

## Oral Treatment for *Mycobacterium ulcerans* Infection: Results From a Pilot Study in Benin

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***Mycobacterium ulcerans* infection is responsible for severe skin lesions in sub-Saharan Africa. We enrolled 30 Beninese patients with Buruli ulcers in a pilot study to evaluate efficacy of an oral chemotherapy using rifampicin plus clarithromycin during an 8-week period. The treatment was well tolerated, and all patients were healed by 12 months after initiation of therapy without relapse.**

*Mycobacterium ulcerans* is the causative agent of Buruli ulcer, an emerging tropical disease marked by devastating skin lesions [1]. Buruli ulcer affects mainly children in rural areas, where access to health care is often delayed and where lengthy hospital stays are problematic. Until recently, surgery was the only treatment for Buruli ulcer. Significant progress has been made in the past 5 years with the demonstration of the efficacy of rifampicin plus streptomycin (R + S) chemotherapy [2]. Its routine implementation has dramatically improved healing while reducing the frequency of relapses [3]. However, streptomycin is an injectable drug, and the lack of an efficacious oral treatment remains one of the main obstacles to decentralizing care at local level.

A recently published randomized controlled trial from Ghana showed no significant difference in the proportion of patients who achieved cure after receiving the World Health Organization (WHO)-recommended 8-week course of R + S chemotherapy,

compared with the proportion who achieved cure after receiving a treatment consisting of 4 weeks of R + S followed by 4 weeks of rifampicin + clarithromycin (R + C) [4].

This study represents a significant step to improve Buruli ulcer treatment. Indeed, clarithromycin is orally administered and is better tolerated than streptomycin, which requires daily injections and is associated with adverse events, such as vestibular toxicity (which occurs in 0.5%–5% of patients) and nephrotoxicity (which occurs in 5%–10%) [5]. A clarithromycin-based oral treatment would be more easily administered, better accepted by patients, and contribute to limiting the number of injections in the developing world [6]. Implementation of an oral R + C chemotherapy is supported by recent evidence of its bactericidal activity in vivo [7, 8] and by several clinical successes [9, 10].

Here, we report a pilot study involving 30 patients with Buruli ulcer disease treated using an oral combination of rifampicin and clarithromycin over an 8-week period. All patients were successfully healed, and no adverse event was observed.

### MATERIALS AND METHODS

This study was realized at the Centre de Diagnostic et de Traitement de l'Ulcère de Buruli (CDTUB) in Pobè, Benin. Eligible patients had laboratory-confirmed cases of Buruli ulcer, were at least 5 years of age, presented with lesions  $\leq 10$  cm in diameter that had appeared within the past 6 months, agreed to be hospitalized during treatment, and were likely to be followed up for 18 months (ie, had stable habitation). Written informed consent was obtained from the patient and from the patient's parent or guardian if the patient was under 18 years of age.

Noninclusion criteria were lesions  $> 10$  cm in diameter, multiple lesions, lesions located over a joint, history of treatment with antimycobacterial drugs, receipt of macrolide or quinolone antibiotics during the previous month, allergy to rifampicin or clarithromycin, pregnancy, or human immunodeficiency virus infection. All nonincluded patients were treated according to WHO guidelines.

The primary end point was defined as healing of the wound at 12 months, without recurrence 18 months after initiation of chemotherapy. Patients were treated using an oral combination of rifampicin (10 mg/kg) and clarithromycin (12 mg/kg) administered simultaneously, once daily, over 8 weeks.

During treatment, patients were hospitalized and attended to daily by nursing staff, who dispensed chemotherapy with a fatty snack and monitored patients for adverse effects during a 1-h period after treatment intake. Clarithromycin was administered

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as 250-mg tablets completed with syrup to achieve exact dose. Nursing staff cleansed the wound using physiological solution and renewed the wound dressing using simple sterile dressings. Every week, patients were examined by a doctor, who also collected samples at week 4, 6, and 8 if the lesion had not healed, using swabs or aspiration. These samples were sent for *M. ulcerans* culture and polymerase chain reaction (PCR) analysis. After the wound closed, patients were discharged from the hospital and were followed-up every 3 months up to 18 months after starting treatment.

Limited surgery was defined as curettage of the lesion or a minor excision to remove excess granulation tissue and to debride ulcer margins. Extensive surgery was defined as major excision followed by skin-grafting. Surgery was undertaken if: (1) no improvement or an aggravation of the lesion characteristics was observed at week 4 of chemotherapy or after, (2) induration persisted 12 weeks after initiation of chemotherapy, (3) the lesion started bleeding at any time, or (4) the lesion showed signs of improper scarring and risks of functional incapacities at any time. Excised tissue was sent for histological analysis, as well as *M. ulcerans* culture and PCR.

All biological samples were collected and handled identically according to WHO guidelines. Blinded analysis of samples was performed at the laboratory in Angers, France, along with routine microbiological analyses for the CDTUB.

Ethical approval for this study was given by the Ministry of Health in Benin. Technical approval was given by the WHO Buruli ulcer working group on chemotherapy.

## RESULTS

Patients were enrolled consecutively from December 2007 through February 2009. Included patients represented one-third of eligible patients who received a diagnosis during this period. The main reason for nonparticipation was refusal of hospitalization. Twelve of the patients were male, and 18 were female; 11 were >15 years of age. Nine patients presented with non-ulcerative lesions, and 21 presented with ulcerative lesions.

Buruli ulcer diagnosis was confirmed by PCR for all patients, and 13 patients (43%) had a positive culture result at enrolment.

Treatment was well tolerated, and no adverse events were reported. All patients had reached 18 months of follow-up by September 2010.

All 30 patients were successfully treated, with complete epithelialization of wounds 12 months after treatment initiation, and no patient experienced relapse during follow-up. The median duration of healing was 104 days (range, 30–212 days; see Table 1 and supplementary Table 1 for individual data).

Microbiological follow-up was performed at week 4, 6, and 8 on lesions that remained open. Culture results became negative for all sampled patients except 1 after 4 weeks; no culture was positive after 6 weeks. PCR follow-up of lesions that remained open showed that negative PCR results were rarely obtained before scarring (supplementary Table 2).

Among those 30 patients, 15 (50%) healed after chemotherapy without any additional intervention (Table 1), and 11 (37%) of the patients underwent limited surgical procedures, such as curettage ( $n = 9$ ) or excision ( $n = 2$ ). These procedures were mainly undertaken without suspicion of failure to promote a faster and more regular scarring of lesions presenting excessive granulation or risks of functional incapacities.

Four patients, 6–8 years of age, underwent extensive surgical excision followed by skin grafting. Three patients presented with category 2 lesions that were large for their body size and either worsened or showed no improvement (supplementary Table 1). One patient who had previously healed without surgery experienced trauma at the site of the scar and required secondary extensive surgery.

As observed in our routine experience of R + S chemotherapy [3], R + C chemotherapy was also sufficient to cure most (8 of 10) patients with category 1 ulcerative lesions and to cure 5 of 11 patients who presented with larger ulcerative lesions. A majority of nonulcerative lesions (7 of 9) required additional surgical procedures.

Tissue specimens were collected from the 15 patients who underwent surgery. Of 14 samples analyzed in culture, none was

**Table 1. Outcome and Additional Care received in 30 Patients with Buruli Ulcer Disease Treated with Oral Rifampicin plus Clarithromycin (R + C) Chemotherapy over an 8-Week Period**

Lesion type	World Health Organization Category <sup>a</sup>	No. (%) of patients successfully healed at 12 months			Total	Median time to healing (days)
		Without surgery	With limited surgery	With excision and skin-grafting		
Ulcerative	1	8	2	0	10	38
	2	5	4	2*	11	115
Non ulcerative	1	2	1	0	3	109
	2	0	4	2	6	111
<b>Total</b>		15 (50)	11 (37)	4* (13)	30 (100)	104

<sup>a</sup> Category 1, lesion diameter <5 cm; category 2, lesion diameter 5–10 cm.

\* Including one patient healed with chemotherapy alone who required secondary skin-grafting after experiencing trauma on the scar site.

positive for *M. ulcerans*. However, 13 patients had a positive PCR result, suggesting persistence of mycobacterial material, as described by others [2].

## DISCUSSION

In this pilot study, 30 patients with confirmed Buruli ulcer disease were successfully healed using R+C chemotherapy. Microbiological results indicate that persistence of viable mycobacteria was unlikely, even when extensive surgery was required. Histological analysis supports this point, showing that 10 of 11 samples analyzed presented evidence of massive inflammatory infiltration. Local inflammatory reactions and the development of ectopic lymphoid tissue have been observed in Buruli ulcer lesions during healing while receiving chemotherapy [11, 12]. This most likely results from mycobacterial antigens and immunostimulators released during chemotherapy. In our experience, as confirmed by others [13], this reaction is associated with good response to treatment and occurs irrespective of the chemotherapy used (A.C., unpublished data).

We advocate that additional chemotherapy efficacy studies should rely on an improved definition of treatment failure that includes microbiological evidence of viable, treatment-resistant mycobacteria and histological assessment of inflammatory reaction. Clinical criteria do not provide a clear rule to discriminate between patients who experience chemotherapy failure and patients who successfully clear the infection but require surgery to expedite the healing process.

In the Ghanaian trial, treatment failure was defined according to clinical criteria, such as lesion size progression or the need for extensive surgery, for all but one of the patients who experienced treatment failure [4]. The subjective component of such clinical criteria makes it difficult to compare results across different studies. In our study, 3 patients required extensive surgery, but none presented persistence of viable mycobacteria.

Positive cultures were obtained in Ghana after treatment completion in 5 patients who belonged to the 4 weeks of R + S followed by 4 weeks of R + C arm [4]. This result is difficult to interpret without information on drug resistance or possible reinfections. In our study, viable mycobacteria were not observed after 6 weeks of treatment. We hypothesize that our higher dosage of clarithromycin was more efficient at clearing the infection, without causing more adverse effects.

In conclusion, this study provides compelling evidence to support a future randomized controlled trial that compares the standard regimen (8 weeks of R + S) with the complete oral regimen (8 weeks of R + C). Proving the efficacy of the R + C combination could lead to a simpler, less invasive, and less painful treatment that is easier to implement at the local level. Surgery is likely to remain necessary for severe lesions, but a large proportion of patients can be cured through oral

chemotherapy alone, which is a great boon to the rural communities most affected by Buruli ulcer.

## Supplementary Material

Supplementary materials are available at Clinical Infectious Diseases online ([http://www.oxfordjournals.org/our\\_journals/cid/](http://www.oxfordjournals.org/our_journals/cid/)).

Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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## References

1. Wansbrough-Jones M, Phillips R. Buruli ulcer: emerging from obscurity. *Lancet* **2006**; 367:1849–1858.
2. Etuful S, Carbonnelle B, Grosset J, et al. Efficacy of the combination rifampin-streptomycin in preventing growth of *Mycobacterium ulcerans* in early lesions of Buruli ulcer in humans. *Antimicrob Agents Chemother* **2005**; 49:3182–3186.
3. Chauty A, Ardant M, Adeye A, et al. Promising clinical efficacy of streptomycin-rifampin combination for treatment of buruli ulcer (*Mycobacterium ulcerans* disease). *Antimicrob Agents Chemother* **2007**; 51:4029–4035.
4. Nienhuis WA, Stienstra Y, Thompson WA, et al. Antimicrobial treatment for early, limited *Mycobacterium ulcerans* infection: a randomised controlled trial. *Lancet* **2010**; 375:664–672.
5. Chan-Tompkins NH. Toxic effects drug interactions of antimycobacterial therapy. *Clin Dermatol* **1995**; 13:223–233.
6. Hutin Y, Hauri A, Chiarello L, et al. Best infection control practices for intradermal, subcutaneous, and intramuscular needle injections. *Bull World Health Organ* **2003**; 81:491–500.
7. Ji B, Chauffour A, Robert J, Jarlier V. Bactericidal sterilizing activities of several orally administered combined regimens against *Mycobacterium ulcerans* in mice. *Antimicrob Agents Chemother* **2008**; 52:1912–1916.
8. Ji B, Chauffour A, Robert J, Lefrançois S, Jarlier V. Orally administered combined regimens for treatment of *Mycobacterium ulcerans* infection in mice. *Antimicrob Agents Chemother* **2007**; 51:3737–3739.
9. O'Brien DP, Hughes AJ, Cheng AC, et al. Outcomes for *Mycobacterium ulcerans* infection with combined surgery and antibiotic therapy: findings from a south-eastern Australian case series. *Med J Aust* **2007**; 186:58–61.
10. Dossou AD, Sopoh GE, Johnson CR, et al. Management of *Mycobacterium ulcerans* infection in a pregnant woman in Benin using rifampicin and clarithromycin. *Med J Aust* **2008**; 189:532–533.
11. Schütte D, Um-Boock A, Mensah-Quainoo E, Itin P, Schmid P, Pluschke G. Development of highly organized lymphoid structures in Buruli ulcer lesions after treatment with rifampicin and streptomycin. *PLoS Negl Trop Dis* **2007**; 1:e2.
12. Schütte D, Pluschke G. Immunosuppression treatment-associated inflammatory response in patients with *Mycobacterium ulcerans* infection (Buruli ulcer). *Expert Opin Biol Ther* **2009**; 9:187–200.
13. O'Brien DP, Robson ME, Callan PP, McDonald AH. "Paradoxical" immune-mediated reactions to *Mycobacterium ulcerans* during antibiotic treatment: a result of treatment success, not failure. *Med J Aust* **2009**; 191:564–566.