Original Report

Pilot study of treatment of Buruli ulcer with rifampin and dapsone

David K. Espey,[1] Gaston Djomand,[2] Idrissa Diomande,[3] Mireille Dosso,[4] Mathieu Z. Saki,[3] Jean-Marie Kanga,[5] Richard A. Spiegel,[6] Barbara J. Marston,[7] Leo Gorelkin,[6] Wayne M. Meyers,[8] Françoise Portaels,[9] Michael S. Deming,[1] and C. Robert Horsburgh Jr,[10]

Objective: Buruli ulcer disease (BU), caused by *Mycobacterium ulcerans*, is endemic in many regions of Africa and causes substantial physical disability. Surgical resection, currently the mainstay of clinical management of BU, is impractical in many endemic areas. Therefore, the study was undertaken to evaluate an antibiotic regimen for medical management of BU.

Methods: A randomized, placebo-controlled pilot study of dapsone plus rifampin versus placebo was conducted.

Results: Forty-one participants were recruited in a BU-endemic zone of Côte d'Ivoire. Thirty persons completed the 2-month trial: 15 were treated with placebo and 15 with dapsone and rifampin. On blinded evaluation of photographs of the ulcers, clinicians with experience examining BU judged that 82% of ulcers in the treatment group improved compared with 75% in the placebo group (P=0.51). The median change in ulcer size was a decrease of 14.0 cm^2 in the treatment group and a decrease of 2.5 cm^2 in the placebo group (P=0.02), but initial ulcer sizes were larger in the treatment group (median 26.2 cm^2) compared with the placebo group (median 4.8 cm^2) (P=0.04).

Conclusions: Results of this study indicate that larger studies of antimycobacterial therapy of BU are warranted and can be successfully undertaken.

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Mycobacterium ulcerans, a slow-growing, toxin-producing bacterium, causes characteristic skin lesions. These lesions are commonly called Buruli ulcers (BU), after the highly endemic area of Uganda where the first large-scale investigations of the disease took place.[1] Although the ulcers are usually self-limited, healing spontaneously over a period of months to several years, they may lead to severe joint contractures, lymphedema, and disfigurement.

[1]International Health Program Office, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia; [2]Projet Retro-CI, Abidjan, Côte d'Ivoire; [3]Secteur Santé Rurale, Daloa, Côte d'Ivoire; [4]Ministere de la Santé et de la Protection Sociale, Abidjan, Côte d'Ivoire; [5]Service Dermatologie, Centre Hospitalier Universitaire de Treichville, Côte d'Ivoire; [6]Division of Bacterial and Mycotic Diseases and Scientific Resources Program, National Center for Infectious Diseases, CDC, Atlanta, Georgia; [7]Department of Medicine, Emory University, Atlanta, Georgia; [8]Armed Forces Institute of Pathology, Washington, DC; [9]Institute of Tropical Medicine, Antwerp, Belgium; and [10]Department of Epidemiology and Biostatistics, Boston University School of Public Health, Boston, Massachusetts.

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Address correspondence to Dr. C. Robert Horsburgh Jr, Department of Epidemiology and Biostatistics, Boston University, 715 Albany Street T-3E, Boston, MA 02118. E-mail rhorsbu@bu.edu.

Corresponding Editorial Office: New York

Buruli ulcer disease appears to have increased its range in Africa over the past two decades. The earliest reports of endemic foci in Africa came from Uganda and Zaire in the 1950s.[1] Since the 1970s, West African countries including Nigeria, Benin, Ghana, Liberia, and Côte d'Ivoire,[2–6] have experienced an increase in reported cases. From 1987 to 1991, Côte d'Ivoire experienced a 3-fold increase in reported cases, and prevalence reached 16% in some villages.[6] Despite widening geographic distribution, cases tend to be localized to specific regions within affected countries.

The pathophysiology of clinical disease has been well described.[7–9] However, few effective or practical treatments exist for BU. Débridement of necrotic tissue, combined with skin grafting, has long been used for large BU lesions with variable results; recurrences and extension are common. Surgical excision of pre-ulcerative lesions or small ulcers is curative.[10] However, this approach is impractical for many affected individuals, both because early lesions are difficult to identify and because many patients live in remote areas with rudimentary health care. Because *M. ulcerans* requires a temperature of 33°C for growth in the laboratory, direct application of heat to speed healing has been recommended, but results of clinical trials have been inconclusive.[11,12]

Mycobacterium ulcerans isolates are susceptible in vitro to many antibiotic agents,[13–15] but only one con-

trolled clinical trial of antibiotic therapy for BU has been reported. Revill and colleagues found no effect of treatment of BU with clofazimine in 1973.[16] However, many mycobacterial infections require therapy with combinations of antibiotic agents. Such therapy provides increased antimycobacterial activity and decreases the likelihood of failure because of drug resistance. Therefore, a prospective, placebo-controlled pilot study of therapy of BU with a combination of rifampin and dapsone was undertaken. These agents were selected for the present trial because both have shown laboratory evidence of activity against *M. ulcerans* and their safety has been demonstrated through years of use in developing countries in the treatment of tuberculosis and leprosy.

MATERIALS AND METHODS

Enrollment

From March 3 to April 4, 1994, leaders of all villages in a region northeast of Daloa in southwestern Côte d'Ivoire near the Lobo River, extending to the Sassandra River, were interviewed and asked to help identify cases of BU. This geographic area is known to be endemic for BU from a previous study and from ongoing case ascertainment performed by district health officials.[6] Entry criteria into the study included that the person met the clinical case definition for BU; had no known tuberculosis, leprosy, human immunodeficiency virus infection, or hepatic insufficiency (established by verbal query); was not pregnant or breastfeeding; was capable of taking oral medication; and was 4 years of age or older.

The study was approved by the Institutional Review Board of the Centers for Disease Control and Prevention (CDC) and by the Ministry of Health of Côte d'Ivoire. Informed consent, consisting of an explanation of potential benefits, risks and an assurance of patient confidentiality, was obtained from participants who were aged 18 years or older. Consent was obtained from a parent or guardian if the participant was younger than 18.

Case definitions

Buruli ulcer was defined as the presence of a painless or minimally painful cutaneous ulcer with undermined

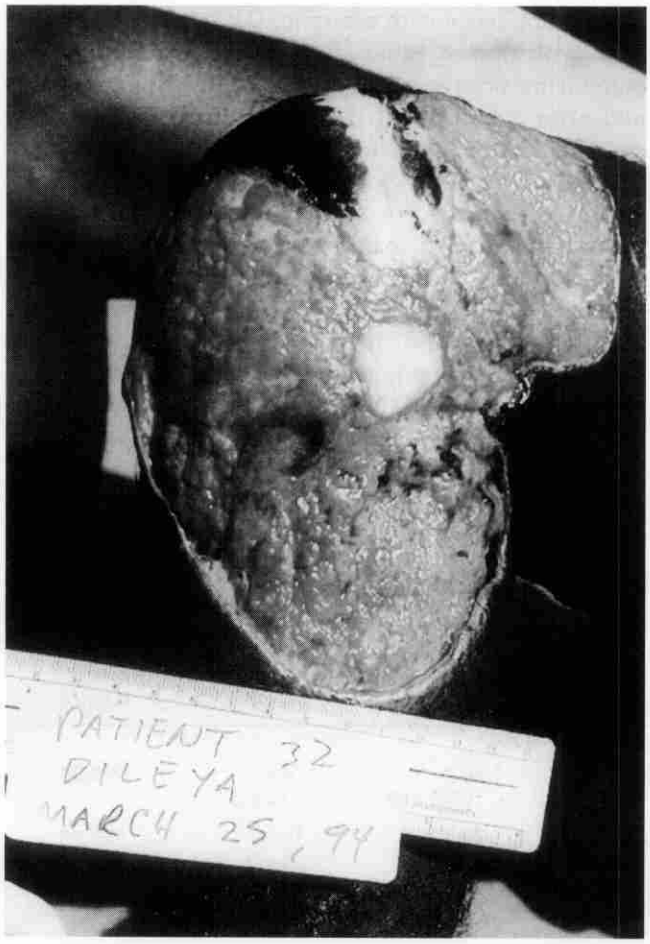

Figure 1. Photograph of a large Buruli ulcer on the lateral aspect of the left knee at enrollment. Name indicates village where examination was performed.

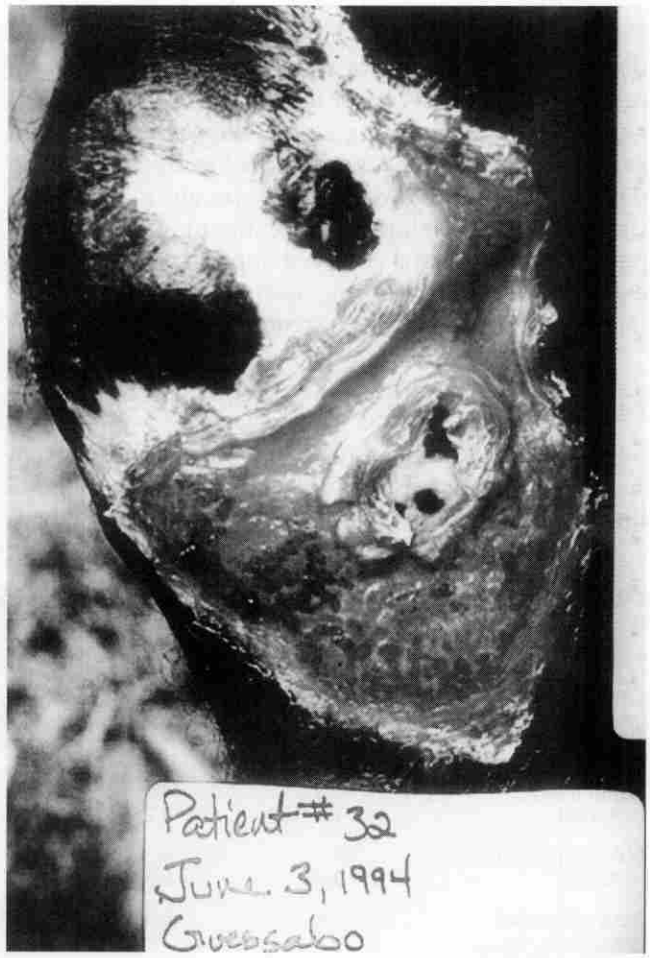

Figure 2. Photograph of the ulcer shown in Figure 1 after 70 days of treatment with dapsone and rifampin. Name indicates village where examination was performed.

margins (Figure 1). A single lesion was chosen as a "study ulcer" in each patient. In the case of multiple ulcers, the largest lesion was chosen as the study ulcer.

Skin Biopsies. A 4- or 6-mm punch biopsy was obtained from each study participant at the recessed margin of the undermined area of the ulcer, identified by probing the lateral extent of the ulcer base with a cotton-tipped swab.

Laboratory examinations

Histologic examination was performed at the CDC and the Armed Forces Institute of Pathology after the specimens were stained with hematoxylin-eosin, Ziehl-Neelsen, Gomori's methenamine silver, and Brown-Hopps stains. At the Belgian Institute of Tropical Medicine, specimens were prepared for culture by maceration and decontamination. They were then placed on Löwenstein-Jensen medium, 1% Ogawa egg yolk medium and Ogawa supplemented with mycobactin. All inoculated media were incubated for up to 12 months at 33°C and observed weekly.

Treatment

Following enrollment and biopsy, participants were randomized by lot to one of two groups. The first group received a combination of dapsone (1.0–1.5 mg/kg/d) and rifampin (10–20 mg/kg/d), whereas the second group received a placebo identical in appearance to dapsone. No placebo for rifampin was given. The investigators were not blinded to treatment status. Participants in both groups received supportive local ulcer care consisting of cleansing and nonsurgical débridement. Both groups received treatment for 2 months.

Follow-up

Subjects were scheduled for follow-up appointments at 1- to 2-week intervals for the first month. Each follow-up visit included an evaluation of compliance with medication by patient query and by pill count. Measurements of the study lesion were taken at enrollment and at each follow-up visit. The lesions were generally round to oval, and ulcer dimensions were obtained by multiplying the greatest perpendicular cross diameters of the study lesion. These diameters were approximated for ulcers having more complex contours. Photographs of the study lesions were taken at enrollment and repeated after 1 and 2 months of participation in the study. A ruler and a label with the date and participant identification number were placed next to the lesion and included in the photographs.

Analysis

Analysis was performed using data from participants of both groups who demonstrated adequate compliance. Adequate compliance was defined as evidence, through patient or guardian query and pill count (with packets and containers present), that at least 75% of the dapsone and dapsone-placebo prescribed for the 2-month period had been taken by the patient. Compliance with dapsone and dapsone-placebo was assessed to facilitate comparison between the two groups, assuming that compliance with dapsone in the treatment group mirrored compliance with rifampin. Determination of clinical evolution of the study ulcers was made by comparing photographs of the lesions at enrollment and after 2 months of treatment. Photographs were examined by two infectious disease specialists with substantial experience examining BU in Africa (CRH, BJM); these specialists were blinded to the treatment status of study participants in the photographs. The specialists scored the paired photographs as "worse," "unchanged," or "improved." The scores were combined and the responses of the two treatment groups were compared by chi-squared test. The Wilcoxon two-sample test was used to compare the two groups, based on change in size of the ulcers at entry compared with size after 2 months of treatment. All data were entered and analyzed using Epi-Info, Version 6.02.[17]

Table 1. Comparison of study participants by inclusion status and treatment group, Buruli ulcer treatment trial

	Participants			*Group*		
Characteristic of group	*Excluded (n=11)*	*Analyzed (n=30)*	*P=Value*	*Treatment* (n=15)*	*Placebo (n=15)*	*P-Value*
Median size of study lesion (cm^2)	5.0	14.0	P=0.73	26.2	4.8	P=0.04
Range	1.5–120	0.25–280		0.25–280	0.25–57.5	
Median age (y)	11	9	P=0.18	13	10	P=0.86
Range	3–29	5–60		5–60	5–60	
Active:Placebo†	5:6	15:15	P=0.80	NA	NA	NA
Gender distribution (M:F)	6:5	18:12	P=1.00	11:4	7:8	P=0.12

*Dapsone and rifampin therapy; †treatment group ratio.

RESULTS

Forty-one patients fulfilled the entry criteria and case definition (Table 1). Seventeen (41.5%) were female and 24 (58.5%) were male. Ten of the 41 patients were lost to follow-up and were excluded from the analysis. One patient was excluded because she became pregnant. There were no significant differences based on initial size of ulcer, treatment status (active vs. placebo), gender, or age between participants who were excluded and those not excluded from the final analysis. The initial median size of ulcers was larger in the active treatment group compared with the placebo group for evaluable participants (26.2 cm^2 [range 0.25–280] vs. 4.8 cm^2 [range 0.25–57.5], P=0.04). No adverse effects of treatment were reported when patients were asked specifically about potential side effects of the medication. Generally, patients from the villages were more likely to comply with the follow-up than the patients from the smaller and more peripheral "campements" (temporary work camps).

Six of the 41 skin biopsies (14.6%) were diagnostic of Buruli ulcer (containing acid-fast bacteria (AFB) or active necrosis of adipose tissue); 29 (70.7%) were indicative of Buruli ulcer (granulomatous changes and necrosis, without AFB); and six revealed nonspecific inflammation. Three specimens (7%) yielded positive cultures. These are the first *M. ulcerans* isolates reported from Côte d'Ivoire.

Thirty participants who demonstrated compliance and returned for follow-up were available for analysis for change in ulcer size. Adequate follow-up photographs were available for 28 of these 30 patients (sample photographs, Figures 1 and 2). The evaluators found that 75% of the placebo group patients and 82% of the active treatment group patients had improved at the 2-month follow-up period; there was no difference between treatment groups with regard to outcome as judged by the photographs (P=0.51) (Table 2). The dapsone and rifampin group (n=15) experienced a decrease in median ulcer size of 14.0 cm^2 (range, 0.0–121); the placebo group (n=15) experienced a decrease in median size of 2.5 cm^2 (range, 0.0–81.0) (P=0.02) (Table 3).

DISCUSSION

In vitro susceptibility testing and trials in animals have provided evidence for activity of several antimycobacterial agents against *M. ulcerans*. Isolates are susceptible in vitro to streptomycin, and this agent inhibited development of lesions in mice when given early in infection.[18–21] Streptomycin has never been evaluated in controlled trials in humans, but it has been used sporadically in endemic areas with encouraging results.[22] However, the need for parenteral administration has limited the usefulness of streptomycin. Laboratory investiga-

Table 2. Comparison of ulcer photographs, Buruli ulcer treatment trial

	Evaluator 1		*Evaluator 2*		*Combined (P=0.51)*	
Grade	*Treatment**	*Placebo*	*Treatment**	*Placebo*	*Treatment**	*Placebo*
Worse	1	0	0	1	1	1
Unchanged	2	4	2	2	4	6
Improved	11	10	12	11	23	21

*Dapsone and rifampin therapy.

Table 3. Comparison of ulcer sizes, Buruli ulcer treatment trial

Median Ulcer Size	*Treatment (n=15)*	*Placebo (n=15)*	*P-Value*
At enrollment median (cm^2)	26.2	4.8	0.04
Range (cm^2)	0.25–280	0.25–280	
After 1 month median (cm^2)	5.3	5.0	0.54
Range (cm^2)	0.0–144.0	0.0–115.0	
After 2 months			
Median (cm^2)	3.0	1.5	0.89
Range (cm^2)	0.0–121.0	0.0–81.0	
Median change in ulcer size			
After 1 month (cm^2)	–8.0 (increase)	1.0 (decrease)	0.26
Range	69.5 to –136.0	112.0 to –30.5	
After 2 months	14.0 (decrease)	2.5 (decrease)	0.02
Median Range	(3.8 to –159.0)	78.0 to –35.0	

tors have demonstrated susceptibility of *M. ulcerans* to cotrimoxazole by disk and minimum inhibitory concentration.[13] The only therapeutic trial of BU using cotrimoxazole, however, was inconclusive because of limitations in study design.[23] Clofazimine is effective against *M. ulcerans* in mice and appeared promising in a pilot study in humans, but a double-blind, placebo-controlled trial failed to confirm its efficacy.[16,24] Rifampin is active in vitro and in mice,[25,26] but has not been evaluated in a controlled clinical trial. *Mycobacterium ulcerans* is also susceptible to dapsone in vitro and in animals,[15,20] but this agent also has not been clinically evaluated.

In the present study, most patients showed clinical improvement, with or without antibiotic therapy. Although a significantly larger decrease in ulcer size was associated with antibiotic treatment with dapsone and rifampin, no discernible differences in outcome were noted between treatment groups, based on evaluation of photographs by experienced clinicians. This discrepancy has several possible explanations. First, since over three-quarters of the patients in both groups showed improvement, the statistical power of the trial to demonstrate differences in improvement was limited. Second, clinical evaluation was limited to interpretation of paired photographs of a single lesion. Since healing in one area of a lesion can occur while progression is observed elsewhere, and a secondary lesion may arise while the primary lesion appears to be healing, some misclassification may have occurred. Thirdly, because infection may progress in subcutaneous tissue without immediate cutaneous manifestations, clinical evolution may be imperfectly reflected by the superficial morphology of the lesion. Finally, despite high rates of improvement at 2 months, it is possible that additional beneficial effects of treatment might have been seen with a longer treatment and follow-up period; such benefits could include fewer relapses and less scarring or deformity.

Measurement of lesion size is a more objective criterion and was planned to be the primary study endpoint. However, differential dropout of patients with larger lesions from the placebo group resulted in a significant imbalance between the two groups with respect to the initial size of the lesions,, with patients in the active treatment group having larger initial ulcers. Although it is unknown whether larger initial lesions are more or less likely to heal spontaneously, it is possible that such a difference, rather than a beneficial effect of antibiotics, was responsible for the greater reduction in median lesion size seen in the antibiotic-treated group. Use of photographs to accurately quantify lesion size provides a practical field tool for a clinical trial, but such methodology is not necessary for routine clinical evaluation of BU lesions.

Diagnosis of BU remains largely clinical. To those accustomed to seeing BU, especially in endemic areas, its clinical diagnosis poses little difficulty; however, for newly or sparsely affected regions, better education of health care workers may be needed to improve recognition of early lesions. Cultures of lesions are infrequently positive; the yield of 7% positive cultures from characteristic lesions in this study is similar to rates reported by others.[1,4] It is thought that tissue necrosis rapidly limits the multiplication of the bacteria, such that cultures taken more than a few days after the lesions ulcerate are less likely to contain organisms. To circumvent the limitations of culture and histologic diagnosis in research settings, newer techniques, such as polymerase chain reaction, may be useful in establishing the diagnosis of BU.[27]

This study did not demonstrate a convincing benefit of therapy of BU with rifampin and dapsone; nevertheless the results indicate a need for further investigation of this and other antimycobacterial regimens for the treatment of BU.[28] This pilot study indicates that such trials are feasible. A substantial number of participants were enrolled in a short time, and 75% of the participants demonstrated compliance with the regimen over a 2-month period. No serious complications of the antibiotic regimen were observed. However, patient adherence was difficult to ensure, and longer follow-up is needed. As frequency of BU continues to increase in West Africa and elsewhere,[29] development of inexpensive, effective, and well-tolerated treatment regimens that can be used in rural areas with limited resources becomes an increasingly important public health priority.

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