Randomized Trial

A Randomized, Double-Blind Study of the Effects of a Sustained Release Formulation of Sodium Nitrite (SR-nitrite) on Patients with Diabetic Neuropathy

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Background: Background: Sodium nitrite has been reported to be effective in reducing chronic peripheral pain.

Objectives: To evaluate the safety and efficacy of 40 and 80 mg, BID, of an oral sustained release formulation of sodium nitrite (SR-nitrite) in patients suffering from diabetic neuropathy, and to determine whether SR-nitrite would reduce the frequency of headaches reported previously by subjects receiving the same doses of an immediate release formulation.

Study Design: Phase II, single-center, randomized, double-blind, placebo controlled clinical trial.

Setting: The Ohio Pain Clinic and Kettering Medical Center.

Methods: Twenty-four patients were randomized to 40 mg or 80 mg SR-nitrite or placebo twice daily for 12 weeks. The primary objective was to determine whether headaches would be reduced using SR-nitrite. The primary efficacy endpoint was the mean difference in the change of the Neuropathic Pain Symptom Inventory (NPSI) pain score from baseline to that reported after 12 weeks of treatment. Secondary endpoints included changes from baseline for the Brief Pain Inventory (BPI) Scale, the RAND 36 questionnaire, Short Form McGill Questionnaire, daily patient reported score for neuropathic pain, changes in HbA1c, PulseOx and quantitative sensory testing.

Results: The number of subjects reporting adverse events and the number of adverse events did not change with dose. There were no reports of treatment-related headaches. Although no significant differences were identified in patient responses to the questionnaires, a trend was observed. In the NPSI assessment, patients in the 40 mg and 80 mg dose group reported a 12.7% and 22.0% reduction in pain, respectively, compared to an 8.4% reduction by patients in the placebo group. A trend was also observed with the BPI total severity score. However, the 40 mg dosing group reported the greatest reduction in pain using the McGill Pain index and via patient logs of daily pain scores, where the mean of pain scores reported by subjects in the 40 mg group dropped by day 41 and generally stayed lower than the mean of scores reported by subjects in either of the other two groups. Patients in the 80 mg SR-nitrite group had an improvement in both Nerve Sensory Conductance and Nerve Sensory Velocity. No changes were observed in HbA1c levels or PulseOx.

Limitations: Small sample size.

Conclusion: Sustained release sodium nitrite prevents the prevalent reports of headaches by patients treated with an immediate release formulation of sodium nitrite. In a previous study of patients with peripheral arterial disease (PAD), 40 mg BID treatment led to a statistically significant reduction in reported pain, similar trends were observed at the end of the trial period for most of the pain questionnaires used in the study. The 80 mg BID treatment had the more pronounced affect on bioactivity (quantitative sensory testing), which was similar to the PAD study, where this dose group had the greatest improvement in FMD {AU: spell out FMD}. The ability to alleviate pain with BID treatment of SR-nitrite offers promise for a new non-addictive, non-sedating treatment of chronic pain and warrants further study.

Key words: diabetes, diabetic neuropathy, neuropathic pain, peripheral neuropathy, sodium nitrite

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Microcirculatory injury, which is common in diabetic patients, can lead to a number of problems. Prominent among these is diabetic peripheral neuropathy (DPN) (1,2). About 10% of patients will have evidence of DPN at the time they are initially evaluated, and almost 50% of diabetic patients will ultimately develop DPN (3,4). Of diabetic patients with DPN, 40% to 50% suffer from chronic pain (4,5), as well as paresthesias, sensory loss, and weakness (6), and have at least an 8-fold increased risk of undergoing a distal lower extremity amputation compared to similar non-diabetics (7).

Endothelial cells play an important part in the regulation of microcirculation, as they maintain vascular tone by secreting both vasodilators and vasoconstrictors (8). A central feature of diabetic microvascular disease (MVD) is endothelial dysfunction, which, in turn, plays an important role in the development and progression of DPN. The pathophysiological factors leading to endothelial dysfunction in diabetes include chronic hyperglycemia and protein glycolation, insulin resistance, inflammation, and increased oxidative stress (3,9-12). Studies have now shown a close relationship between endothelial dysfunction and diminished nitric oxide (NO) bioavailability (13,14).

Endogenously produced NO has a half life measured in seconds, and is rapidly oxidized to nitrite (NO_2–) and nitrate (NO_3—) end products, the latter of which is biologically inert (15). In the presence of microcirculatory ischemia and endothelial cell dysfunction, however, endogenous NO production by eNOS is much more limited. In such circumstances, circulating NO2– can be non-enzymatically reduced to increase NO availability (16). In addition to serving as a circulating NO reservoir, nitrite itself has also been shown to have direct and potent vasodilatory effects in vitro and in vivo (17-19). The findings that NO_2– mediates vasodilatation, both directly and through NO generation, has led to growing interest in the potential effectiveness of nitrite as a therapeutic agent in conditions associated with DPN and endothelial dysfunction (20,21). Such conditions include diabetic microvascular disease, DPN, and retinopathy, in which low levels of NO and NO_2–, as well as elevated levels of nitrate (NO_3), suggest that the complete oxidation of NO occurs during diabetes with insufficient NO_2– reserves to restore NO bioavailability (22-24).

Previous human studies with an oral formulation of $NaNO_2$ have shown that administration twice daily improves vascular function (25-27). In the peripheral arterial disease study (25), subjects who received the lower dose of $NaNO_2$ reported a significant reduction in pain. Although side effects were minimal, headaches and dizziness were reported by a large number of subjects, likely due to the rapid release of $NaNO_2$ leading to vasodilation. An oral sustained-release formulation of $NaNO_2$ (SR-nitrite) was developed in an attempt to overcome these problems and was tested in a porcine model of metabolic syndrome with critical limb ischemia. SR-nitrite-treated animals showed increased myocardial NO bioavailability, diminished oxidative stress, and cytoprotection in ischemic tissue (21,28). Importantly, 24-telometry recordings of blood pressure showed no evidence of vasodilation.

In the present study, we hypothesized that the SR-nitrite would reduce or eliminate headaches reported in patients following administration of the immediate release formulation. Given the promising results on reducing pain in diabetic patients with peripheral arterial disease reported in the previous study (25), patients with diabetic neuropathy were utilized in this study to determine whether any trends in reducing pain could be observed.

Methods

Study Design

A randomized, placebo controlled, double-blind phase II study was carried out to investigate the safety and potential biological activity of multiple doses of an oral, sustained-release formulation of sodium nitrite (SR-nitrite; Theravasc Inc., Cleveland, OH, USA), BID in doses of 40 mg and 80 mg over a 12-week treatment period, in human subjects with diabetes and neuropathic pain in the lower extremities and feet. The trial was approved by the Copernicus IRB and listed on ClinicalTrials.gov: www.clinicaltrials.gov/ct2/show/NCT02412852. The study was funded by Theravasc Inc.

Study Subjects

Subjects 18 years of age or older with Type I or Type II diabetes mellitus complicated by DPN and neuropathic foot pain were candidates for the study. The inclusion and exclusion criteria are listed in Table 1.

Study Protocol

SR-nitrite (40 mg), as well as matching placebo tablets, were provided by the sponsor. Following screening, subjects were randomized to one of 4 groups: 80 mg of SR-nitrite (two tablets twice a day for 12 weeks)

Table 1. Study subject inclusion and exclusion criteria. Females must be post-menopausal or using suitable measures to prevent pregnancy; they must not be nursing. DPN, diabetic peripheral neuropathy; NPRS, Numerical Pain Rating Scale.

Inclusion requirements	Patient exclusion
Age >18 years	Fibromyalgia
Male or female*	Pain from cervical or lumbar compression
Diabetes with HbA1c >6.0	Underlying unrelated neurological disease
Diagnosis DPN with pain in feet >3 months	Significant psychological disorder
Pain score ≥4 on NPRS	Liver disease
Understand and provide written consent	Poorly controlled diabetes
	Hypersensitivity to NaNO2
	Life expectancy <6 months
	Chronic illness or active malignancy
	Pregnant or nursing women
	Diagnosis substance abuse
	Use of phosphoesterase type 5 inhibitors
	History of methemoglobinemia ≥15%
	Inability to speak English

(8 subjects), 40 mg of SR-nitrite (one tablet twice a day for 12 weeks) (7 subjects), and two separate placebo groups (one group receiving one tablet twice a day and the other receiving 2 tablets twice a day for 12 weeks) (4 and 5 subjects, respectively). Randomization was completed by an independent CRO (clinical research organization) using sequential numbers to conceal the identity of the patients. The CRO (Kettering Health Network) generated the random allocation sequence, enrolled participants, and assigned participants to the intervetions. Since all subjects were assigned numbers, the subjects, the physicians, and anyone involved in the patient care process were blinded.

Subject screening included demographics, medical history, physical examination, blood samples for laboratory studies, and assessment using the Numerical Pain Rating Scale. Laboratory studies included clinical chemistry (AG ratio, albumin, alkaline phosphatase, ALT, anion gap, AST, BUN, calcium (serum), serum chloride, CO2, serum creatinine, glucose, potassium, sodium, total bilirubin, total protein, and hematology (globulin, WBC, RBC, Hb, Hct, MCV, MCH, MCHC, Platelets, RDW) panels, eGFR and Hb1Ac. A urine pregnancy test was obtained at that time.

Subjects who met inclusion criteria were randomized and on visit 1 (day 0) completed the RAND 36 quality of life questionnaire (29), Brief Pain Inventory (BPI) (30), Neuropathic Pain Symptom Inventory (NPSI) (31), and the Short Form McGill Pain Questionnaire (SFMP) (32). Quantitative nerve sensory testing was also conducted at this visit. Study drug was then administered and blood samples were collected pre-dosing and 60 minutes post-dosing for analysis of methemoglobin levels. A logbook featuring patients' daily reports of pain was explained and given to subjects, study medication was provided, and the subject was released.

Subjects returned to the clinic on day 42 (visit 2) and day 84 (Visit 3). During each visit, information regarding the clinical course, compliance, and possible AEs was collected. Each patient's history was updated and a physical examination was completed. Every visit included blood draws for clinical laboratory testing, completion of questionnaires described above, review of the daily pain and medication use logbook, and blood draws for pre-dose and 60 minutes post-dose methemoglobin testing.

Safety Monitoring

Safety parameters were assessed at each visit and included medical and medication histories, concomitant medication usage, physical examination, vital signs, comprehensive metabolic panel, complete blood count, pre- and post-dose methemoglobin levels, and adverse events. Evaluation for acute adverse events (i.e., a drop in blood pressure, dizziness) was done following the visit dose of the investigational product or placebo. Dose-limiting toxicity (DLT) was defined as grade 3 and clinically significant hemato-

logical events, including close scrutiny of methemoglobin levels.

Quantitative Sensory Testing

Quantitative sensory testing was assessed using a quantitative nerve conduction testing machine, where nerves in the distal extremity such as the sural nerve or peroneal nerves (for example) are subjected to electrical stimulation to determine the sensory threshold in the skin. For each subject, the mean conduction measurements were determined by averaging the results from the 3 study visits. Velocity means were also determined in that manner.

Statistical analyses

Results for a total of 29 different laboratory test values were summarized and compared using a 3 by 3 Analysis of Variance with one between-subjects factor (Combined placebo, 40 mg TV1001, or 80 mg TV1001) and one within-subjects factor (Visit 1, Visit 2, or Visit 3). The functional status (pain and quality of life questionnaires) measures were analyzed using a 3 by 3 Analysis of Variance with one between-subjects factor (Group: both placebo groups combined, 40 mg TV1001, and 80 mg TV1001); and one within-subjects factor (Visit: Visit 1, Visit 2, and Visit 3).

Demographic and clinical test results were summarized and compared using descriptive statistics and two-tailed t-tests. Statistical significance was defined by *P* - values less than 0.05.

Results

Since only one dose of SR-Nitrite was available (40 mg) to maintain the blind, subjects randomized to the placebo group were further randomized to either one or 2 tablets twice daily, then pooled for analysis of data. Twenty-six subjects were randomized, but 2 of these subjects withdrew within 3 weeks of initial dosing and are therefore not included in the analysis. Of the 24 subjects who completed testing, 7 were in the 40 mg group, 8 in the 80 mg group, and 9 in the combined placebo recipients. Demographic data showed no significant differences between the groups (Table 2).

Safety Monitoring and Adverse Events

Two subjects withdrew from participation in the study, one on low dose SR-nitrite informed the study site on the first phone call 14 days after beginning dosing because of undefined side effects; and the second, on high dose SR-nitrite, had a drop in blood pressure that met termination criteria. Both subjects had an Early Termination visit approximately 3 weeks after initial dosing. There were no abnormal safety findings and given the early withdrawal, the functional data were not included in the analysis.

There were no significant treatment effects for any of the laboratory values and only 2 instances of out-of-range laboratory values that were considered to be clinically significant, one for low sodium levels and the other for high glucose, neither of which were thought to be treatment related. Special attention was given to hemoglobin, white blood cells, red blood cells and platelet values in assessing hematologic parameters. Platelet values at baseline were significantly lower for the placebo group, but they did not change for any group with treatment, nor was any treatment affect observed for any hematologic assessment.

Table 2. *Selected subject characteristics by study group.*

	Placebo	40 mg group	80 mg group
Number	9	7	8
Age, years (SD)	60.0 (13.1)	63.1 (11.8)	50.2 (9.8)
Sex, male	5 (55.6%)	4 (57.1%)	3 (37.5%)
Race, white	100%	100%	100%
Ethnicity, white	100%	100%	100%
Smoking Status			
Former	6 (66.7%)	3 (42.9%)	3 (37.5%)
Current	0	1 (14.3%)	0
Peripheral Vascular Disease	0	1 (14.3%)	0
Cardiovascular Disease	6 (66.7%)	6 (85.8%)	3 (37.5%)
HbA1c at screening (SD)	7.7 (1.4)	7.7 (1.2)	8.5 (2.4)

Table 3. *Serious Adverse Events. SAEs, severity, relatedness, date began and ended and action taken are shown. Subject 22 had multiple SAEs occurring at different times throughout the study period.*

ID	Serious Adverse Event	Onset Date	Date Resolved	Severity	Related?	Action Taken
Placebo						
S04	hyperglycemia (glucose 531)	8/12/15	8/12/15	Mild	Not	None
S23	chest pain	9/27/15	9/28/15	Not Recorded	Not	Hospitalization
40 mg SR-nitrite						
S13	Fall	6/25/15	6/25/15	Mild	Not	None
S22	GI bleed	9/19/15	9/20/15	Moderate	Not	Hospitalization
	orthostatic hypotension	9/21/15	9/23/15	Moderate	Possibly	Hospitalization
	hyperglycemia	11/5/15	11/7/15	Moderate	Not	Hospitalization
	hyponatremia	11/5/15	11/7/15	Moderate	Not	Hospitalization
80 mg SR-nitrite						
S02	hyponatremia	7/19/15	7/21/15	Mild	Not	Hospitalization
S24	febrile illness	10/12/15	10/14/15	Moderate	Not	Hospitalization

There were no deaths in the study. Six different patients had significant adverse events (SAEs), 2 from each of the 3 treatment groups. Nine SAEs were reported during the study, including 4 by one subject at three different times. Seven of the 9 reported SAEs resulted in hospitalization. Of the 9 SAEs, only one was recorded as possibly treatment related (Table 3).

A total of 75 AEs were documented during the study period. The frequency of observed moderate and severe AEs, along with the most frequently reported AEs, is shown by treatment group in Table 4. All subjects in the placebo and 40 mg treatment group, and 7 of the 9 subjects in the 80 mg treatment group, reported at least one AE. The total number of reported AEs was slightly higher for the placebo group, but not really different from those in the SR-nitrite groups. Importantly, headaches, which was the most common AE reported in the previous trial that utilized the immediate release formulation of sodium nitrite, were not reported by any subjects in the 40 mg treatment group and were reported by the same number of subjects in the 80 mg treatment group as in the placebo group (2 subjects each). Dizziness was another commonly reported AE when immediate release sodium nitrite was used. In this study, when the sustained release formulation of sodium nitrite was administered to patients, there was no difference in reports of dizziness between the 3 groups.

Vital Signs

There were no consistent drops in baseline blood pressure or changes in baseline pulse rates for any of the groups. Blood pressure changes immediately post-treatment administration did fall fairly dramatically for the 40 mg treatment group on Visit 1, but did not show much difference when the 80 mg group was compared to the placebo group at this visit. The small sample size may make interpretation difficult, since at Visit 2, the placebo group showed a mean drop of 9.0 Hg for systolic blood pressure and 9.9 mm Hg for diastolic blood pressure post-dosing.

Methemoglobin Levels

Methemoglobin levels were assessed pre-dosing and 1 hour post-dosing at each visit and at 6 hours post-dosing at Visit 1. There was no change in methemoglobin levels following dosing at any visit or time.

Quantitative Sensory Changes

The mean sensory velocity did not differ between treatment groups at baseline (41.7, 39.9 and 40.5 meters/second for placebo, 40 and 80 mg groups respectively) but was lower than normal nerve velocity (50-60 meters/second), which is consistent with subjects exhibiting nerve damage due to peripheral neuropathy. Nerve sensory conductance showed very little change

Table 4. *Adverse Events. Summary of Adverse Events, including severity and relatedness are show, along with those AEs reported by 3 or more of the subjects.*

	Placebo	40 mg SR-nitrite	80 mg SR-nitrite
	N=9	N=8	N=9
Number of Subjects with at least 1 AE	9	8	7
Total Number of AEs	29	23	23
Number of AES by Severity			
Mild	23	14	17
Moderate	2	5	4
Severe	1	4	1
Not Recorded	3		1
Number of AEs by Relatedness			
Not related	24	19	19
Possibly	5	4	4
Probably	0	0	0
AEs reported by >10% subjects			
Dizziness, inc. shakiness	2	2	2
Headaches	2	0	2
Swelling	3	0	0
Increase in pain	2	1	0
Difficulty urinating	0	2	1

between baseline testing and Visits 1 and 2 for the placebo and 40 mg treatment groups, but trended toward decreasing with the 80 mg treatment group ($P = 0.154$). Similarly, nerve sensory velocity remained stable for the placebo and 40 mg treatment groups but exhibited a trend towards increasing ($P = 0.116$) with 80 mg treatment (Table 5). This suggests that the 80 mg dose of SR-nitrite was most effective at improving nerve function.

Functional Status and Pain Assessment

Evaluation of the quality of life subscores of the RAND 36 showed little effect of treatment with the exception that the baseline level of fatigue/energy was significantly worse in subjects in the 80 mg group.

This trial was not powered to show any significant results from questionnaires and as expected, there was a great deal of variability across groups and visits. Instead, the questionnaires were used to determine whether there was a trend in reducing pain or improving quality of life and to help in selecting the appropriate vehicles to use and in powering subsequent trials. In each of the major categories in the three different pain questionnaires, subjects reported less pain on visit 3 than on visit 1. This was not necessarily true for visit 2, particularly for the 80 mg group, which reported more pain on a number of the questionnaires. There was also variability between visits within a treatment group, with the average reduction of pain at visit 2 often being greater than the average reduction at visit 3. When visit 3 over-all data from each questionnaire were compared to baseline data for each treatment group, there were apparent trends in reducing pain based on the Neuropathic Pain Symptom Inventory, with subjects in the 40 mg dose group reported approximately 1.5 fold less pain and subjects in the 80 mg dose group approximately 2.6 fold less pain at the end of the study when compared to subjects in the placebo group (Table 6). The same trend was observed in the Severity Score of the Brief Pain Inventory, but not in the Interference Score of the same questionnaire. In this section of the questionnaire, the placebo group responded better than either treatment group. Results from the McGill Pain Index were even more inconsistent, with the 40 mg treatment group reporting slightly less pain in each of the three major sections at the end of the treatment period than did the placebo group, however, the subjects in the 80 mg treatment group reported very little reduction in pain using this questionnaire.

Table 5. *Nerve Sensory Testing. The mean and SD for Nerve Sensory Conductance and Nerve Sensory Velocity are shown for Visit 1 (baseline), Visit 2 and Visit 3 (end of study) for the placebo group (n=9), 40 mg SR-nitrite treatment group (n=7) and 80 mg SR-nitrite treatment group (n=8).*

		Visit 1	Visit 2	Visit 3
Conduction Mean (SD)	Placebo	3.9 (1.4)	4.3 (1.8)	3.5 (1.2)
	40 mg group	3.4 (0.7)	3.1 (0.9)	3.2 (0.6)
	80 mg group	6.0 (2.9)	5.2 (3.0)	4.2 (1.5)
Velocity (meters/second) Mean (SD)	Placebo	44.7 (6.5)	40.1 (5.3)	43.2 (6.4)
	40 mg group	41.1 (4.3)	39.3 (7.8)	37.7 (3.2)
	80 mg group	39.4 (8.2)	42.3 (10.7)	46.8 (4.2)

Table 6. *Percent changes in pain scores from study visit 1 to visit 3 (+ numbers indicate improvement) for the placebo group (n=9), 40 mg SR-nitrite treatment group (n=7) and 80 mg SR-nitrite treatment group (n=8).*

Questionnaire		Subject group	Improvement between Visits 1 and 3 (%)
Test	Score parameter		
Brain Pain Inventory	Interference	Placebo	14.0%
		40 mg	4.5%
		80 mg	10.9%
	Severity	Placebo	5.9%
		40 mg	11.6%
		80 mg	13.6%
NPSI*	Total Score	Placebo	8.4%
		40 mg	12.7%
		80 mg	22.0%
McGill Pain Index	Total Score	Placebo	29.4%
		40 mg	35.9%
		80 mg	4.2%
	Continuous Pain	Placebo	36.0%
		40 mg	48.6%
		80 mg	2.3%
	Intermittent Pain	Placebo	31.7%
		40 mg	39.1%
		80 mg	10.2%

*NPSI, Neuropathic Pain Symptom Index

A number of questionnaires were used to assess patients' pain, the most important being the widely accepted Neuropathic Pain Symptom Inventory (NPSI). In the NPSI assessment, patients in the high dose SR-Nitrite group reported a 22% reduction in pain, patients in the low dose group reported a 12.7% reduc tion in pain compared to a 8.4% reduction by patients in the placebo group at the end of the trial compared to baseline. There was a statistically significant effect for Group. The mean score for the 40 mg treatment group was significantly lower than for the 80 mg treatment group. There were no significant effects for Visit or for the Group by Visit. (Figure 1). Although the difference between the SR-nitrite groups and placebo was not as

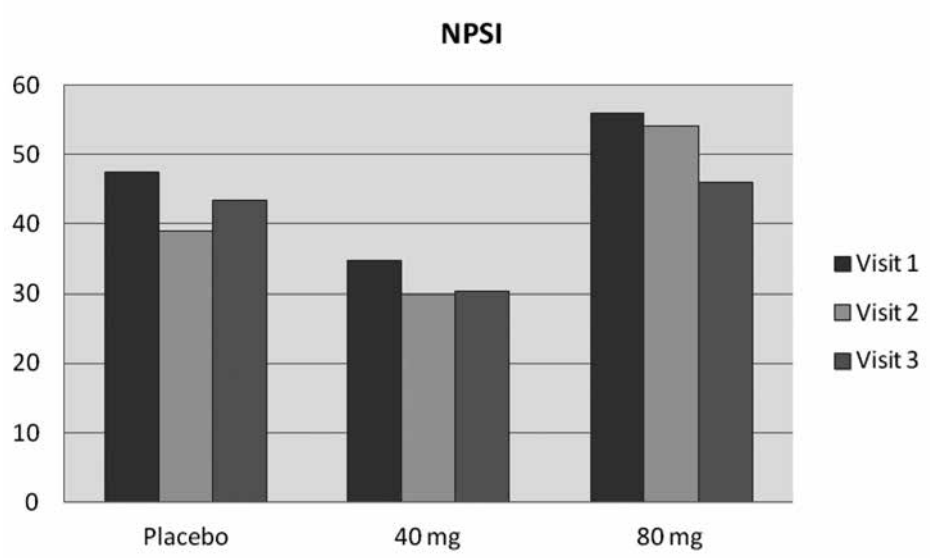

Fig. 1. *Mean scores for NPSI Questionnaire. The mean scores for Total Score Section of the NPSI Questionnaire are shown for Visit 1 (baseline), Visit 2 and Visit 3 (end of study) for the placebo group (n=9), 40 mg SR-nitrite treatment group (n=7) and 80 mg SR-nitrite treatment group (n=8).*

pronounced based on patient reported pain using the BPI Severity Score, a dose dependent trend was still observed at the end of the trial period, although no dose effects were seen when the BPI Interference Scores were compared. There was a statistically significant effect for Group for both the Severity and the Interference score. For both measures, the mean score for the 40 mg treatment group was significantly lower than for the 80 mg treatment group. There were no significant effects for Visit or for the Group by Visit Interaction (Fig. 2). The results from the McGill Pain Index showed a pattern of lower intermittent, continuous, and total pain scores by visit 3 for those subjects taking 40 mg tablets, as compared to those taking placebo, with the subjects in the 80 mg group reporting less benefit than the placebo group. There was a statistically significant effect for Group for each of the Total and Intermittent scores. For each of these measures, the mean score for the 40 mg treatment group was significantly lower than for the 80 mg treatment group. There were no significant effects for Visit or for the Group by Visit Interaction (Figure 3).

As with most studies, there was a placebo effect on pain but on the key NPSI questionnaire the low dose SR-nitrite group reported approximately 1.5 times the reduction in pain and the high dose approximately 2.6 times the reduction in pain from what they experienced initially, compared to that reported by the placebo group at the conclusion of the study. Perhaps most striking, the 40 mg group consistently demonstrated the largest improvements in multiple pain parameters between visit 1 and visit 3 when compared to the other groups on all but the BPI Interference Questionnaire (Table 6).

Daily pain scores according to treatment group are seen in Figure 4. There was a great deal of variability in the number of subjects reporting scores on any given day, making statistical analysis of the data not practical. For example, only 5 of 9 and only 4 of 7 subjects in the placebo group and the 40 mg treatment group self-reported pain scores on Day 1 and on Day 90. Although not surprisingly, there is a good deal of variability over time, the mean of pain scores reported by subjects in the 40 mg group did seem to drop by day 41 and generally stayed lower than the mean of scores reported by subjects in either of the other two groups. When the means of the final visit were subtracted from the baseline value for each group, the 40 mg treatment group reported the greatest reduction in pain, a mean drop of 4.0, while the placebo group reported a mean drop of 2.1. Very little change in pain perception was reported by subjects in the 80 mg treatment group, with a mean drop of only 0.5.

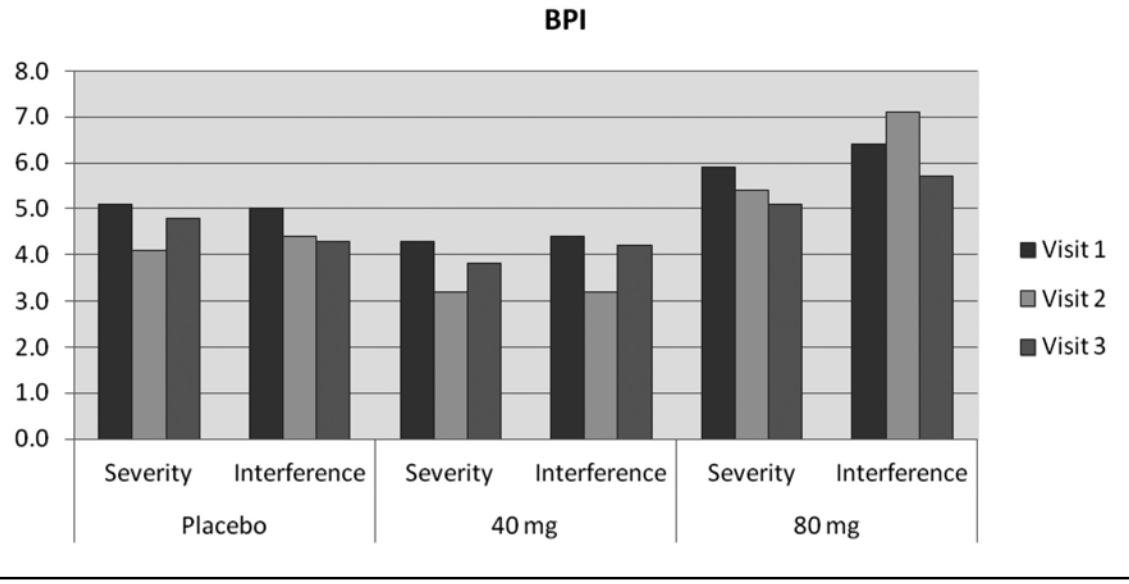

Fig. 2. *Mean scores for BPI Questionnaire. The mean scores for the Severity and the lnterference Score Sections of the BPI Questionnaire are shown for Visit 1 (baseline), Visit 2 and Visit 3 (end of study) for the placebo group (n=9), 40 mg SR-nitrite treatment group (n=7) and 80 mg SR-nitrite treatment group (n=8).*

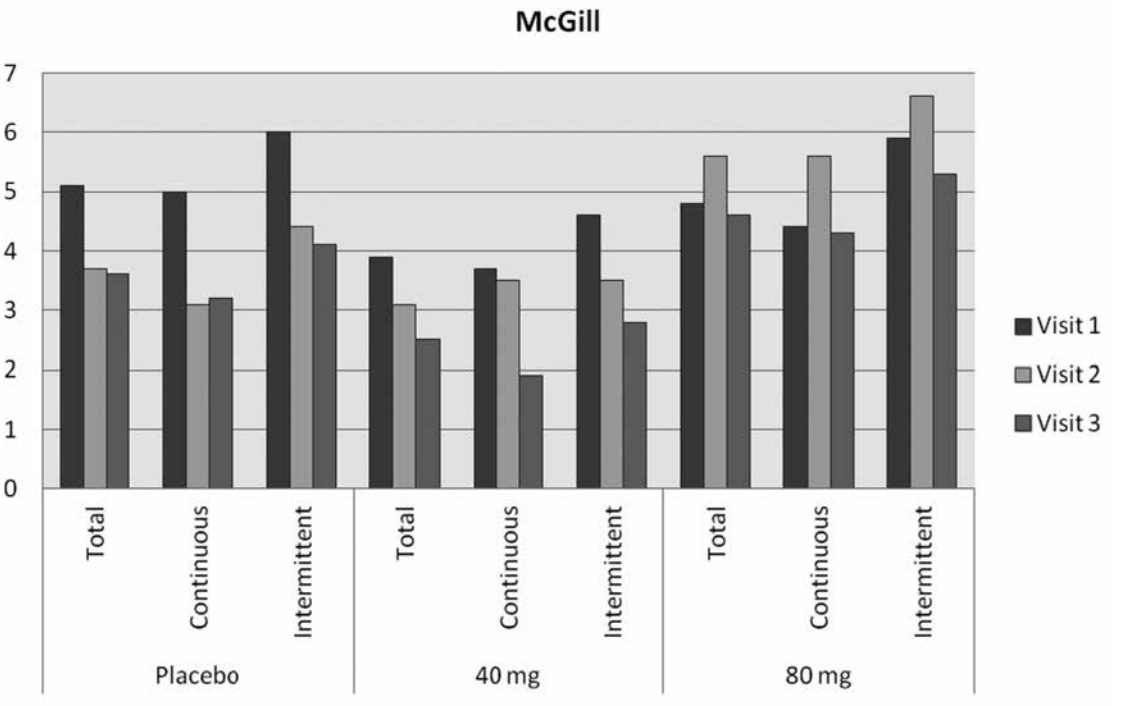

Fig. 3. *Mean scores for McGill Short-Form Questionnaire. The mean scores for the Total, the Continuous and the Intermittent Score Sections of the McGill Short-Form Questionnaire are shown for Visit 1 (baseline), Visit 2 and Visit 3 (end of study) for the placebo group (n=9), 40 mg SR-nitrite treatment group (n=7) and 80 mg SR-nitrite treatment group (n=8).*

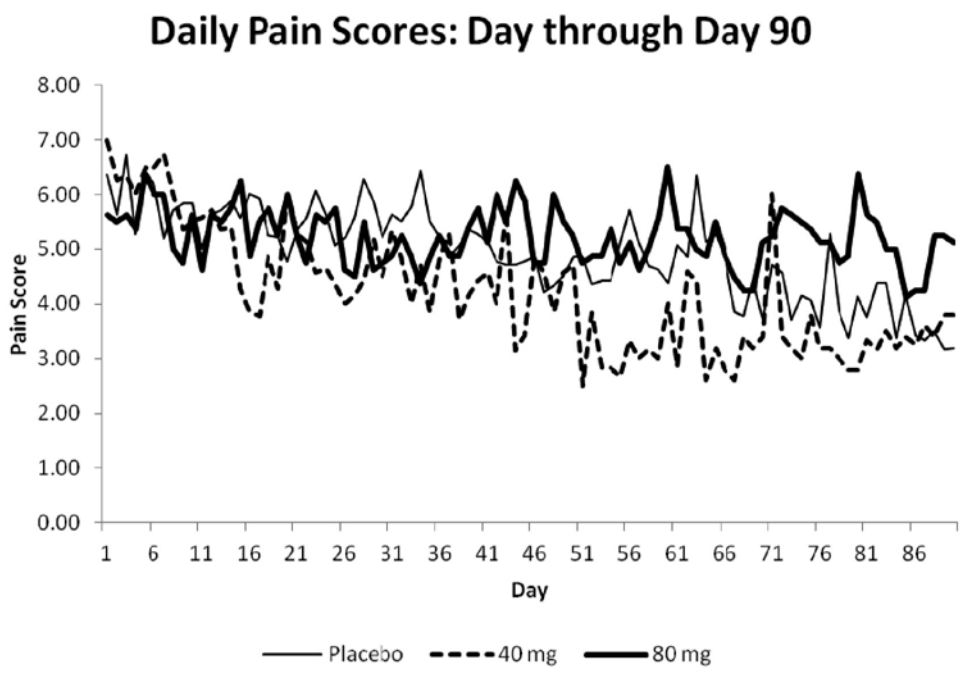

Fig. 4. *Mean scores for Daily Pain Logs. The mean scores for each day recorded in the Daily Pain Logs are shown for Visit 1 (baseline), Visit 2 and Visit 3 (end of study) for the placebo group, 40 mg SR-nitrite treatment group and 80 mg SR-nitrite treatment group.*

PulseOx and HbA1c

The mean levels of HbA1c did not change significantly during the trial period. Levels at baseline and at Visit 3 were 7.7±1.4 and 7.6±2.0 for the placebo group, 7.7±1.2 and 7.7±1.6 for the low dose SR-nitrite group and 8.5±2.4 and 8.2±1.2 for the high dose SR-nitrite group. PulseOx levels also did not change over the treatment period for any of the groups.

Discussion

The relationship between nitric oxide and pain is well known (8). NO has been reported to regulate pain at both the central and peripheral levels. Anatomically, nitric oxide synthase expression (namely nNOS) is observed throughout the nervous system and NO regulation of central sensations is known to occur in the dorsal horn of the spinal cord by modulating release of various neurotransmitters such as glutamate, substance P, neurokinin A, and calcitonin-gene related peptide (8). NO can facilitate nociception involving both analgesic and/or hyperalgesic responses. The duality of NO effects on these responses is governed in part by several variables (time after injury, dose, model, and administration route) that have been examined in experimental models. Of these variables, dose and administration routes provide insight into nociception responses that could be exploited for therapeutic purposes (8). NO has also been shown to be an important mediator of analgesic drugs such as acetylcholine, morphine, opioids, loperamide, cannabinoids and other agents (8). During diabetes, nNOS expression, function and neuronal NO bioavailability are significantly diminished, resulting in hyperalgesia and neuron damage (33,34); suggesting that targeted NO therapy might be beneficial during diabetic neuropathy. Importantly, mediators of neuropathic pain are complex and involve tissue ischemia, inflammation and oxidative stress that provide ideal conditions for nitrite reduction back to NO to alleviate pain.

Thus, NaNO2 therapy may represent a novel strategy for treating pain in diabetic neuropathy by acting on the underlying reason for the pain, poor microvasculature, inflammation and oxidative stress. In addition, NaNO2 is non-addictive and non-constipating, with very few side effects reported. An oral formulation of immediate release NaNO2 used both in a study involv-

ing patients with peripheral arterial disease (25) and normal, aged healthy subjects (26,27) demonstrated improved vascular function, but also reported a significant number of subjects complaining of head aches and dizziness, likely due to the vasodilatory properties of NaNO2 . Significant decreases of systemic systolic blood pressures in the 5 to 10 mmHg range were regularly observed during the 30 to 60 minutes following administration of a 80 mg dose (35). Although most studies consider this to be clinically insignificant, it is interesting that its occurrence coincides with the reported headaches and dizziness.

To overcome these issues, a sustained release formulation of NaNO2 was developed. In a porcine model of metabolic syndrome with peripheral vascular disease using this SR-nitrite formulation, positive effects on angiogenesis were observed after 30 days but more importantly, 24 hour telemetry showed no drop in blood pressure at any time following administration of an 80 mg dose (28). This SR- nitrite formulation was tested in the current study primarily to determine whether headaches and dizziness could be reduced. Since pain had been alleviated in the PAD study (25), a secondary endpoint of the current study was to assess pain in diabetic patients with neuropathy.

Side effects were minimal with no difference between placebo and either treatment groups. Importantly, treatment with SR- NaNO2 was not associated with reported headaches and dizziness. Headaches were reported by 4 subjects, 2 in the placebo group and 2 in the 80 mg group, while dizziness, including shakiness, was reported by two subjects in each group.

Data from the current study also show a significant reduction of DPN-associated pain on the Neuropathic Pain Symptom Inventory (NPSI) in those subjects taking 40 mg and 80 mg of SR-nitrite twice daily during the treatment period. The pain reduction in the 40 mg group was consistently superior to that of the placebo in all but the BPI Interference analysis when subject reporting at the end of the study was compared to baseline reported pain levels. The 80 mg dose group, while showing a reduction in pain from baseline to Visit 3 in the NPSI and BPI Severity assessment compared to placebo, was not as effective in the other assessments. These differences in dose effect are consistent with the complex and seemingly dual functions of the role of NO in pain. Studies have demonstrated that NO can promote or inhibit nociception, thus affecting pain and analgesia, even in the same injury (36). Some data suggest that this, at least in part, may be the result of local NO levels or NaNO2 doses (36,37).

Nerve sensory velocity and conductance, indicators of biological activity, were improved in the 80 mg dose group, which suggests improved nerve function. These data are consistent with the previous observations of the higher dose group having a better effect on FMD (25,26), another indicator of biological activity, than either the placebo or 40 mg dose groups.

In this study, a trend was observed for most of the pain assessments, in that the low dose group was better at alleviating pain, which is similar to the results seen in the previous study (25). While the higher dose may be better at improving biological activity, it may also lead to NO-induced acute pain, thus masking the effects on chronic pain associated with microvascular disease. More work needs to be conducted to better understand this.

Conclusions

This study demonstrates that SR-nitrite eliminates the headaches and dizziness observed with an immediate release formulation of NaNO2 , and the potential of SR-nitrite to alleviate pain in patients with diabetic neuropathy with few side effects. While these results are encouraging, a larger study should be carried out to confirm the ability of SR-nitrite to reduce pain.

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