers. Although the full mechanism for the pathogenesis of DPN and diabetic ulcers is complex, a mechanism involving a lack of nitric oxide has been identified as a significant factor for the development of DPN, and nitric oxide has already been administered in clinical experiments for treatment of painful symptoms by addressing local vascular function of the neuropathy. Further, the development of diabetic foot ulcers can also be explained by decreased vascular function, poor delivery of nutrients, and therefore, insufficient support of dermal tissue leading to poor healing and risk for infection and amputation.

Based on an analysis and synthesis of the literature, a mechanism for the development of DPN has been identified. A transdermal administration of nitric oxide is likely to ameliorate the painful symptoms of neuropathy and address the underlying pathology.

4. Need for improved treatments for DPN

Currently, approximately ten percent of U.S. adults suffer from diabetes. The condition kills more Americans each year than AIDS and breast cancer combined [31]. More concerning still is the CDC's estimate for the future incidence level of diabetes in the U.S. One CDC analysis suggests that in the next few decades, as many as one in three Americans may develop diabetes by 2050 [6]. It is also expected that 50% of diabetic patients will experience DPN at some point in their lives [32]. Millions of Americans already suffer from DPN, and millions more are expected to develop this condition in the coming years. An effective treatment is needed to address their condition and recover lost nerve and vascular function.

5. Discussion: potential of nitric oxide therapy

The amount of nitric oxide synthesized by eNOS and released into the peripheral vasculature is reduced in patients with DPN, and it has been demonstrated that the resulting poor vascular function may lead to DPN and diabetic foot ulcers. Replacing the deficient quantities of the molecule should improve both DPN and diabetic ulcer conditions. Low levels (80 ppm) of the inhaled form of nitric oxide are commonly used to treat neonatal pulmonary hypertension. This illustrates the clinical safety and vasodilatory, hypoxia-reducing effects of nitric oxide which could be utilized to improve the conditions that contribute to nerve dysfunction and diabetic ulcers in DPN patients.

Nitric oxide is safe for human treatment if dosed and delivered properly. Although commonly used in neonates, the inhaled form of nitric oxide would not effectively reach the site of a peripheral neuropathy due to the extremely short half-life of nitric oxide: 0.05–1.8 ms [33]. Since nitric oxide is capable of absorption through the skin [34], the topical route of delivery is therefore the most convenient for patients and allows the direct treatment of affected tissues.

Transdermal nitric oxide therapy is likely to produce many significant benefits in patients. First, the microvasculature is expected to improve by increased vasodilation and blood flow. Because of improved perfusion, neuropathy would be less likely to develop, and existing neuropathies may improve. One study examined the pain-reducing effects of a nitric oxide-generating isosorbide dinitrate spray and found a significant reduction in both overall neuropathic pain and the characteristic burning sensation in patients when using the spray, though other symptoms such as numbness (possibly due to irreversible damage) were not found to be significantly altered by the 4-week therapy (Fig. 2) [29]. In addition to a reduction in painful sensations, it is likely that regular sensory nerve function would be improved significantly by the same mechanism of restoring nerve health by providing adequate blood supply to the affected tissues. Severe infections in the foot would be prevented by increasing perfusion to the distal tissues and allowing sufficient levels of immune cells to access these distal tissues. Finally, diabetic foot ulcers would be less likely to develop, and existing ulcers would heal at an accelerated rate due to the restored vasodilatory function of the microvasculature. Thus, a transdermal application of nitric oxide has the potential to address many significant components of the pathogenesis of DPN and diabetic foot ulcers, leading to enhanced blood flow, restored nerve functions, and healed diabetic foot ulcers. As there are currently 29 million diabetic patients in the U.S., and considering that 70% of these patients will develop neuropathy, the potential impact of an effective treatment for diabetic peripheral neuropathy and diabetic foot ulcers is very significant [1]. Also, considering the immense projected growth in the diabetic population, it is essential that nitric oxide be evaluated further for its potential for treating

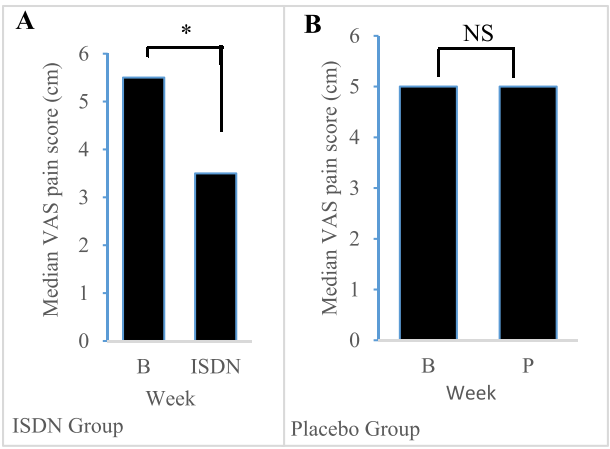

Fig. 2. A: Subjects applying ISDN NO-releasing spray once daily reported significantly lower pain scores compared to their baseline pain level before treatment. **B:** Subjects receiving a placebo spray did not report a significant difference in pain level compared to their baseline measurement after applying the placebo spray for the same duration as the ISDN group. P=NS, *P < 0.01.

DPN and diabetic foot ulcers.

6. Conclusions

A transdermal nitric oxide treatment shows strong potential as a treatment for DPN and diabetic foot ulcers. Endothelial nitric oxide synthase levels are reduced in neuropathic patients, and a replacement of the resulting NO deficiency has been demonstrated to reduce painful symptoms for human DPN patients. Compared to existing treatments which treat painful symptoms, nitric oxide has the potential to reduce the overall disease burden and cost of managing an increasingly diabetic and neuropathic American population by directly addressing the underlying pathology. Diabetic foot ulcers are also expected to improve from this treatment.

Future research could assess the histological changes in the peripheral nerves of a diabetic animal model after treatment with nitric oxide. Nerve conductance tests should be performed using this model to assess potential changes in the velocity of nerve signals. Larger and longer duration studies must also be conducted with human patients to determine whether the effects of nitric oxide treatment are sustained, and whether nitric oxide treatment prevents or contributes to healing of diabetic skin ulcers. Later studies could be performed to investigate combining nitric oxide treatments with medications to target free radicals or complement nitric oxide in addressing the multifactorial pathogenesis of DPN. Tens of millions of current patients may benefit significantly from this nitric oxide treatment. Potentially, amputations and mortality could be reduced, along with cost. Transdermal nitric oxide treatment has the potential to allow many patients to achieve an improved quality of life and regain control over their painful and debilitating DPN and diabetic wound ulcers.

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References

[1] CDC. More than 29 million Americans have diabetes; 1 in 4 doesn't know. 2014. http://www.cdc.gov/media/releases/2014/p0610-diabetes-report.html. [Accessed 19 February 2018].
[2] NIH. Nerve damage (diabetic neuropathies). 2013. https://www.niddk.nih.gov/health-information/diabetes/preventing-diabetes-problems/nerve-damage-diabetic-neuropathies. [Accessed 19 February 2018].
[3] Gordois A, Scuffham P, Shearer A, Oglesby A, Tobian JA. The health care costs of diabetic peripheral neuropathy in the US. Diabetes Care 2003;26(6):1790–5.
[4] American Diabetes A. Economic costs of diabetes in the U.S. in 2012. Diabetes Care 2013;36(4):1033–46.
[5] Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010;33(10):2285–93.
[6] CDC. Number of Americans with diabetes projected to double or triple by 2050. Centers for Disease Control and Prevention; 2010.
[7] Rice JB, Desai U, Cummings AK, Birnbaum HG, Skornicki M, Parsons NB. Burden of diabetic foot ulcers for medicare and private insurers. Diabetes Care 2014;37(3):651–8.
[8] Boulton AJM. Management of diabetic peripheral neuropathy. Clin Diabetes 2005;23(1):9–15.
[9] Waldfogel JM, Nesbit SA, Dy SM, et al. Pharmacotherapy for diabetic peripheral neuropathy pain and quality of life: a systematic review. Neurology 2017;88(20):1958–67.
[10] Cakici N, Fakkel TM, van Neck JW, Verhagen AP, Coert JH. Systematic review of treatments for diabetic peripheral neuropathy. Diabet Med 2016;33(11):1466–76.
[11] Groninger H, Schisler RE. Topical capsaicin for neuropathic pain #255. J Palliat Med 2012;15(8):946–7.
[12] Derry S, Wiffen PJ, Moore RA, Quinlan J. Topical lidocaine for neuropathic pain in adults. Cochrane Database Syst Rev 2014;7. CD010958.
[13] Hearn L, Moore RA, Derry S, Wiffen PJ, Phillips T. Desipramine for neuropathic pain in adults. Cochrane Database Syst Rev 2014;9. CD011003.
[14] Lunn MP, Hughes RA, Wiffen PJ. Duloxetine for treating painful neuropathy, chronic pain or fibromyalgia. Cochrane Database Syst Rev 2014;1. CD007115.
[15] Vadivelu N, Kai A, Maslin B, Kodumudi G, Legler A, Berger JM. Tapentadol extended release in the management of peripheral diabetic neuropathic pain. Therapeut Clin Risk Manag 2015;11:95–105.
[16] Aiyer R, Barkin RL, Bhatia A. Treatment of neuropathic pain with venlafaxine: a systematic review. Pain Med 2017;18(10):1999–2012.
[17] Park J, Park HJ. Botulinum toxin for the treatment of neuropathic pain. Toxins 2017;9(9).
[18] Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. World J Diabetes 2015;6(1):37–53.
[19] Moulik PK, Mtonga R, Gill GV. Amputation and mortality in new-onset diabetic foot ulcers stratified by etiology. Diabetes Care 2003;26(2):491–4.
[20] Skinsight Neurogenic Ulcer (Diabetic Ulcer); https://www.skinsight.com/skin-conditions/adult/neurogenic-ulcer-diabetic-ulcer. Accessed 19 February 2018.
[21] Veves A, Akbari CM, Primavera J, et al. Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease, and foot ulceration. Diabetes 1998;47(3):457–63.
[22] Ii M, Nishimura H, Kusano KF, et al. Neuronal nitric oxide synthase mediates statin-induced restoration of vasa nervorum and reversal of diabetic neuropathy. Circulation 2005;112(1):93–102.
[23] Schindelin JA-C I. & Frise, E. et al. Fiji: an open-source platform for biological-image analysis. Nat Methods.9(7): 676–682.
[24] Schneider CA, R WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2017;9(7):671–5.
[25] Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res 2010;89(3):219–29.
[26] Bukharaeva EA, Salakhutdinov RI, Vyskocil F, Nikolsky EE. Spontaneous quantal and non-quantal release of acetylcholine at mouse endplate during onset of hypoxia. Physiol Res 2005;54(2):251–5.
[27] Nishimura M. Factors influencing an increase in spontaneous transmitter release by hypoxia at the mouse neuromuscular junction. J Physiol (London) 1986;372:303–13.
[28] Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 2006;113(13):1708–14.
[29] Yuen KC, Baker NR, Rayman G. Treatment of chronic painful diabetic neuropathy with isosorbide dinitrate spray: a double-blind placebo-controlled cross-over study. Diabetes Care 2002;25(10):1699–703.
[30] Ylitalo KR, Sowers M, Heeringa S. Peripheral vascular disease and peripheral neuropathy in individuals with cardiometabolic clustering and obesity: national Health and Nutrition Examination Survey 2001-2004. Diabetes Care 2011;34(7):1642–7.
[31] ADA. Fast Facts - Data and Statistics About Diabetes | American Diabetes Association. http://professional.diabetes.org/content/fast-facts-data-and-statistics-about-diabetes. [Accessed 19 February 2018].
[32] Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. Lancet Neurol 2012;11(6):521–34.
[33] Rassaf T, Preik M, Kleinbongard P, et al. Evidence for in vivo transport of bioactive nitric oxide in human plasma. J Clin Invest 2002;109(9):1241–8.
[34] Bath PM, Pathansali R, Iddenden R, Bath FJ. The effect of transdermal glyceryl trinitrate, a nitric oxide donor, on blood pressure and platelet function in acute stroke. Cerebrovasc Dis 2001;11(3):265–72.