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Topical sodium nitrite for chronic leg ulcers in patients with sickle cell anaemia: a phase 1 dose-finding safety and tolerability trial

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Summary

Background—Well-tolerated and effective treatments are needed for chronic leg ulcers in sickle cell anaemia. Topical sodium nitrite, a known nitric oxide donor, enhances blood flow in ulcers and has known bacteriostatic effects. We aimed to assess the safety, tolerability, and pharmacokinetics of topical sodium nitrite in patients with sickle cell disease and chronic leg ulcers.

Methods—We enrolled adult patients from an ambulatory clinic at the National Institutes of Health (Bethesda, MD, USA) with sickle cell anaemia with leg ulcers (with a surface area of 2.5–100 cm²) persisting for at least 4 weeks into a safety and tolerability phase 1 dose-escalation trial of topical sodium nitrite. Increasing concentrations of sodium nitrite cream were applied twice weekly for 4 weeks to one ulcer per patient at five dose levels (0.5%, 1%, 1.5%, 1.8%, and 2%). The primary endpoints were safety and tolerability, with secondary endpoints of pharmacokinetics, blood flow, and wound healing. Pain relief was analysed post hoc. Endpoints

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Contributors

CPM, AMG, YYH, and GJK contributed to the design of this study. K-MD, MP-L, JSN, and AC recruited patients and gathered data. MS and NM gathered and analysed cutaneous blood flow imaging data. GG formulated the sodium nitrite cream and provided investigational pharmacy services. DX did data analysis, with contributions from CPM, AMG, MS, CC, and GJK. YYH did pharmacokinetic modelling analysis. LM provided biospecimen processing and assays. CPM, DX, and GJK wrote the report. All authors helped to write the final report, provided comments, and have seen and approved the final version.

Declaration of interests

We declare no competing interests.

See **Online** for appendix

For **Common Terminology Criteria for Adverse Event standards** see <http://evs.nci.nih.gov/ftp1/CTCAE>

For **NIH natural history protocol** see <http://clinicaltrials.gov/show/NCT00081523>

were analysed over time for the whole study population and according to dose level. This study is registered with ClinicalTrials.gov, number NCT01316796.

Findings—Between April 4, 2011, and March 19, 2013, we enrolled 18 adult patients with sickle cell anaemia and leg ulcers into our trial. We assigned three patients into each cohort, and each cohort was treated with a different concentration of sodium nitrite cream (cohort 1: 0.5%, cohort 2: 1.0%, cohort 3: 1.5%, and cohort 4: 2.0%). Patients were not enrolled into the next cohort dose until we were able to establish that no dose-limiting toxicities were observed. An additional six patients were enrolled to cohort 3a: 1.8%, after two patients in cohort 4 had asymptomatic drops in diastolic blood pressure. No grade 3–4 adverse events were observed, and there were no serious adverse events or dose-limiting side-effects. Pharmacokinetic analysis showed that systemic absorption of sodium nitrite was very low. Application of topical sodium nitrite was associated with a significant increase in peri-wound cutaneous blood flow measured by laser speckle contrast imaging ($p=0.0002$), corroborated by increased peri-wound skin temperature by infrared thermography ($p=0.0119$). We recorded a dose-dependent decrease in leg ulcer size ($p=0.0012$) and pain ($p<0.0001$). Ulcers healed completely in three patients who received the highest concentrations of topical sodium nitrite (the 1.8% and 2% cream). In our post-hoc analysis of pain, brief pain inventory scores improved in pain severity ($p=0.0048$) and pain interference ($p=0.0013$).

Interpretation—Our results indicate that topical sodium nitrite 2% cream is suitable for additional clinical trials in adults with sickle cell anaemia to promote healing of leg ulcers.

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Introduction

Morbidity from chronic leg ulcers remains a substantial clinical burden in patients with sickle cell disease, despite advances in care including the use of disease-modifying agents such as hydroxycarbamide and blood transfusions, and improved supportive care.¹ Patients with sickle cell disease and leg ulcers have biomarkers of more severe haemolytic anaemia, a state associated with low bioavailability of nitric oxide. Existing therapeutic approaches for sickle cell disease ulcers are unsatisfactory, and are mostly based on treatments for venous and arterial ulcers in the general population. A recent Cochrane review² identified only six prospective, randomised therapeutic trials in sickle cell disease leg ulcers in the past 30 years—four in Jamaica^{3–6} and two in the USA.^{7,8} The results were mixed, with statistically significant increases in wound closure reported with topical Arg-Gly-Asp (RGD) peptide⁷ and intravenous arginine butyrate.⁸ These agents remain in early-phase drug development, and patients have few therapeutic options available.⁹ Sodium nitrite was selected for clinical development on the basis of the extensive published literature about its safety profile when administered intravenously^{10,11} and orally,¹² its vasodilating properties,^{10,11,13,14} and preliminary reports of acidified nitrite in other patient populations with chronic skin ulcers.^{13,14}

In animals, sodium nitrite has been shown to promote revascularisation of ischaemic limbs; to protect against ischaemic infarction of the heart, liver, and brain; and to have a protective effect against cardiac arrest-mediated heart and brain injury.¹⁵ The nitrite anion acts as a

vasodilator in vivo by generating nitric oxide in tissues with low oxygen tension and pH,^{15,16} which are conditions that are likely to be present in chronic wounds. The mechanism involves an oxygen-dependent and pH-dependent nitrite reductase activity of haemoproteins or xanthine oxidoreductase.^{15,16} Experimental models have suggested beneficial effects of nitric oxide in the early and late phases of wound healing, including increased extracellular matrix production, immune response modulation, and stimulation of keratinocyte cell proliferation, angiogenesis, and bactericidal properties.^{16–23} Nitric oxide mediates essential vascular homeostasis, including vasodilation, and antiplatelet activity, and affects several growth factors involved in endothelial homeostasis.²⁴

We report the results of the first multi-dose exposure of topical sodium nitrite in human beings, in patients with sickle cell anaemia and chronic leg ulcers.

Methods

Study design and patients

In this single-centre, open-label, phase 1 study of increasing doses of sodium nitrite cream, patients 18 years of age and older with confirmed sickle cell disease and a leg ulcer present for at least 4 weeks with a surface area greater than 2.5 cm² but smaller than 100 cm² were eligible for inclusion. Patients were recruited from the ambulatory clinic at the Clinical Center of the National Institutes of Health (NIH), National Heart Lung and Blood Institutes (Bethesda, MD, USA). Hydroxycarbamide and chronic transfusions were allowed. Patients with acute infections were excluded. A full list of the exclusion criteria is available in appendix p 2.

We originally planned to enrol four cohorts with three patients in each, to give a total of 12 participants. A cream containing four different increasing concentrations of sodium nitrite (0.5% for patients in cohort 1, 1.0% in cohort 2, 1.5% in cohort 3, and 2.0% in cohort 4) was applied twice weekly to one ulcer (the study ulcer) per patient for 4 weeks (eight applications). The first two doses of cream were administered as inpatients, and the remaining six doses in the outpatient day hospital at the NIH Clinical Center. Around 4 days later, patients returned to the outpatient clinic for removal of the cream and end of study assessments, so the total duration of the study was about 32 days per cohort, which the US Food and Drug Administration judged to be sufficient to assess the safety and pharmacokinetics of sodium nitrite. All three patients in each cohort were assessed for the 32-day duration of the study to ensure no dose-limiting toxicities before patients were enrolled into the next cohort. After 12 participants had been enrolled and treated, we noted occasional asymptomatic, self-resolving decreases in diastolic blood pressure below the predetermined limit of 50 mm Hg, in two patients in cohort four (the 2% sodium nitrite cream cohort). To further assess the safety of the cream, we enrolled an additional six participants (cohort 3a) who received cream containing an intermediate dose of 1.8% sodium nitrite, to create a total of 18 participants.

We planned to remove participants from the study if they experienced dose-limiting toxicities or progression of the ulcer to more than 50% of initial size, and the sodium nitrite dose was to be reduced if the ulcer size decreased by more than 50% of initial size. Toxicity

was monitored with clinical laboratory testing at the time of each twice-weekly treatment in the first 12 patients, and weekly for the final six patients who were treated with 1.8% sodium nitrite, including methaemoglobin levels, complete blood counts, and comprehensive serum chemistry panels of liver and kidney function.

All patients provided written informed consent in accordance with the Declaration of Helsinki to a clinical research protocol approved by the Institutional Review Board of the National Heart, Lung and Blood Institute.

Procedures

In patients with more than one ulcer, all ulcers were treated similarly with standard wound care (see appendix p 2), with the application of sodium nitrite cream only to the study ulcer. The study ulcer was selected by the wound care nurse and principal investigator on the basis of ulcer duration, size, and accessibility for digital imaging. Patients completed a brief pain inventory and a 7-day diary of opioid use the week before and after completion of treatment, and identified the areas contributing to their pain. Patients were admitted to hospital (at the NIH Clinical Center) for the first two applications of topical sodium nitrite, and stayed in hospital from Monday morning to Friday afternoon (ie, for 4.5 days), to help pharmacokinetic sampling and clinical monitoring; thereafter, they were treated in the outpatient clinic (the NIH Clinical Center Day Hospital) twice weekly for 3 further weeks. Sodium nitrite cream was applied on day 3 and day 5 of week 1 at the same time (normally around 10:00 h). Patients were not confined to their bed except for 6–8 h on day 3 when most of the pharmacokinetic sampling and imaging was done. A visual analogue scale pain score was obtained at each treatment during the study (ie, a total of eight times) for the study ulcer and each of the non-study ulcers. Pain scores were not adjusted for analgesic use. Ulcers were measured manually with a disposable ruler (longest length and widest width) at each encounter and by digital photography three times: before the first application of cream (baseline), and after 2 weeks and after 4 weeks of treatment. ENVI software (version 4.3) was used for digital image analysis of the wound surface area. The ulcer area was calculated by converting the number of pixels representing the ulcer to cm^2 using a calibrated square ($35.48 \text{ mm} \times 35.62 \text{ mm} \times 6.52 \text{ mm}$) that was placed near the ulcer for reference. A plain radiograph of the long bones and feet and MRI scan of the lower extremities were obtained to ensure the absence of occult osteomyelitis. Wound cultures were obtained at screening and at the end of the study.

Sodium nitrite 0.5%, 1%, 1.5%, 1.8%, and 2% cream was compounded and provided by the NIH Clinical Center Pharmacy Department Pharmaceutical Development Section (Bethesda, MD, USA). The amount of sodium nitrite contained in 1 cm of the 0.5%, 1%, 1.5%, 1.8%, and 2% cream was 1.38 mg, 2.75 mg, 4.13 mg, 4.95 mg, and 5.50 mg, respectively. These doses were chosen on safety grounds so that each dose remained well within the intravenous infusion dose already approved by the US Food and Drug Administration for the treatment of cyanide poisoning, in case the sodium nitrite was quickly absorbed systematically. The cream was applied with a sterile spatula over the wound in a thin layer. The proportion of cream used was 0.1 cm of cream for each cm^2 of wound surface area.

Plasma and whole blood nitrite and nitrate concentrations were collected at the following timepoints: pre-dose; immediately after application; 5, 10, 15, 20, 30, 45, 60, and 90 min post-dose; 2, 3, 6, 8, 12, 24, and 48 h post-dose; then weekly and at the end of the study. All processing was done at the bedside. Plasma and whole blood nitrite and nitrate were measured with a gas-phase chemiluminescence nitric oxide analyser, as previously reported.²⁵ Methaemoglobin was measured predose, and then 12 times after cream application: immediately afterwards (0 min [baseline]), and then at 15, 30, 45, 60, and 90 min post-application, and 2, 3, 6, 8, 24, and 48 h post-application. Afterwards, for weeks 2–4, met haemoglobin was monitored weekly.

Adverse events were recorded and assessed for their association with the study drug at every encounter and between visits by telephone, and were classified according to the Common Terminology Criteria for Adverse Event standards (CTCAE) version 4.0, 2010. Dose-limiting toxicity included clinically significant hypotension (blood pressure lower than 85/50 mm Hg, and/or symptomatic dizziness, tachycardia, or hypoxia, according to the judgment of the principal investigator). The mean of at least three blood pressure measurements recorded 24 h before the first application of the cream was viewed as the “baseline” value. Pulse oximetry was recorded continuously during inpatient stay and at each outpatient visit.

We took digital photographs to measure ulcer surface area and to draw the peri-wound area—the region of interest—before the first cream application, at week 3, and at the end of the study. The internal and external border of each region of interest was drawn around the ulcer by the image processing toolbox of MATLAB 7.10. The resulting annular region of interest, roughly 1.5–2 cm in size, was used to analyse changes in peri-wound blood flow and temperature. Images were collected after acclimatisation at a room temperature of around 23°C. A calibrated infrared camera (3–5 µm wavelength, temperature resolution of 0.015°C), measured peri-wound temperature at a sampling rate of about 1.9 Hz. In conjunction with infrared thermography, a laser speckle contrast imaging camera (moor FLPI Full-Field Laser Perfusion Imager (Moor Instruments Ltd, Wilmington, DE, USA) with flux measurement accuracy of within 10% was used to record red blood cell flux at a sampling rate of about 5 Hz (775–795 nm laser wavelength, 113 × 152 pixels per image, 16 bits). Infrared cameras were positioned roughly 42 cm from the ulcer and laser speckle contrast imaging cameras were placed about 32 cm from the ulcer.

Outcomes

The primary outcome was the safety and tolerability of topical sodium nitrite. The secondary outcomes were changes in ulcer size and pain (in which pain was analysed post hoc, although all the data were a prospectively obtained at predefined timepoints), and the pharmacokinetics of topical sodium nitrite cream and methaemoglobin, with exploration of the possible mechanisms of action such as changes in regional blood flow and local temperature after cream application.

Statistical analysis

We compared laboratory parameters using a pairwise *t* test. Analyses of the blood pressure considered measurement within 24 h before and after initial cream application; we did two-sample *t* tests by cohort to assess any differences. Correlation analyses are reported using Spearman's rank correlation coefficient. The statistical significance was set at a two-sided *p* value lower than 0.05. Analyses were done with R version 2.13.1. This study is registered with ClinicalTrials.gov, number NCT01316796.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between April 4, 2011, and March 19, 2013, we screened 23 adult patients with sickle cell anaemia for inclusion, 18 of whom enrolled and completed the trial. Reasons for disqualification of the five patients who were not eligible were enrolment in another trial (one patient) and the ulcer being too small or acutely infected (four patients). Table 1 and appendix p 4 show the baseline patient demographics and ulcer characteristics. Chronic daily opioid use was almost universal at study entry (16 [89%] of 18 patients) and attributed to localised pain at the ulcer site in 15 patients. Half of the patients were taking hydroxycarbamide and three of 18 (17%) were receiving chronic transfusions. Both treatments are used frequently in adults with sickle cell anaemia disease with several complications, such as the patient population with chronic leg ulcers, as confirmed in a recent survey of treatment patterns for sickle cell disease and ulcers in North America.^{9,26}

A full list of the organisms recovered from cultures at the beginning and at the end of treatment is presented in appendix p 6. MRI of the lower extremities was done at study entry in 17 of 18 patients (one patient did not have an MRI scan because of the presence of brain surgical clips). One patient had evidence of resolved osteomyelitis. Lower extremity Doppler ultrasound clinical examinations showed one partially resolved venous clot in one patient and a clot in each popliteal vein in a second patient.

For the primary endpoint of safety and tolerability, no serious adverse events or withdrawals from the study occurred. One patient in the 1.8% concentration cohort received a pre-planned 50% dose reduction after 2 weeks of topical sodium nitrite treatment because the ulcer size decreased to smaller than half the starting size, and was healed by week 5. Two additional patients in the 1.8% concentration cohort (cohort 3a) were treated at the 1.5% concentration after the first application of the cream, because of asymptomatic decreases in diastolic blood pressure. These patients were assessed for toxicity in the 1.8% cohort and for efficacy in the 1.5% cohort. No treatment-related or other deaths occurred during the study, and no patient discontinued the trial because of drug-related toxicities. Grade 1 adverse events were reported by 12 (67%) of the 18 patients; table 2 lists those events that were possibly or probably related to topical sodium nitrite. The most frequently reported adverse

event possibly related to study drug was a grade 1 decrease in diastolic blood pressure below a predetermined threshold of 50 mm Hg. Before study drug application, four patients (one in cohort 3, two in 3a, and one in cohort 4) had transient asymptomatic episodes of diastolic blood pressure falling to lower than 50 mm Hg, which also occurred after study drug application in three of these patients and in four additional patients. Attribution to study drug was classified as “possibly related” for the latter four patients (one in cohort 2, two in cohort 3a, and one in cohort 4) (table 2). None of the patients had a clinically significant decrease in systolic blood pressure or mean arterial pressure, nor needed any intervention to increase their blood pressure. All events were asymptomatic, resolved spontaneously within 1 h (ie, at the next blood pressure check), and were judged to be not clinically significant. An examination of all blood pressures 24 h before and after first application showed no statistically significant changes in diastolic blood pressure by cohort. We recorded no association between plasma levels of nitrite and nitrate and changes in blood pressure.

No clinically significant changes in laboratory parameters occurred during the study (appendix p 5). We recorded statistically significant decreases in mean white blood cell count, from $9.9 \times 10^9/\text{L}$ (SD 4.5) to $8.7 \times 10^9/\text{L}$ (SD 3.1) ($p=0.0102$), and mean total protein, from 80 g/L (SD 0.5) to 78 g/L (SD 0.5) ($p=0.0302$). Methaemoglobin did not change significantly ($p=0.82$)—it peaked at 4.1% in one patient in cohort 3 (the 1.5% concentration cohort) 90 min post-application, and decreased spontaneously (appendix p 3).

Assessment of plasma nitrite, nitrate, and whole blood nitrite concentrations in the first 48 h showed that only patients receiving hydroxycarbamide had consistent whole blood nitrite concentration–time profiles (appendix p 3). Therefore, we did a subsequent pharmacokinetic analysis with respect to the time and dose of hydroxycarbamide intake instead of nitrite cream administration, using a population approach. We fitted a two-compartment model with first-order absorption and elimination to whole blood nitrite concentrations using first-order conditional estimation method by Phoenix NLME 1.2 (Certara USA Inc, St Louis, MO, USA), and we used an exponential model to describe the inter-individual variability and a mixed-ratio model to describe the intra-individual variability (appendix p 3). No such profile was recorded in the eight patients who were not receiving hydroxycarbamide (appendix p 3).

We recorded significant reductions in ulcer size and pain during treatment with topical sodium nitrite, generally with signs of dose dependence. Mean ulcer surface area at study entry was 5.97 cm² (SD 3.40; range 2.51–14.66 cm²) measured by digital photography and 7.50 cm² (SD 4.65; range 2.09–16.50 cm²) according to manual measurements, and decreased to 3.26 cm² (SD 2.72; range 0–9.39 cm², $p=0.0003$) on digital photography, and 3.67 cm² (SD 3.97; range 0–14.07 cm², $p<0.0001$) with manual measurements after treatment (figure 1A, 1B). 17 (94%) of 18 ulcers decreased in size by the end of the study, and 13 (72%) of 18 of ulcers had a greater than 25% decrease in surface area by digital photography (14 [78%] of 18 had this decrease according to manual measurements). From baseline to the end of the study, the study ulcer size decreased by a mean of 29.9% for cohort 1 (0.5% sodium nitrite), 7.7% for cohort 2 (1% sodium nitrite; one patient in this cohort progressed), 32.5% for cohort 3 (1.5% sodium nitrite), 69.7% for cohort 3a (1.8% sodium nitrite; one complete closure), and 88.3% for cohort 4 (2% sodium nitrite, two

complete closures; table 3). These reductions in wound size correlated with nitrite dose cohort (Spearman's correlation coefficient 0.7, $p=0.0012$; figure 1C). Hydroxycarbamide use and fetal haemoglobin level were not associated with ulcer healing ($p=0.96$ and $p=0.38$, respectively). Chronic transfusion therapy (in three of 18 patients) had a weak association with percent closure (ie, percentage reduction in wound surface area from baseline to the end of the study) at the end of the trial ($p=0.0126$).

Pain at the study ulcer decreased significantly during the study: the mean visual analogue scale score before application of sodium nitrite was 3.7 cm (SD 3.4; range 0–10) and decreased to 1.0 cm (1.2; 0–4.2) at the end of the study (figure 2A) ($p=0.0006$). Figure 2C shows the changes in visual analogue scale score according to cohort. In the nine patients who had more than one ulcer, visual analogue scale scores for non-treated ulcers did not change significantly during the trial (mean 3.4 cm [SD 3.3; range 0–9.6] at the beginning of the trial, and mean 2.1 cm [2.8; 0–6.4] at the completion of the trial; $p=0.38$) (figure 2B). Opioid analgesic use decreased over time, but this reduction was not significant (1379.2 mg morphine equivalents the week before treatment vs 804.8 mg the week after [$p=0.26$]; figure 2D).

Brief pain inventory scores for severity and interference improved significantly at the end of the study ($p=0.0048$ for severity and $p=0.0013$ for interference; figure 3A, 3B). Baseline brief pain inventory pain severity and interference scores correlated with C-reactive protein level at baseline (with severity score: Spearman's correlation coefficient 0.55 [$p=0.0182$], and with interference score: Spearman's correlation coefficient 0.47 [$p=0.0488$]), but not with ulcer age, initial size, hydroxycarbamide use, transfusions, and percentage of haemoglobin S or haemoglobin F (data not shown). The changes in pain severity, pain interference, and visual analogue scale scores did not correlate with sodium nitrite cohort, nor did they correlate with ulcer size reduction (data not shown).

Laser speckle contrast imaging showed a significant increase in regional blood flow after the first cream application (from 272.0 [SD 137.3] to 312.1 [150.6], $p=0.0002$) (figure 4B), and a smaller, non-significant increase after the second application (237.5 [SD 117.5] to 262.8 [137.4], $p=0.10$) (data not shown). This treatment effect of increased cutaneous peri-wound blood flow by laser speckle contrast imaging was corroborated by increased peri-wound skin temperature measured by infrared thermography (figure 4A) after the first dose (mean 34.2°C [SD 0.9] to 34.5°C [SD 0.7], $p=0.0119$) and after the second dose (34.1°C [SD 0.8] to 34.5°C [SD 0.8], $p=0.0008$; data not shown). The mean temperature in the wound bed itself increased significantly, from 32.8°C (SD 1.4) to 34.9°C (0.7) after the first cream application ($p<0.0001$), and from 33.0°C (SD 1.1) to 34.8°C (0.7) after the second ($p<0.0001$) and increased in parallel in the peri-wound region (Spearman's correlation coefficient 0.72, $p=0.0010$). The increase in skin peri-wound temperature and regional blood flow did not correlate with sodium nitrite dose cohort (figure 4C, 4D).

Discussion

Our findings from this small phase 1–2 safety and tolerability dose-finding study show that sodium nitrite 2% cream is well tolerated, with preliminary indications of efficacy, and is

suitable for further clinical trials. Evidence of efficacy is not usually expected from an early phase trial. Surprisingly, however, several of the prospectively specified secondary outcome efficacy variables showed statistical significance. This encouraging efficacy signal in an early phase trial should be interpreted with caution because all early phase trials are mainly designed to answer questions of safety and tolerability with small numbers of patients, and efficacy is not as reliable as it is in phase 3 trials, which are designed specifically and primarily to determine efficacy (panel).

Our pharmacokinetic analysis showed that topical sodium nitrite was minimally absorbed, which is consistent with the absence of systemic effects. Whole blood nitrite concentrations increased after ingestion of hydroxycarbamide and not after sodium nitrite application, which confirms previous reports of hydroxycarbamide as a donor of nitric oxide that is converted to nitrite.^{27,28} We had anticipated the possibility of clinically significant systemic absorption as a possible issue, but in reality this turned out to be negligible and clinically insignificant. We detected asymptomatic events of diastolic blood pressure below a predetermined limit of 50 mm Hg in some patients both before and after topical sodium nitrite application, with no association with plasma levels of nitrite and nitrate, all of which suggest that blood pressure changes are not a side-effect of these concentrations of topical sodium nitrite. Although our statistical power is too low to detect small changes in blood pressure related to plasma levels of nitrite or nitrate, clinically significant systemic blood pressure changes are not expected with our poorly absorbed topical doses of sodium nitrite that, even if fully absorbed, would provide only half of the dose amounts reported to cause systemic effects when administered intravenously.^{11,29} Patients with sickle cell disease have lower blood pressure than do healthy African-American controls,³⁰ and patients with sickle cell disease and leg ulcers have an even lower diastolic blood pressure than do those without leg ulcers.¹ In a post-hoc analysis to establish the frequency of diastolic blood pressure lower than 50 mm Hg in an existing database of blood pressure measurements from adults with sickle cell disease at the same clinical site, for the purposes of comparison, diastolic blood pressures were not significantly lower in 18 patients with sickle cell disease and leg ulcers treated with sodium nitrite than in 15 adults with sickle cell disease and leg ulcers followed in a separate NIH natural history protocol (ClinicalTrials.gov identifier NCT00081523) using standard of care treatments. In fact, during 12 days of blood pressure monitoring for the two sets of patients, only 30 (7%) of the 403 diastolic blood pressure measurements in the nitrite group fell below 50 mm Hg, compared with 11 (13%) of the 82 blood pressure measurements in the sickle cell disease natural history group ($p=0.005$). Occurrences of low diastolic blood pressure seem to be a previously unappreciated characteristic of adults with sickle cell disease and leg ulcers, which might warrant additional investigation, but do not seem to be induced by topical sodium nitrite.

Our results suggest that topical sodium nitrite has a dose-dependent effect on wound healing. We recorded a rapid decrease in wound size with complete closure of three ulcers treated with the highest concentrations of sodium nitrite cream, and a mean reduction in ulcer size that ranged from 30% at the lowest concentration to 88% with the highest. This apparent dose-response association between topical sodium nitrite concentration and the rate of healing helps to mitigate the acknowledged potential confounding effects of bed rest during the patients' 4.5-day stay in hospital and consistent wound care in promoting wound

healing during the protocol. This preliminary indication of therapeutic effect is further supported by the decrease in pain rating only at the ulcers treated with topical sodium nitrite and not at ulcers treated only with standard of care, although this subjective finding is subject to placebo effect.

The apparently increased rate of wound healing in this initial open-label dose-finding study is encouraging because these 18 patients had chronic wounds that were established for 10–300 months before the study, and wounds in patients with sickle cell anaemia have previously been reported to often heal very slowly, over periods of months to years.³¹ Improvement in wound size after 4 weeks has been shown to be predictive of eventual healing in chronic wounds in the general population.³² These preliminary efficacy data should be interpreted with caution, but the results encourage the development of a randomised, placebo-controlled phase 2 clinical trial.

The decrease in ulcer pain in this trial is intriguing. Pain at the ulcer site is a characteristic of sickle cell disease ulcers, which differentiates them from typical venous ulcer and contributes substantially to the morbidity of sickle cell leg ulcers,³³ affecting patients' quality of life, ability to work, mood, and sleep pattern.^{34,35} Affected patients often need chronic systemic opioid analgesics for pain, as was the case in our patient population. The improvement in pain might be related to improved regional blood flow and reduced ischaemia, or to decreased inflammation caused by the known bacteriostatic effects of nitrite, or both these effects. Of great interest is the finding that the decrease in pain was not related to the reduction in ulcer size, but occurred independently of it, even in patients who did not experience notable healing. Our results add to existing data from animals and human beings that suggest a role for nitric oxide and nitric oxide synthase in pain signalling. In future topical sodium nitrite trials, it will be worthwhile to examine whether our observed nonsignificant reduction in opioid analgesia use might be significant in a larger trial with greater statistical power.

The increases in both laser speckle contrast imaging and peri-wound and wound skin temperature after application of sodium nitrite cream provide evidence of a quantitative physiological response and serve as proof of principle that topical nitrite penetrates the wound and modifies local vascular physiology within minutes of its application. Nitrite acidified by ascorbic acid has been used as a nitric oxide generator system, probably providing bursts of nitric oxide over short periods by a disproportionation reaction that can cause tissue and cell death, DNA damage, and changed mitochondrial function.^{15,36,37} Our results support our hypothesis that acidification of nitrite is not needed to promote wound healing. The non-acidified preparation used in this study is stable, and hypothetically acts as a nitric oxide prodrug specifically after slow absorption into ischaemic wounds. The established property of nitrite to deliver nitric oxide targeted to ischaemic tissue provides an attractive rationale for its mechanism of action in sickle cell leg ulcers.

The increase in regional blood flow recorded after cream application supports the hypothesis that nitric oxide functions as a local vasodilator and causes beneficial improvements in microcirculation. The physiological and clinical responses to topical sodium nitrite reported in this open-label dose-finding trial, coupled with its apparent safety and tolerability,

provide encouragement to develop placebo-controlled phase 2 trials in patients with sickle cell disease who have non-healing leg ulcers. In the general US population, the cost for medical care of chronic wounds is estimated to be more than US\$25 billion.³⁸ Therefore, it is thought-provoking to consider the potential effect of extending future trials of topical sodium nitrite for chronic wounds in these other populations, such as patients with diabetes, who share similar problems with those affected by sickle cell anaemia including low nitric oxide bioavailability, vasculopathy, poor cutaneous blood flow, and skin ulceration with slow healing.

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Panel: Research in context**Systematic review**

Rigorous clinical trial data regarding the treatment of leg ulcers in patients with sickle cell anaemia are sparse. We searched PubMed on Oct 16, 2014, with the terms “sickle and ulcer,” and restricted our search to article type “clinical trial.” We did not use any language restrictions. In the past 30 years, only six prospectively designed trials of interventions for leg ulcers in patients with sickle cell disease have been published: two from the USA^{7,8} and four from Jamaica.^{3–6} Three trials investigated systemic interventions and three a topical treatment, and together involved a total of 198 participants and 250 ulcers. None of these have yet led to follow-up trials.

Interpretation

Our clinical trial tests a prodrug for production of nitric oxide, a vasoregulatory molecule shown to be depleted in sickle cell disease. Nitrite, which is normally present in low concentrations in plasma and tissue, has rarely been tested as a topical agent. Our phase 1 trial shows a concentration of sodium nitrite in topical cream that is safe and well tolerated in patients with sickle cell disease. Unusually for a small phase 1 clinical trial, the results from this trial also show an immediate physiological response and short-term clinical response to application of topical sodium nitrite. These results are encouraging and support the development of more advanced clinical trials for treatment of ulcers.

Figure 1. Changes in leg ulcer surface area before and after application of sodium nitrite cream (A) Wound size measured by digital planimetry. (B) Wound sizes measured manually (longest length and widest width). (C) Changes in wound size according to sodium nitrite concentration. Arrows in (A) and (B) represent first application of cream. Plotted points or bars are mean values and error bars are standard error of the mean. *p=0.0058 compared with first application. †p<0.0001. ‡p=0.0001. §p=0.0008.

Figure 2. Changes in leg ulcer pain during the topical sodium nitrite treatment period

Patients were asked to score pain from the ulcer treated with topical sodium nitrite and any additional ulcers not treated with topical sodium nitrite. (A) Changes in VAS pain scores for the ulcer treated with topical sodium nitrite at all doses, from screening to the end of the study. (B) Changes in VAS score for additional ulcers not treated with topical sodium nitrite (n=9). (C) Changes in VAS scores for the study ulcer treated with sodium nitrite cream at at different concentrations. (D) Mean weekly use of opioid analgesics (total morphine equivalents) by all patients at the beginning of treatment compared with the end of treatment. Error bars are standard deviations. VAS=visual analogue scale.

Figure 3. Changes in the brief pain inventory scores during treatment of leg ulcers with topical sodium nitrite

Significant reductions were recorded in (A) the pain severity subscore and (B) the pain interference subscore averaged from all treated patients after eight treatments over 4 weeks (n=18, paired *t* test). (C, D) Changes in scores according to sodium nitrite concentration. Bars indicate mean values and error bars are standard error of the mean.

Figure 4. Cutaneous imaging before and after first application of topical sodium nitrite cream Changes after the first application of sodium nitrite cream for all 18 trial participants in (A) cutaneous peri-wound temperature by infrared thermography and (B) cutaneous peri-wound blood flow by laser speckle contrast imaging. The infrared thermography and laser speckle contrast measurement of cutaneous blood flow data are calculated by spatial averaging of the region of interest followed by time averaging over the 5 min before application of sodium nitrite cream and the final 5 min of the imaging 30 min after the application. (C, D) Subgroup analysis results categorised according to the concentration of sodium nitrite cream.

Table 1

Baseline characteristics

	Value
Sex	
Men	8 (44%)
Women	10 (56%)
Age (years)	39 (12); range 20–59
Ulcer size (cm ²)	
Measured manually	7.50 (4.65); range 2.09–16.50
Measured digitally	5.97 (3.40); range 2.51–14.66
Number of ulcers	1.5 (1–3.25)
Age of ulcers (months)	10 (3.75–18.5)
Hydroxyurea therapy	9 (50%)
Chronic transfusion	3 (17%)
Taking daily opioid	16 (89%)
Current anti-coagulation therapy	6 (33%)
Hospital admission in past 12 months for vaso-occlusive crisis	9 (50%)
History of priapism (men only)	5/8 (63%)
History of pulmonary embolism or deep vein thrombosis	8 (44%)
Mean arterial pressure (mm Hg)	81.1 (8.2)
Systolic blood pressure (mm Hg)	118.6 (13.0)
Diastolic blood pressure (mm Hg)	64.0 (7.3)
Pulse oximeter on room air	96.8 (1.1)
Previous ulcer treatments per patient	5 (7–9)
Number of patients who had each previous treatment	
Surgical/sharp debridement	18 (100%)
Hyperbaric chamber	7 (39%)
Skin graft	6 (33%)
MIST	4 (22%)
Oral/parenteral antibiotics	11 (61%)

Data are n (%), mean (SD), or median (IQR), unless otherwise indicated.

Table 2

Adverse events probably or possibly related to study cream, by dose cohort

	Patients (n)	Cohort (sodium nitrite concentration, %)	Outcome Grade
Pruritus	2	1 (0.5%), 4 (2.0%)	Resolved 1
Burning	3	3 (1.5%), 3a (1.8%), and 4 (2.0%)	Resolved 1
Hypoxia (91%)	1	3 (1.5%)	Resolved 1
Diastolic blood pressure <50 mm Hg after application only	4	One patient in cohort 2 (1.0%), two in 3a (1.8%), and one in cohort 4 (2.0%)	Resolved 1

Hypotension describes episodes of decreases in diastolic blood pressure below a predefined threshold of 50 mm Hg. Mean arterial blood pressure and systolic blood pressure did not change significantly before and after application of the study drug. One patient in cohort 4 had a significant change in diastolic blood pressure (diastolic blood pressure mean of 58 mm Hg fell to a mean of 51 mm Hg after 24 h). Appendix p 5 provides additional details.

Table 3

Percentage reduction in ulcer surface area from baseline in each cohort

	Sodium nitrite concentration	Patients (n)	Reduction at week 3	Reduction at end of study
Cohort 1	0.5%	3	31.1%	29.9%
Cohort 2	1.0%	3	17.4%	77% (one ulcer progressed)
Cohort 3	1.5 %	5	17.3%	32.5%
Cohort 3a	1.8%	4	56.1%	69.7% (one ulcer healed)
Cohort 4	2.0%	3	54.9%	88.3% (two ulcers healed)

Measurements of ulcer surface area were done by digital planimetry. Reduction in wound size correlates with nitrite dose cohort (Spearman's correlation coefficient 0.7; p=0.0012).