Phase Change Material for Thermotherapy of Buruli Ulcer: A Prospective Observational Single Centre Proof-of-Principle Trial

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Abstract

Background: Buruli ulcer (BU) is an infection of the subcutaneous tissue leading to chronic necrotizing skin ulcers. The causative pathogen, Mycobacterium ulcerans, grows best at 30°C–33°C and not above 37°C. We explored the safety, tolerability and efficacy of phase change material (PCM), a novel heat application system for thermotherapy of BU.

Methodology/Principal Findings: In a prospective observational single centre proof-of-principle trial in Ayos/Cameroon, six laboratory reconfirmed patients with ulcerative Buruli lesions received 28–31 (ulcers ≤2 cm) or 50–55 (ulcers >2 cm) days of thermotherapy with the PCM sodium acetate trihydrate as heat application system. This PCM is widely used in commercial pocket heat pads, it is easy to apply, rechargeable in hot water, non-toxic and non-hazardous to the environment. All patients enrolled in the trial completed treatment. Being completely mobile during the well-tolerated heat application, acceptability of the PCM bandages was very high. In patients with smaller ulcers, wounds healed completely without further intervention. Patients with large defects had skin grafting after successful heat treatment. Heat treatment was not associated with marked increases in local inflammation or the development of ectopic lymphoid tissue. One and a half years after completion of treatment, all patients are relapse-free.

Conclusions/Significance: Our reusable PCM-based heat application device appears perfectly suited to treat BU in endemic countries with limited resources and infrastructure.

Trial Registration: Controlled-Trials.com ISRCTN88392614

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Introduction

Buruli ulcer (BU) is a chronic necrotizing disease of skin and soft tissue caused by Mycobacterium ulcerans [1]. The disease starts as a subcutaneous nodule, papule or plaque that eventually ulcerates and progresses over months to years. In BU lesions, clumps of extra-cellular acid-fast organisms surrounded by areas of necrosis are found primarily in subcutaneous fat tissue [2]. M. ulcerans produces a macrolide toxin, mycolactone, which is associated with tissue destruction and local immunosuppression [3]. BU has been reported in >30 countries, but the major burden lies on children living in remote areas of West Africa associated with swamps and stagnant water bodies. Traditionally wide excision of the infected tissue alone was the standard treatment for BU. This is hampered by traumatic interventions, high cost and very high recurrence rates [4]. Chemotherapy with streptomycin and rifampicin is currently re-evaluated as an adjunct treatment to surgery and as a therapy in its own right [5,6,7,8].

M. ulcerans differs from most other pathogenic mycobacteria in that it grows best at 30–33°C and not above 37°C [9]. This characteristic feature of the pathogen was first used for therapeutic purposes in the early 1970s. Meyers et al. treated 8 patients from Zaire maintaining a temperature of approximately 40°C in the ulcerated area for a mean duration of 68 days [10]. There was no evidence of local recurrence during follow-up periods of up to 22 months. Based on this impressive success rate, WHO guidelines listed the application of heat as a treatment option for BU [11]. However, the heat application devices employed so far were impractical in most endemic countries. Here we describe the use of a cheap and easy to apply phase change material (PCM) device suitable for thermotherapy of BU in countries with limited resources.
Buruli ulcer is an infection of the subcutaneous tissue leading to chronic necrotizing skin ulcers. The causative pathogen, *Mycobacterium ulcerans*, grows best at 30°C–33°C and not above 37°C, and this property makes the application of heat a treatment option. We achieved a breakthrough in heat treatment of Buruli ulcer by employing the phase change material sodium acetate trihydrate as a heat application system for thermotherapy, which is widely used in commercial pocket heat pads. It is easy to apply, rechargeable in hot water, non-toxic and non-hazardous to the environment. Six laboratory confirmed patients with ulcerative Buruli lesions were included in the proof-of-principle study and treated for four to six weeks. In patients with small ulcers, wounds healed completely without further intervention. Patients with large defects had skin grafting after successful heat treatment. Heat treatment was not associated with marked increases in local inflammation or the development of ectopic lymphoid tissue. One and a half years after completion of treatment, all patients are relapse-free. The reusable phase change material-based heat application device appears perfectly suited for use in remote Buruli ulcer–endemic areas of countries with limited resources and infrastructure.

Methods

**Study participants**

The design of a study to assess thermotherapy of Buruli ulcer was described in detail elsewhere. Inclusion criteria comprised patients between the ages of 6 and 30 years with a single ulcer. Patients were excluded if they had any of the following: (1) clinical signs and symptoms non-communicable diseases (myocardial, pulmonary, renal, CNS) and (2) inability to confirm BU using laboratory methods.

A BU case was defined as a patient with an ulcer diagnosed as BU on clinical grounds in the catchment area of the Buruli treatment center Ayos/Cameroon and were candidates for inclusion in the study. They were not admitted to the study if any of the following criteria were present: (1) clinical signs and symptoms of communicable diseases other than BU (fever, weight loss, night sweats, persistent cough, jaundice, pulmonary or CNS involvement, ascites, pleural effusion), (2) clinical signs and symptoms of non-communicable diseases (myocardial, pulmonary, renal, CNS) and (3) inability to confirm BU using laboratory methods.

**Laboratory confirmation of clinical diagnosis.** On day 0 four swabs from the undermined edges and one diagnostic biopsy were taken from all patients enrolled into the trial on clinical grounds. A second set of biopsies was taken in week 4 of thermotherapy to assess histopathological changes in response to heat treatment. All samples were investigated by microscopy for acid-fast bacilli (AFB) after Ziehl Neelsen (ZN) staining and by IS2404 real-time PCR [12]. Histopathological changes typical for BU were recorded in the initial biopsies and the follow-up biopsies in week 4 of thermotherapy.

Immediately after performing the punch biopsies, tissue samples were fixed in 4% neutral-buffered PFA (paraformaldehyde) for 24 h and subsequently transferred to 70% ethanol for short term storage and transport. Biopsies were dehydrated, embedded in paraffin, cut into 5 μm thin sections and retrieved on glass slides. After dewaxing and rehydration, sections were stained with haematoxylin/eosin (HE) and ZN. Immunohistochemistry (IHC) was performed with antibodies against Elastase (polymorphonuclear neutrophils [PMNs]; Dako) and CD3 (T lymphocytes; Dako). Staining was performed using Vector NovaRED and haematoxylin.

**The setting and location where the data were collected.** Volunteers were recruited in the catchment area of the Buruli treatment center at the hospital Ayos/Cameroon, identified by active and passive case detection. The treatment center has a longstanding collaboration with and is supported by Leprosy Relief Emmanus-Switzerland (ALES). It maintains a very well equipped and functioning operation theatre, wards for pre- and postsurgical care, physiotherapy and a school which is of importance because the majority of patients with this disease are children and convalesce after excision of ulcers and skin grafting takes many months in the majority of patients. Dr. A. Um Boock, the director of the ALES Bureau Régional pour l’Afrique, and his team are very experienced in the diagnosis and management of patients with Buruli ulcer, including surgery and skin grafting.

**Ethical approval and informed consent.** The protocol was approved by the National Ethics Committee of Cameroon and the Ethics Committee of the University Hospital, Heidelberg, Germany. Patients were enrolled in the study only after informed written consent was obtained from them or their care providers.

**Interventions**

**Heat application.** Commercially available plastic bags filled with the PCM sodium acetate trihydrate were used. Starters were placed in the bags to initiate the crystallisation process (Fig. 1). Size of filled bags is 21 cm x 15 cm x 2 cm with an average weight of 800 g. The melting temperature of the PCM sodium acetate trihydrate is 58°C. The unique feature of PCM is its thermal energy storing capacity combined with an almost constant temperature during the liquid-solid phase transition. This property is widely used in commercial pocket heat pads.

After cleaning and sterile dressing of the ulcers a heat sensor connected to a data logger (testo 177-T3, testo AG, Lenzkirch, Germany) was placed on healthy skin at the edge of the ulcer. The area of contact between skin and PCM packs was protected by tube gauze and a layer of elastic bandage to lower the PCM working temperature from 58°C to the therapeutic target temperature of 40°C at skin surface (Fig. 1). Temperatures of ≤58°C do not cause burns when not applied for prolonged periods of time. Skin temperatures of up to 43°C were accepted and well tolerated for short intervals of time immediately after mounting the PCM bandage. The affected skin (ulcer/oedema/induration) plus a safety margin of several centimeters was covered by one to four PCM packs per session depending on the size of the total area to be treated (Fig. 2). The PCM packs were fixed with several layers of elastic bandage. A thermal insulation layer, commercially available to insulate hot water pipes, was used to reduce heat loss to the environment and to reinforce positioning of the PCM packs (Fig. 1). This allowed patients to move around freely. The 24 hours protocol was as follows: 8.00: Clinical progress assessment, cleaning and dressing of ulcers and renewal of PCM-packs, photo documentation at, on average, 3 day intervals. 12.00: Removal of PCM-packs, dressing of the wound to protect from contamination during a 5 hour pause of heat treatment, skin care with fatty cream. 17.00: Additional wound cleaning and dressing, if needed, renewal of PCM-packs. 22.00: Renewal of PCM-packs.

Clinical observations (appearance of the ulcer and the surrounding heat exposed skin, overall clinical assessment of the patient) were recorded daily at the above mentioned time points on case record forms (CRF). Temperature at the skin surface was automatically...
recorded at 10 minute intervals and stored in a small data logger carried by the patients. Temperature data were transferred daily to a notebook and checked for therapeutic and safety margins (testo software ComSoft 3.4, testo AG, Lenzkirch, Germany).

Patients with small ulcers and without significant oedema (patients 1, 2, 3) received heat treatment for 28–31 days, patients with large ulcers and/or significant oedema (patients 4, 5, 6) for 50–55 days.

Study objectives
In the current study we tested the hypotheses that

1. PCM-based heat application is safe and comfortable for patients
2. with PCM based heat application the results of the thermotherapy study of Meyers et al [10] can be reproduced, i.e. primary healing of Buruli ulcer without relapse can be achieved

Primary outcomes
(1) Proportion of patients completing 28–31 days of heat treatment in patients with small ulcers (≤2 cm) or 50–55 days in patients with large ulcers (>2 cm) and ulcers with prominent surrounding oedema
(2) Proportion of patients cured 6 months after completing heat treatment (including skin grafting where necessary). Cure is defined as complete closure of the wound by epithelialisation or scarification or by skin graft.
(3) Proportion of patients who are recurrence free 18 months after completing heat treatment

Secondary endpoint
Histopathological responses in week 4 of thermotherapy compared to reference samples at day 0.

Results
Participant flow
Seven patients with ulcers suggestive for BU on clinical grounds were recruited by active and passive case detection. In six of the seven patients enrolled the diagnosis was laboratory confirmed.
Protocol deviations

We extended the total duration of heat application of large ulcers (>2 cm) and ulcers with prominent surrounding oedema from 4 weeks to 50–55 days and did not, as originally planned, treat small and large ulcers equally for 4 weeks only. This was done even though all ulcers appeared clinically healed after 4 weeks of heat treatment, independent of size and surrounding oedema. This decision was taken on the basis of the results of the punch biopsies in week 4 of thermotherapy showing residual AFB with intact rod-shaped appearance.

Recruitment and follow-up

Eligible patients were recruited between February 28, 2007 and March 3, 2007. Patients stayed in the hospital during the course of heat treatment and thereafter until the wound was closed (patients with small ulcers; patients 1, 2, 3, 4) or skin grafted (patients with large ulcers; patient 5 and 6). All patients were followed up until 18 months after completion of heat treatment.

Baseline data

The age range of the seven patients enrolled was six to 21 years. Three patients had single ulcers on the upper and four had single ulcers on the lower extremities. Medical history and physical examination revealed no significant health problem other than BU. In six out of seven patients enrolled in the study on clinical grounds, diagnosis was laboratory confirmed. The unconfirmed patient was excluded from the analysis (Fig. 2).

Outcomes

All patients enrolled into the trial completed treatment. In all patients temperatures at the lesion and over a wide margin of healthy looking skin were maintained above $39^\circ C$ for between 8.4 and 13.2 hours and $40^\circ C$ for between 4.4 and 9.3 hours per day (Fig. 2). Undermined margins collapsed between day 1 and day 3. Epithelialization started in all patients between 4 and 11 days after the start and was almost completed in patients 1, 2, and 3 at the end of heat treatment (Fig. 2 and Fig. 3). In particular in patients with oedematous lesions (patients 4, 5) white discharge

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**Figure 2.** Baseline data, heat treatment schedules and results.

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from ulcers was observed during initial treatment for various lengths of time. The two patients with large defects (patients 5 and 6) had skin grafting after completion of heat treatment (Fig. 3B).

All six reconfirmed patients were healed and relapse-free 18 months after completion of treatment.

In the punch biopsies taken prior to start of treatment, histopathological changes characteristic for BU, such as fat cell ghosts, deep dermal necrosis and/or psoriasiform epidermal hyperplasia, were found in six patients (Fig. 2). All patients yielded positive semi-quantitative IS2404 real-time PCR results. AFBs
were detected in swabs or punch biopsies of 4 out of 6 patients included in the study.

Analysis of serial sections of punch biopsies taken at day 0 and in week 4 of thermotherapy showed, that heat treatment was not associated with marked increases in local inflammation, the development of ectopic lymphoid tissue or haemorrhages. At both time points small numbers of both polymorphonuclear cells as members of the innate and T cells as members of the adaptive immune system were present, with polymorphonuclear cells mainly located around necrotic areas and T cells more confined to areas close to vessels in the upper dermis. Only the lesion of patient 3 contained both on day 0 and in week 4 of thermotherapy mixed cellular infiltrates, which were much more pronounced than in typical untreated BU lesions.

Safety and tolerability of PCM-based heat treatment, adverse events

The heat treatment procedure was very well tolerated by all patients. Patients with one (patients 1, 2, 3, 4) and with two PCM packs (patient 5) could move around freely and did not feel unacceptably disturbed during their daily activities or during sleep at night. Patient 6 with four PCM packs also walked with acceptable restrictions and slept largely undisturbed. None of the patients and their guardians requested termination of treatment at any time. Temperatures between 40–43°C were observed only for short intervals of time immediately after mounting of the PCM packs without causing unacceptuable discomfort. Only initially a few small blisters were occasionally observed. With a simple patient-controlled method the therapeutic target temperature of 40°C at skin surface was quickly reached and maintained without further side effects.

Discussion

Successful treatment of BU with heat has been reported in individual patients and small case series since 1950 [10,13,14,15]. This has not been carried further into clinical research and practice due to the fact that available heat application systems were cumbersome and not suited for use in developing countries. We achieved a break through by employing PCM packs as a cheap heat application system which is rechargeable in hot water, non-toxic and non-hazardous to the environment. In this proof-of-principle study we demonstrated that our heat application system is easy to use and allows the patient to move freely.

Family members and the hospital community accepted the treatment very well and favoured it over other treatments currently offered (surgery, antibiotics). Nurses quickly adopted the techniques of mounting the PCM packs and of recharging the packs in boiling water. The only side effects observed were sensation of excessive heat for a short period after applying the PCM packs. Lowering of the temperature at the skin surface by an elastic bandage interposed between tube gauze and PCM packs reliably prevented skin irritation and development of blisters, which may occur if the initial temperature at skin surface is less rigorously controlled.

With our PCM-based heat application system we reproduced the excellent results of the thermotherapy study of Meyers’ group in 1974 [10] with significantly shorter heat application times both with respect to length of heat treatment per day (close to 24 hours [39°C–40.5°C] vs a mean of 10 hours, range 8.4–13.2 hours [≥39°C]) and to total heat application time (28 to 115 days vs 28 to 55 days). Since both systems worked at the same temperature range measured at skin surface, the minimum length of heat application to achieve healing of BU appears to be in the range of our heat treatment schedule or even shorter.

The initial clinical improvement of ulcerative lesions in our series was as fast as in the patient series of Meyers et al. As early as three days after initiation of heat treatment undermined ulcer margins collapsed and the skin attached to the underlying subcutaneous tissue with re-epithelialization starting at the edges. Discharge of the wound decreased over various lengths of time. Firm attachment of the affected skin was complete only after discharge stopped. By using heat treatment alone no viable tissue is lost and even the overarched margins at undercutting edges are often rescued. Lesions were clinically inactive in all of our patients with very good granulation and re-epithelialization responses after 28 days of heat treatment. In one of our patients (patient 6) non-viable tissue extended far beyond the ulcerated area, which had to be excised before skin grafting. In this patient and one other patient with a large defect (patient 5) skin grafting was performed after a good granulation response had been achieved. Currently, all our patients are relapse-free 18 months after completion of heat therapy.

Rifampicin/streptomycin chemotherapy of BU is associated with the development of ectopic lymphoid tissue in the lesions [16]. In some patients, effects reminiscent of the immune reconstitution syndromes observed in tuberculosis and leprosy patients after highly active antiretroviral therapy [17] are observed. In contrast, heat treatment did not lead to massive increases in local inflammation and this less vigorous response may favour rapid re-epithelialization. Also haemorrhages, which are regarded as negative indicators for uncomplicated wound healing [18] were not observed.

Results of two pilot studies, the study of Meyers et al. in the 1970s [10] and our study, demonstrate that heat is a highly efficacious therapy for M. ulcerans disease. Use of PCM packs represents a break through for thermotherapy with respect to its practicality in endemic areas with poor infrastructure. Further optimization of the heat treatment schedule should make it suitable for community application.

Supporting Information

Checklist S1  CONSORT checklist
Found at: doi:10.1371/journal.pntd.0000380.s001 (0.06 MB DOC)

Protocol S1  Study protocol “Phase change material to treat Buruli ulcer through heat treatment”
Found at: doi:10.1371/journal.pntd.0000380.s002 (0.09 MB DOC)

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Author Contributions

Conceived and designed the experiments: TJ AUB DS HW GP. Performed the experiments: TJ AUB MV DS HW GP. Analyzed the data: TJ AUB
MV DS HW GP. Wrote the paper: TJ AUB MV DS HW GP. All co-authors participated in various aspects of the study, analysis, and interpretation of data, and to the development of the manuscript. The final version was seen and approved by all authors. First author of the manuscript: TJ. Developed the idea of using PCM as a heat delivery system to treat Buruli ulcers (together with the engineer Dr. M. Hellmann), contributed to the conception of the study design, enrollment and monitoring of the study patients, data interpretation and led the development of the manuscript: TJ. Contributed to the conception of the study design and monitoring of the study patients (laboratory component), data interpretation and the development of the manuscript: GP. Was the local principal investigator in cooperation with TJ and MV and contributed to the conception of the study design, enrollment and monitoring of the study patients, data interpretation and the development of the manuscript: AUB. Contributed to the conception of the study design and monitoring of the study patients, data interpretation and the development of the manuscript (laboratory component): DS. Contributed to the conception of the study design and monitoring of the study patients, data interpretation and the development of the manuscript with regard to the PCM-based heat delivery device: HW.

References

1. Johnson PD, Stinear T, Small PL, Pluschke G, Merritt RW, et al. (2005) Buruli ulcer (M. ulcerans infection): new insights, new hope for disease control. PLoS Med 2: e108. doi:10.1371/journal.pmed.0020108.
2. Hayman J (1993) Out of Africa: observations on the histopathology of Mycobacterium ulcerans infection. J Clin Pathol 46: 5–9.
3. George KM, Chatterjee D, Gunawardana G, Welty D, Hayman J, et al. (1999) Mycolactone: a polyketide toxin from Mycobacterium ulcerans required for virulence. Science 283: 854–857.
4. Walsh SD, Portela F, Meyers WM (2008) Buruli ulcer (Mycobacterium ulcerans infection). Trans R Soc Trop Med Hyg 102: 969–978.
5. Sizaire V, Nackers F, Comte E, Portaels F (2006) Mycobacterium ulcerans infection: control, diagnosis, and treatment. Lancet Infect Dis 6: 280–286.
6. Eddyani M, Portaels F (2007) Survival of Mycobacterium ulcerans at 37 degrees C. Clin Microbiol Infect 13: 1033–1035.
7. Meyers WM, Shelly WM, Connor DH (1974) Heat treatment of Mycobacterium ulcerans infections without surgical excision. Am J Trop Med Hyg 23: 924–929.
8. WHO (2000) Buruli Ulcer (eds. K. Asiedu, R Scherphof, M Raviglione) WHO/CDs/CPE/GBUI/2000.1.
9. WHO (2001) Buruli Ulcer. Diagnosis of Mycobacterium ulcerans disease (eds F. Portaels, P. Johnson, WM. Meyers). WHO/CDS/CPE/GBUI/2001.4.
10. Reid IS (1967) Mycobacterium ulcerans infection: a report of 15 cases at the Port Moresby General Hospital, Papua. Med J Aust 1: 427–431.
11. Schuette D, Um-Boock A, Mensah-Quainoo E, Itin P, Schmid P, et al. (2007) Development of highly organized lymphoid structures in buruli ulcer lesions after treatment with rifampicin and streptomycin. PLoS Negl Trop Dis 1: e2. doi:10.1371/journal.pntd.0000002.
12. Lipman M, Breen R (2006) Immune reconstitution inflammatory syndrome in HIV. Curr Opin Infect Dis 19: 20–25.
13. Waldorf H, Fewkes J (1995) Wound healing. Adv Dermatol 10: 77–96; discussion 97.