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A randomized phase II trial of Arginine Butyrate with standard local therapy in refractory sickle cell leg ulcers

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Summary

Sickle cell leg ulcers are often debilitating, refractory to healing, and prone to recurrence. Healing of leg ulcers was incidentally observed during dose-ranging trials of Arginine Butyrate in beta haemoglobinopathies. Here, a controlled Phase II trial was performed in sickle cell patients who had lower extremity ulcers refractory to standard care for at least 6 months. Patients were randomized to receive standard local care alone (Control Arm) or standard care with Arginine Butyrate administered 5 d/week (Treatment Arm), for 12 weeks. Ulcers were photographed weekly, traced, and ulcer areas were calculated by computerized planimetry and compared between the two study arms. Twenty-seven study courses were evaluated. Control Arm subjects had 25 ulcers with a mean area of 25·7 cm^2 initially and 23·2 cm^2 after 12 weeks; 2/25 (8%) healed completely. Treatment Arm subjects had 37 ulcers with a mean area of 50·6 cm^2 initially and 28·3 cm^2 at 12 weeks; 11/37 of these (30%) healed completely. After 3 months, proportions of ulcers which healed were 6/25 (24%) and 29/37 (78%), in the Control and Treatment Arms respectively ($P < 0{\cdot}001$). These findings strongly suggest that Arginine Butyrate merits further evaluation for the treatment of refractory sickle cell leg ulcers in larger trials.

Keywords: randomized controlled trial, arginine butyrate, wound healing, leg ulcer, sickle cell anaemia.

Lower extremity ulcers in patients with sickle cell disease are reported in 25–63% of adult sickle cell patients, are often refractory to treatment, and are prone to recurrence (Walshe & Milner, 1967; Gueri & Serjeant, 1970; Karayalcin *et al*, 1975; Oluwasanmi *et al*, 1980; Morgan, 1982; Ademiluyi *et al*, 1988; Koshy *et al*, 1989; Eckman, 1996; Perrine *et al*, 2010). Healing of lower extremity ulcers in sickle cell disease occurs 20-times more slowly than healing of other types of refractory vascular lower extremity wounds (Gueri & Serjeant, 1970). The average duration of leg ulcers in sickle cell patients is >3 years in the United States and >9 years in patients in the Caribbean (Walshe & Milner, 1967; Gueri & Serjeant, 1970; Serjeant *et al*, 1970; Karayalcin *et al*, 1975; Oluwasanmi *et al*, 1980; Morgan, 1982; Ademiluyi *et al*, 1988; Koshy *et al*, 1989; Eckman, 1996; Perrine *et al*, 2010). The current treatment for sickle cell leg ulcers is generally supportive, including protection from trauma, antibiotics for secondary infection, wet-to-dry dressings, surgical debridement, grafting where possible, and use of an Unna boot in some. A number of topical agents have been studied. Wethers *et al* (1994) demonstrated that an RGD matrix accelerated healing and closure of chronic sickle cell leg ulcers. Despite this, there is no currently US Food and Drug Administration (FDA)-approved treatment specific for refractory sickle cell leg ulcers, and patients suffer debility, pain, and a decline in quality of life. Recent evidence indicates that development of leg ulcers is related to the degree of haemolysis and associated with serious morbidity, including pulmonary hypertension (Steinberg, 2005).

During Phase I/II dosing trials of Arginine Butyrate to stimulate production of fetal haemoglobin (HbF) in patients with haemoglobinopathies, complete healing of refractory leg ulcers was incidentally observed in seven patients with sickle cell disease or beta thalassaemia (Perrine *et al*, 1993; Sutton *et al*, 1994; Atweh *et al*, 1999). Retrospectively, it was noted that healing of these long-standing leg ulcers began *prior* to any significant changes in HbF or total haemoglobin during the

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early Butyrate trials. Healing appeared to occur more rapidly with weekly Arginine Butyrate therapy, than when the treatment was reduced to 1 or 2 weeks per month in a 'pulsed' regimen. In contrast, the pulsed, intermittent regimen was more effective in inducing HbF (Atweh *et al*, 1999). Both components of Arginine Butyrate (L-arginine and butyric acid) have been considered to have healing actions in other types of refractory wounds (Agarwal & Schimmel, 1989; Barbul *et al*, 1990; Dobson *et al*, 1990; Roediger, 1990; Regan *et al*, 1991, 1993; Holt *et al*, 1992; Scheppach *et al*, 1992; Sutton *et al*, 1994; Chapman *et al*, 1995; Vernia *et al*, 1995; Eckman, 1996; Shearer *et al*, 1997; Ulland *et al*, 1997; Roberts *et al*, 1998).

Because of these intriguing incidental observations and the reported actions of the individual drug components on wound healing, a Phase II trial was undertaken to prospectively evaluate whether Arginine Butyrate infusions, in addition to standard local care, could increase the proportion of refractory sickle cell leg ulcers which heal partially or completely compared to standard local care alone. A higher rate of closure and healing was observed in subjects with refractory ulcers who received (previously prescribed) standard local care and Arginine Butyrate therapy for 3 months, compared to the patients' standard local care regimen given alone. Further, long-standing refractory leg ulcers of many years' duration healed when Arginine Butyrate treatment was extended beyond the brief study evaluation period.

Materials and methods

Patients and study protocol

Eligibility criteria required a subject to be aged >18 years, have sickle cell anaemia or sickle beta thalassaemia, and the presence of one or more leg ulcers which had been refractory to healing with National Institutes of Health (NIH)-defined standard local care, consisting of twice daily cleaning and wet-to-dry dressing changes, for at least 6 months. The use of an Unna boot was considered best therapy for one Control Arm subject, and this was continued. Patients were also eligible to participate if an ulcer had recurred and not healed with at least 3 months of standard care. Many of the enrolled subjects had suffered from refractory ulcers for many years and had tried multiple therapies. Exclusion criteria included renal or hepatic compromise or current chronic transfusion therapy. All ulcers were examined for infections weekly and treated with antibiotics; surgical debridement was performed when indicated in a Control Arm subject. Patients were randomly assigned to study arms, following a table of random numbers prepared by a blinded statistician. Randomized subjects received either their prescribed standard local care alone (Control Arm) or standard local care and Arginine Butyrate for 5 d/week for 12 weeks (Treatment Arm). If healing was objectively documented during the first 12-week treatment cycle, as determined by a decrease in measured ulcer area by at least 25% of the baseline area, Arginine Butyrate could be continued for two additional courses of 8-week cycles, although the responses to the extended treatment were not analysed as study endpoints. Control Arm subjects could cross-over to the Treatment Arm if their ulcer did *not* heal after 12 weeks of closely monitored and supervised standard local care. Their remaining ulcers were then assessed on the Treatment Arm for 12 weeks. During the course of the study, three patients randomized initially on the Control Arm elected to cross-over to the Treatment Arm after completing 12 weeks as a Control subject, when no significant healing of their refractory ulcers had occurred; healing rates in 11 ulcers therefore were evaluated on both Control and Treatment Arms in these subjects.

Participants in the Treatment Arm received their prescribed best standard local care with the addition of Arginine Butyrate at a total daily dose of 500 mg/kg/dose given 5 d/week. The drug formulation consisted of 5% butyric acid (50 g/l) and 7·5% L-arginine (75 g/l). The Arginine Butyrate was administered by intravenous infusion through a port-a-cath or peripheral pass-port in one subject, generally over 6–8 h/d. The administration rate for Arginine Butyrate did not exceed 85 mg/kg/h and those patients infused with rates >60 mg/kg/h were pre-medicated with acetaminophen or ibuprofen and an anti-emetic to prevent headache or nausea. Patients randomized to the intravenous therapy were encouraged and assisted to ambulate frequently during the 6-h infusion.

The study (ClinicalTrials.gov identifier NCT00004412) was approved by the Institutional Review Boards of all participating institutions, and all subjects gave informed consent.

Assessment of ulcer healing and statistical analyses

During the weekly clinical visits, the ulcer was traced on acetate film and photographed. All ulcer areas were then calculated by computerized planimetry, using the IMAGEJ software (NIH, Bethesda, MD, USA) at the central site, separately from personnel who performed the tracings and photography. Partial healing was defined as a decrease in ulcer area by at least 25% of the baseline ulcer area; complete healing was defined as complete closure of the ulcer (to an area of 0 cm^2).

Statistical tests were performed by two-sided methods as applicable, and the level of significance used was $P < 0{\cdot}05$. Descriptive statistics were calculated and the percentage of ulcers healing partially and completely, and the percentage of patients undergoing partial and complete healing, were compared statistically only at 12 weeks on each study arm, or at the time of complete ulcer closure, if it occurred earlier than 12 weeks. The Fisher exact test was used to compare the percentage of patients whose ulcer areas were reduced from their initial ulcer area by 25% or more. The Wilcoxon test, with normal approximation and a continuity correction of 0·5, was used to compare the distribution of the areas. All analyses were performed using the Statistical Analysis System (SAS)®, version 8.2 (SAS Institute Inc., Cary, NC, USA).

Table I. Characteristics of the study subjects at baseline.

Parameter	SLC	AB + SLC
No. of patients studied	12	14*
No. of treatment courses	12	15†
Age (years)‡	34·7 (20–57)	36·6 (21–60)
% Female	58	57
Mean baseline ulcer area (cm^2) ± SE	25·7 ± 8·1	50·6 ± 13·9
Total number ulcers	25	37
No. ulcers per patient	2·1	3·5
Ulcer location in lower extremities (%)		
Lateral	46	28
Medial	39	52
Other	15	20
Total haemoglobin (g/l) ± SD	77 ± 20	77 ± 10
Fetal haemoglobin (%) ± SD	11·6 ± 12·0	9·5 ± 9·7
Genotype		
HbSS	11	13
HbS/β^+-thalassaemia	1	1

No., number; SLC, standard local care; AB, arginine butyrate; SE: standard error; SD: standard deviation.
*Includes three cross-over patients.
†Includes a patient who underwent two treatment courses after a recurrence.
‡Range in age is shown in parentheses.

Results

Prospective clinical trial

The baseline characteristics of the study subjects are shown in Table I. A total of 23 different patients enrolled and entered the study, 11 patients were initially randomized to the Treatment Arm and 12 patients were randomized to the Control Arm. In addition, three study courses were analysed in three subjects (with 11 ulcers) who crossed-over from Control to Treatment Arms. The genotypes of the patients enrolled included HbSS and two subjects with HbS/β^+-thalassaemia. There were no significant differences in age, gender, genotype, total haemoglobin, or HbF at baseline between the subjects randomized to these groups. Only one subject received concommittant hydroxycarbamide (in the Control Arm). The distribution between location laterally or medially of the lower extremity ulcers did not differ significantly between the two groups. In the Treatment group, 28% of ulcers were located laterally and 52% were medially located on the lower extremities, with 20% located on the foot or extending circumferentially around the entire lower extremities. The Control group had 46% located laterally and 39% medially, with 15% in other locations.

There were 25 refractory leg ulcers present in the randomized Control Arm subjects and 37 leg ulcers in the Treatment Arm subjects. In the Control group, three *new* ulcers developed *during* the course of the study; these are not included in the analysis, as they were not present for at least 6 months prior to beginning the study. The mean initial (baseline) ulcer area in the Treatment group (50·6 cm^2) was nearly twice that of the Control group subjects (25·7 cm^2), although the difference was not significant in this small sample size ($P = 0{\cdot}823$). After treatment for 12 weeks, the mean ulcer area was reduced to 23·2 cm^2 in the Control Arm and was 28·3 cm^2 in the Treatment Arm. These differences in mean ulcer area from baseline to week 12 between the two randomized Arms, Treatment and Control, were significant, $P = 0{\cdot}028$.

Complete healing (closure) occurred in 30% (11/37) of ulcers in the Treatment group and in 8% (2/25) of ulcers in the Control Arm subjects within 12 weeks of study; the difference in closure rates approached significance ($P = 0{\cdot}056$). Some healing, as defined by partial closure with a decrease in ulcer area of at least 25% of initial baseline area (but not complete closure) was observed in 78% (29/37) of ulcers on the Treatment Arm compared with 24% (6/25) of ulcers on the Control Arm ($P < 0{\cdot}001$). However, most patients had more than one ulcer at study entry and the differences between the proportion of patients with healed ulcers in the two study Arms did not reach significance in this small group: 10/15 (67%) on the Treatment Arm and 4/12 (33%) on the Control Arm had partial healing and one patient on each arm had complete healing at 12 weeks. Many of the partially healed ulcers closed completely with extended arginine butyrate therapy (Figs 1 and 2). Overall, 75% of Treatment Arm subjects demonstrated some significant ulcer healing.

There was a wide range in initial ulcer area in the subjects, from >300 to 1 cm^2. As smaller ulcers had healed more rapidly in other studies, the percentage changes in ulcer area were also

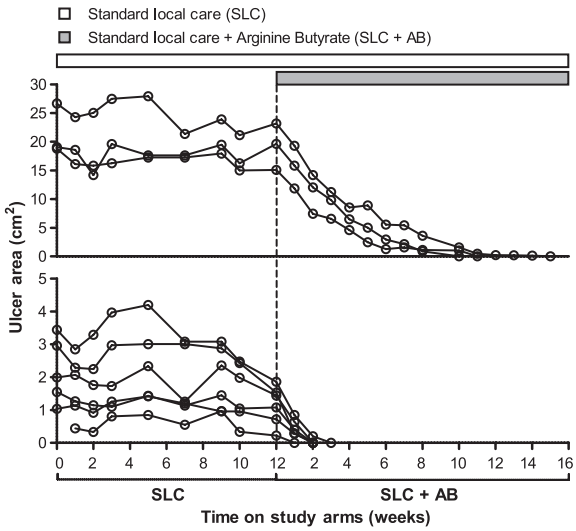

Fig 1. Rates of ulcer healing in one participant with nine ulcers treated with standard local care (designated by the light horizontal bar above the graph) and Arginine Butyrate (AB) with standard local care (shown by the dark horizontal bar above the graph). The institution of AB is designated by the dotted vertical line. This patient had nine ulcers of long-standing duration, which eventually completely healed with Arginine Butyrate infusions, as shown by the decrease in ulcer area. The time on the two study arms is shown.

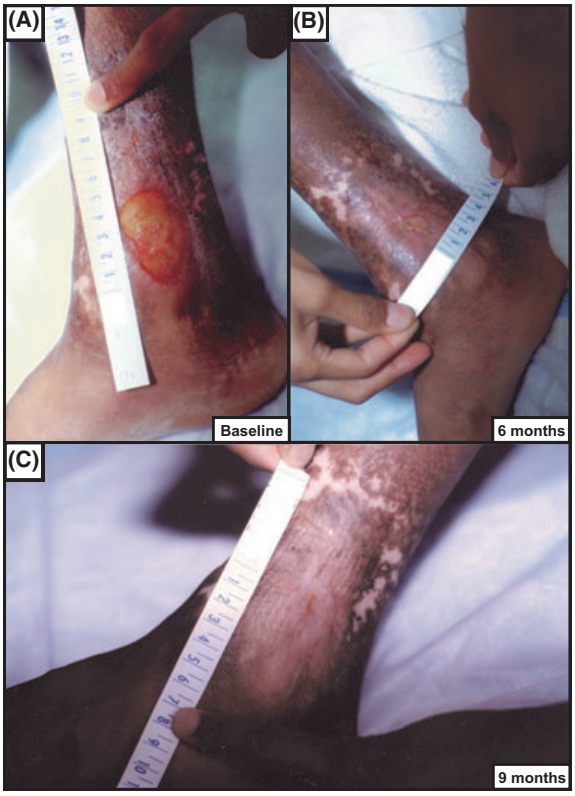

Fig 2. Photograph of representative ulcers at study entry, with ulceration above the right medial malleolus (panel A), after 24 weeks of Arginine Butyrate therapy (panel B), and after 41 weeks of Arginine Butyrate therapy (panel C) in a patient who had previously suffered refractory and recurrent lower extremity ulcers for 10 years. Panel (A) shows the patient's ulcer on the right medial ankle at baseline (13 cm^2). Panel (B) shows the same ulcer 6 months (24 weeks) after beginning study protocol (0·21 cm^2). Although this ulcer completely healed at 25 weeks, the patient had other ulcers and continued on the study protocol for a total of 47 weeks. Panel (C) shows sustained healing of this ulcer after 9 months (41 weeks).

compared according to baseline ulcer area. Addition of the Arginine Butyrate treatment resulted in healing of both large (>40 cm^2) and smaller (<10 or 10–40 cm^2) leg ulcers, at similar rates of closure (Fig 3). As in prior trials, smaller ulcers (<10 cm^2) completely healed more readily with Arginine Butyrate therapy within the study time frame than did larger ulcers. However, the mean decrease in ulcer area was nearly four times greater for the Treatment Arm in the 10–40 cm^2 category (53% vs. 12% closure) and nearly twice as many ulcers in the Treatment Arm healed in the larger >40 cm^2 group (43% vs. 23% in the Control arm), as shown in Fig 3B.

Continuation phase

The courses of two subjects who were treated as cross-overs (one is shown in Fig 1), first on the Control Arm and then the Treatment Arm, were particularly striking. In one cross-over subject treated first as a Control Arm subject in hospital (with an ulcer >40 cm^2), no healing occurred despite rigorous local care. With institution of Arginine Butyrate therapy, the ulcer healed completely. A year later this subject developed a new ulcer, was randomized and treated again on the Control Arm. She did not respond significantly to standard local care provided in hospital, but had complete closure of the new ulcer when Arginine Butyrate was resumed. Recurrence of an ulcer was later reported more than a year following the study in a second subject who had complete ulcer healing on the Treatment Arm.

Figure 1 shows rates of ulcer healing (closure) in a subject with nine non-healing ulcers and in whom amputation had been recommended. Eight of these ulcers were chronic, and this subject was randomized and crossed-over from the Control to the Treatment Arm. The ulcers did not change significantly during the Control Arm study period of closely supervised standard local care alone, particularly in the three larger ulcers shown in the top panel of Fig 1 (left panel relative to the dotted line). Ulcer healing in the subject is illustrated by the decrease in ulcer area when Arginine Butyrate therapy was begun (as shown to the right of the vertical dotted line). A sharp decline in the areas for all the subject's ulcers was observed after Arginine Butyrate therapy was begun.

Representative photographs of the long-standing refractory ulcers studied on this trial are shown in Fig 2, which illustrates ulcer healing in a representative subject who had had multiple recurrent and refractory leg ulcers for >10 years. Treatment with Arginine Butyrate in addition to standard local care resulted in partial (>90%) healing (closure) of these refractory leg ulcers at 12 weeks, at the time of study evaluation, although complete closure occurred following the formal study period. The most extensive leg ulcers were observed in a subject with sickle cell disease who also had diabetes and suffered from constant refractory leg ulcers for >30 years, with ulcers extending circumferentially (>300 cm^2) around both lower extremities, with severe pain and chronic disability. The ulcers partially healed at 12 weeks and eventually healed completely following the formal period of the study evaluation.

No serious adverse events were reported to be directly related to the study drug. Drug-related adverse events related to Arginine Butyrate included headache and nausea, which were usually preventable or controlled with anti-emetics and acetaminophen or ibuprofen therapy given prior to and during the infusions. Port-a-cath infections did occur in two subjects who had ports for their standard sickle cell care, prior to Arginine Butyrate treatment on this study. The incidence of port infection was not different from rates of infection in implanted port devices generally reported in sickle cell patients (Phillips *et al*, 1988; McCready *et al*, 1996; Jeng *et al*, 2002).

As patients treated with Arginine Butyrate received high doses (750 mg/kg/dose) of L-arginine within the 6-h infusions of Arginine Butyrate, and L-arginine is a precursor for the vasodilator nitric oxide (NO), vascular flow studies designed to detect changes in skin flow rates were performed in four

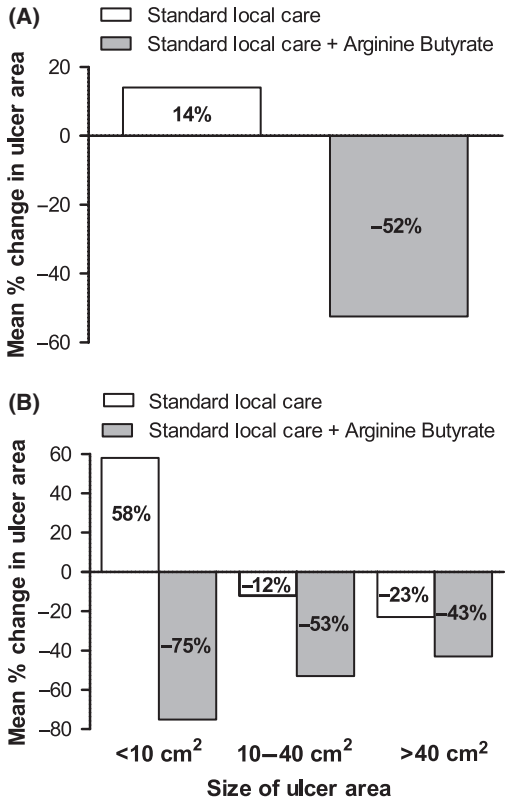

Fig 3. The percentage changes in ulcer area after 12 weeks on study are shown graphically. Ulcers in patients treated with standard local care (SLC) alone (Control Arm) are shown by the white bars; ulcers in patients treated with SLC + Arginine Butyrate are shown by the darker shaded bars. (Panel A) The mean percentage change in area relative to baseline area after 12 weeks of treatment with local care with and without Arginine Butyrate treatment is shown. (Panel B) Mean ulcer healing is shown by initial ulcer size at baseline, for the Control and Treatment groups, illustrating that ulcers healed with Arginine Butyrate regardless of initial size. 78% of ulcers healed partially and 30% healed completely in the Treatment Arm in 12 weeks, compared to 24% partially and 8% completely healed in the Control Arm, who received local care alone.

patients prior to and after 3 h of Butyrate infusions. These studies did not reveal significant changes in acute vascular flow patterns during 3 h of Arginine Butyrate infusions, albeit a brief time duration.

An intensive regimen of Arginine Butyrate was previously found to be less effective than an intermittent or pulse regimen of Butyrate in stimulating fetal haemoglobin (HbF) production in sickle cell disease subjects, and was suspected to be related to the anti-proliferative effects of Butyrate on erythropoiesis (Atweh *et al*, 1999). In the Butyrate cycles observed here, an increase in HbF levels compared to baseline levels was observed in 10/15 treated patients during the therapy, with eight patients achieving peak HbF levels of 17%, but changes often required 2 months of treatment and declined from the peak response with continued treatment. However, ulcer healing was noted to begin within 3 weeks of treatment and *prior* to any changes in HbF levels, and ulcer healing occurred in five patients who did *not* exhibit any increases in HbF. These changes did not appear temporally related to the ulcer healing. These observations dissociate the effects of HbF from the ulcer healing.

Discussion

An incidental observation noted in previous dose-ranging studies in which Arginine Butyrate was administered to attempt to stimulate HbF production was coincident healing of chronic lower extremity ulcers, of 2–6 years duration, in both sickle cell and beta thalassaemia intermedia subjects. In those prior observations, complete ulcer healing occurred in five of seven patients who had refractory leg ulcers, and substantial healing (>80% reduction in ulcer area) occurred in the remaining two patients within 8–12 weeks of initiating Butyrate treatment (Perrine *et al*, 1993; Atweh *et al*, 1999). Similar case reports of coincident chronic sickle cell ulcer healing with Arginine Butyrate have been reported. These observations prompted this trial to prospectively evaluate the potential activity of Arginine Butyrate on wound healing of refractory leg ulcers in sickle cell patients who had not responded to other standard measures. As Arginine Butyrate requires administration through an indwelling vascular device, the study was not offered to subjects who had not had a reasonable trial of local therapy alone. In this controlled trial in patients with long-standing sickle cell-related ulcers, treatment with Arginine Butyrate in addition to the described standard local ulcer therapy produced an overall 52% reduction in average ulcer area and a 30% complete closure rate within a 12-week period in a highly refractory group of subjects, while only 8% healed completed in the control group.

Both ulcer size and duration are thought to be important determinants of healing potential (Serjeant & Howard, 1977). Small ulcers tend to heal more rapidly, as do acute lesions (Caputi *et al*, 1984). Although not included in the statistical analysis, regression of ulcer area continued throughout prolonged treatment for those patients who elected to extend treatment beyond the study endpoint. Ulcers with areas even >300 cm^2 which had been present and debilitating for more than 10 years healed with continued therapy over many months. Standard therapy alone was less effective in both large and small ulcers. With extended treatment beyond the formal study period, 16/37 (46%) of the refractory ulcers in our Treatment Arm subjects completely healed with Arginine Butyrate treatment.

The Arginine Butyrate regimen was reasonably well-tolerated. The most common treatment-associated side effects of nausea and headache responded to premedication with mild analgesics or anti-emetics. Nonetheless, intravenous administration of Arginine Butyrate requires indwelling vascular devices, which are associated with small risks of infection and thrombosis (Phillips *et al*, 1988; McCready *et al*, 1996; Jeng *et al*, 2002). The dose of Arginine Butyrate used was

empirically based on prior trials of the drug for induction of fetal globin expression, and may have provided more drug than necessary for healing leg ulcers.

All subjects received increased attention to local wound care and regular surveillance for wound infection during this study. Super-infection inhibited healing of the ulcers significantly, often resulting in rapid enlargement of ulcers in both Control and Treatment study Arms. In the subjects studied here, care was taken to evaluate changes in ulcer healing independent of potentially confounding differences in ambulation or bed rest. Most subjects were treated as out-patients and remained ambulatory during infusions. Patients were assisted with ambulation, if necessary, during the infusions. One subject was treated in hospital for both Control and Treatment Arms. Only one subject was not completely ambulatory; this subject had had refractory ulcers for more than 30 years and was unable to walk due to excruciating pain. This subject eventually had dramatic healing of circumferential ulcers around both lower extremities. However, some reduced mobility compared to the Control Arm cannot be ruled out and is a potential limitation of the study.

While significant differences in healing between Treatment Arms were observed and included large ulcers, this study was potentially biassed against the Treatment Arm, as the mean ulcer area was twice that of the Control Arm at baseline. Our observations suggest that more definitive or pivotal trials could probably test the activity of the study drug better if initial ulcer areas between Control and Treatment groups were more closely matched, perhaps by limiting the initial ulcer area to <25 cm^2, and if the study was continued for a duration longer than 3 months. Such provisions would allow a more reasonable comparison of the effects of Arginine Butyrate therapy on wound healing compared to local treatment measures alone. As Arginine Butyrate therapy requires administration through long vascular catheters or port devices, its practical potential benefit is most likely for patients who do not respond to topical measures and can attend an outpatient clinic for treatment. However, home administration of Arginine Butyrate therapy is an alternative, which has been used by some thalassaemia patients for many years.

The mechanisms underlying the pathophysiology of chronic ulcer formation in sickle cell disease are poorly understood. Potential roles of vaso-occlusion and venous incompetence in the genesis of leg ulcers are controversial. Emerging evidence indicates that leg ulceration is probably linked with hyperhaemolytic phenotypes of this disease (Steinberg, 2005; Kato *et al*, 2007). Other conditions with chronic haemolysis, such as β-thalassaemia, are also associated with refractory leg ulcers (Pascher & Keen, 1957; Pope & Hodgson, 1968; Taher *et al*, 2010). Recent studies to elucidate genetic modifiers have identified an association between single nucleotide polymorphism (SNPs) in or near the *KL* gene and the occurrence of leg ulcers (Nolan *et al*, 2006). Klotho, the protein product of the *KL* gene, promotes endothelial NO production, and polymorphic variants decrease NO bioavailabilty. Further elucidation of the pathology of the refractory leg ulcers in this population may help to determine targets of this therapeutic.

The mechanisms of the wound-healing activity observed with Arginine Butyrate are not clear, but both components of the drug formulation could theoretically provide benefit. Prior evidence suggests both L-arginine and butyric acid have activity in healing ulcers and wounds in other medical conditions. L-arginine alone has been tested as a supplement for chronic surgical wounds that do not heal and has been reported to promote wound healing in debilitated and elderly patients (Kirk *et al*, 1993; Shi *et al*, 2007). Some of the wound healing properties of arginine include stimulation of collagen production, prevention of restenosis after vascular injury, and improved immune function (Barbul *et al*, 1990; Regan *et al*, 1991, 1993; Holt *et al*, 1992; Shearer *et al*, 1997; Ulland *et al*, 1997; Roberts *et al*, 1998; Angele *et al*, 2002; Tong & Barbul, 2004; Loehe *et al*, 2007) (Ahrendt *et al*, 1994). In addition, L-arginine may increase nitric oxide production, as it is a substrate for nitric oxide synthase, and might thereby indirectly cause vasodilation and improved oxygen delivery to the lower extremities (Eckman, 1996; Schaffer *et al*, 1997; Faller, 1998) (Schaffer *et al*, 1997; Faller, 1998; Rizk *et al*, 2004). Excessive arginase activity is reported in refractory wounds. It may be interesting to attempt to determine if a topical formulation of the therapeutic could also facilitate wound healing, if a suitable non-irritating formulation could be developed for these typically painful ulcers.

Still, it is more likely that the Butyrate is the predominant active agent. A derivative of Butyrate (monobutyrin) has been reported to have angiogenic activity in some experimental systems (Dobson *et al*, 1990), and topical application of a short-chain fatty acid cocktail is reported to facilitate healing of intestinal ulcers (Agarwal & Schimmel, 1989; Roediger, 1990; Scheppach *et al*, 1992; Chapman *et al*, 1995; Vernia *et al*, 1995). Mechanisms of action of butyric acid contributing to wound healing may include the ability of Butyrate to stimulate platelet-derived growth factor (PDGF) production, which has an important function in wound healing (Tang *et al*, 1990). The role of unregulated production of inflammatory mediators, including transforming growth factor-β and tumour necrosis factor-α in non-healing wounds is well established, and Butyrate has been shown to block the production, or signalling activity, of these and other inflammatory cytokines in a variety of systems (Segain *et al*, 2000; Menzel *et al*, 2004; Diakos *et al*, 2006; Matsumoto *et al*, 2006; Wang *et al*, 2006; Eming *et al*, 2007; Park *et al*, 2007). Butyrate may also down-regulate matrix metalloproteinases (MMPs), as other reports have shown it to be capable of altering MMPs gene expression (Emenaker & Basson, 1998; Mort, 2005; Young *et al*, 2005). MMPs are proteolytic enzymes which destroy tissue and matrix proteins, healing growth factors (PDGF and vascular endothelial growth factor), and cytokines in the wound, and increased *MMP2* gene expression has been found in the wound fluid of refractory non-healing ulcers, but was not found in fluid from wounds which heal normally (Wysocki *et al*, 1993;

Saito *et al*, 2001). However, our current understanding of the role of Arginine Butyrate in inducing wound healing, and indeed wound healing in general, does not permit any conclusions at this point. Other studies would be required to elucidate the mechanism of action.

It was initially hypothesized that increases in HbF were responsible for the incidentally-observed wound healing, as elevated levels of HbF protect patients with sickle cell disease from many complications including leg ulceration (Steinberg, 2005). Leg ulcers are not observed in Indian and Saudi Arabian sickle cell disease patients who have high levels of HbF (Perrine *et al*, 1978; Steinberg, 2005). However, the Arginine Butyrate dosing regimen used in this study protocol is not optimal for inducing HbF production (Perrine *et al*, 1993; Brauer *et al*, 1995; Atweh *et al*, 1999). In addition, ulcer healing was observed to begin within 3 weeks of initiating Arginine Butyrate therapy, while HbF levels typically do not increase for 6–8 weeks in those patients where an effect on HbF was observed. This lack of temporal correlation with HbF induction and ulcer healing in several cases strongly suggest that the mechanisms of action of Arginine Butyrate on wound healing are independent of, and distinct from, the effect of the drug on HbF, and the more rapid onset of healing cannot be explained by changes in red cell mass.

Chronic leg ulcers in sickle cell disease have long been difficult to manage. The results of this randomized, controlled study, although small, provide evidence that Arginine Butyrate treatment can promote healing of long-standing refractory ulcers that have not responded to standard local care. Further extended trials of Arginine Butyrate in larger patient populations of refractory leg ulcers associated with haemolytic anaemia appear warranted.

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