Outcomes for *Mycobacterium ulcerans* infection with combined surgery and antibiotic therapy: findings from a south-eastern Australian case series

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ABSTRACT

Objective: To describe the effect of antibiotics on outcomes of treatment for Buruli or Bairnsdale ulcer (BU) in patients on the Bellarine Peninsula in south-eastern Australia.

Design: Observational, non-randomised study with data collected prospectively or through medical record review.

Patients and setting: All 40 patients with BU managed by staff of Barwon Health's Geelong Hospital (a public, secondary-level hospital) between 1 January 1998 and 31 December 2004.

Main outcome measures: Epidemiology, clinical presentation, diagnosis, treatment and clinical outcomes.

Results: There were 59 treatment episodes; 29 involved surgery alone, 26 surgery plus antibiotics, and four antibiotics alone. Of 55 episodes where surgery was performed, minor surgery was required in 22, and major surgery in 33. Failure rates were 28% for surgery alone, and 19% for surgery plus antibiotics. Adjunctive antibiotic therapy was associated with increased treatment success for lesions with positive histological margins ($P < 0.01$), and lesions requiring major surgery for treatment of a first episode ($P < 0.01$). The combination of rifampicin and ciprofloxacin resulted in treatment success in eight of eight episodes, and no patients ceased therapy because of side effects with this regimen.

Conclusions: Adjunctive antibiotic therapy may increase the effectiveness of BU surgical treatment, and this should be further assessed by larger randomised controlled trials. The combination of rifampicin and ciprofloxacin appears the most promising.

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Buruli or Bairnsdale ulcer (BU) is caused by *Mycobacterium ulcerans*. It occurs mainly in tropical regions of sub-Saharan Africa, but it is also endemic to tropical northern Queensland and temperate south-eastern Victoria in Australia.[1,2] It usually causes a localised destructive skin and subcutaneous lesion resulting from a destructive toxin called mycolactone.[3] The mode of transmission and risk factors for acquisition remain unknown.

Recommended treatment for BU has been wide surgical excision, but this often causes significant morbidity, and it is technically difficult, resource intensive, expensive, and often not easily accessible in endemic areas.[4-6] Furthermore, BU recurs after treatment in 16%–47% of cases.[7,8] As most cases of BU occur in resource-limited settings, there is an urgent need to simplify treatment and improve outcomes.[5,6,9] Despite the known sensitivity of *M. ulcerans* to many antibiotics in vitro,[10,11] until recently there has been little clinical evidence of their effectiveness in vivo,[12,13] and thus antibiotic therapy has not generally been recommended for treatment. However, recent observational studies from Africa[14] have led to a provisional recommendation by the World Health Organization to initiate treatment for BU with rifampicin and streptomycin.[5]

In south-eastern Australia, there has been a sustained outbreak of *M. ulcerans* infection, which began in 1998 in a newly recognised endemic area on the Bellarine Peninsula. During this outbreak, most cases were managed by the staff of Barwon Health, and antibiotics were used in a proportion of cases. Therefore, we performed an observational study to assess the impact of antibiotics on the outcomes of BU treatment.

METHODS

We collected data on all patients diagnosed with *M. ulcerans* who were managed by staff of Barwon Health's Geelong Hospital between 1 January 1998 and 31 December 2004. Cases were identified from a prospective clinical database of all patients managed by the infectious diseases service of Barwon Health, or from patients on the database of the pathology service (PathCare) who had histopathology samples or cultures positive for *M. ulcerans*.

A case of *M. ulcerans* skin infection was defined by the presence of a lesion clinically suggestive of BU, plus any of:

- culture of *M. ulcerans* from the lesion; or
- a positive result on polymerase chain reaction (PCR) for *M. ulcerans* on a biopsy or swab of the lesion; or
- a histopathology sample of the lesion showing a necrotic granulomatous ulcer with the presence of acid-fast bacilli (AFB).

The PCR method has been reported previously,[15,16] and has a reported 96% sensitivity and 100% specificity for *M. ulcerans*.

As patients could have more than one lesion, the treatment of each lesion was analysed separately as a single "treatment episode", unless a number of lesions underwent the same treatment simultaneously, in which case they were considered together. A "treatment episode" was completed when either the treatment was ceased by the clinician because the outcome was clinically satisfactory, or the patient developed a recurrence and a new treatment episode was started. To account for possible correlation of outcomes between episodes from the same patient, we also analysed treatment outcomes for only the first treatment episode for each individual.

Treatment success was defined as clinical resolution of the original BU lesion in the absence of subsequent local or distant recurrent lesions in the 12 months after completion of treatment. If this definition was not met, the treatment was deemed to have failed.

A recurrence was classified as local if it occurred in the wound margins or within 3 centimetres of the wound. Otherwise it was termed distant.

1 Association with treatment outcomes in 55 surgically treated lesions, and complication rates among 59 treatment episodes, for antibiotics used in treating Buruli or Bairnsdale ulcer

Drug	Treatment success (episodes)	Treatment failure (episodes)	Complications*
Rifampicin	20 (80%)	5 (20%)	6/27 (22%)
Clarithromycin	10 (91%)	1 (9%)	9/15 (60%)
Ciprofloxacin	8 (100%)	0	1/8 (13%)
Ethambutol	8 (80%)	2 (20%)	6/14 (43%)
Amikacin	3 (43%)	4 (57%)	3/8 (38%)
Azithromycin	0	1 (100%)	1/2 (50%)

* Surgery was not performed in four patients. ◆

Positive margins were defined as the presence of granulomatous inflammation or necrotic tissue extending to one or more of the surgical excision margins of the lesion on histopathological examination. Minor surgery was defined as excision of the *M. ulcerans* lesion with primary closure of the wound. Major surgery involved either a split skin graft or a vascularised skin and tissue flap. Operations were performed by multiple surgeons.

Antibiotics were used if the patient had been referred to the infectious diseases unit by the treating surgeon or general practitioner. As there was no formalised protocol for antibiotic use, we describe the referral and treatment choices of the medical practitioners involved.

An antibiotic complication was defined as a side effect severe enough to warrant either a reduction in the dose or the cessation of antibiotic therapy.

Statistical analysis

Data were collected and analysed with Epi Info 6 (Centers for Disease Control and Prevention, Atlanta, Ga, USA). Statistical comparisons of proportions were determined using the 2-tailed Fisher's exact test.

RESULTS

Epidemiology and clinical findings

There were 40 patients, all of whom lived in the city of Geelong or on the Bellarine Peninsula. Twenty-three patients were female and their median age was 66 years (range, 4–88 years).

The initial lesion was located on the lower limb in 27, the upper limb in 11, and the trunk in two patients. Thirty-four lesions were on the distal limb (elbow/knee and below), and 16 occurred over a joint. Thirty-three people developed only a single lesion, while two people had two lesions, two had three lesions, and the remaining three had four, six and seven lesions, respectively. No patient had a history of previous BU.

Diagnosis

In addition to a clinically suggestive lesion, *M. ulcerans* was cultured from the lesion in 15 patients, and PCR of tissue or a swab from the lesion was positive for *M. ulcerans* in a further 21. In the remaining four patients, a necrotic granulomatous ulcer containing AFB was present on histopathological examination.

Treatment

There were 59 treatment episodes; 29 involved surgery alone, 26 surgery plus antibiotics, and four involved antibiotics alone.

In the 55 episodes where surgery was performed, primary closure of the wound was achieved in 22 episodes, skin grafting was required in 27, and a vascularised flap in nine episodes (three patients had both a skin graft and a vascularised flap). The type and frequency of the antibiotics used in these episodes are shown in Box 1.

Outcomes

The mean follow-up period after completion of treatment was 40 months (range, 13–92 months).

Treatment failed in all four patients who received antibiotics alone. In one, antibiotic therapy was stopped because of side effects at Day 7 with no clinical improvement. In another, antibiotic therapy was stopped because of side effects at Day 21, with subsequent clinical progression of the disease. In the other two patients, despite antibiotic therapy for 65 and 75 days, respectively, there was clinical progression of the disease. The episodes in these four patients were not considered further in this analysis.

Box 2 shows results for those who had surgery, with all episodes in a patient included. The overall failure rate was 24%, with local recurrence in five episodes and distant recurrence in nine episodes (one patient had both a local and a distant recurrence). For the distant recurrences, three occurred on a separate body part on the same limb, and two occurred on different limbs. Local recurrences were not more likely to occur in those with positive margins (compared with distant recurrences; ($P = 0.5$) or those who underwent minor surgery ($P = 0.7$). The failure rate for surgery alone was higher if there were positive margins (compared with negative margins; 71% v 14%; $P < 0.01$), with similar rates for major and minor surgery (33% v 21%; $P = 0.68$).

We compared first episodes (Box 3) and all episodes (Box 2) treated with surgery and antibiotics with those treated with surgery alone. Antibiotics were significantly associated with higher treatment success rates for first treatment episodes overall ($P = 0.03$), if the margins were positive ($P < 0.01$), or if the first treatment episode required major surgery ($P < 0.01$).

2 Outcome comparison for all surgically excised Buruli or Bairnsdale ulcer episodes in patients treated with and without antibiotics

	Total episodes	Total treatment successes	Antibiotics*	No antibiotics*	*P* value†
All episodes	55	42 (76%)	21/26 (81%)	21/29 (72%)	0.47
Minor surgery	22	17 (77%)	6/8 (75%)	11/14 (79%)	1.0
Major surgery	33	25 (76%)	15/18 (83%)	10/15 (67%)	0.42
Negative margins	30	24 (80%)	5/8 (63%)	19/22 (86%)	0.30
Positive margins	25	18 (72%)	16/18 (89%)	2/7 (29%)	< 0.01

* Number of treatment successes/number of treatment episodes.
† Comparison of antibiotics versus no antibiotics, determined by 2-tailed Fisher's exact test. ◆

3 Outcome comparison for first episodes of surgically excised Buruli or Bairnsdale ulcer in patients treated with and without antibiotics

	Total episodes	Total treatment successes	Antibiotics*	No antibiotics*	*P* value†
All episodes	38	32 (84%)	16/16 (100%)	16/22 (73%)	0.03
Minor surgery	16	15 (94%)	4/4 (100%)	11/12 (92%)	1.0
Major surgery	22	17 (77%)	12/12 (100%)	5/10 (50%)	< 0.01
Negative margins	22	19 (86%)	4/4 (100%)	15/18 (83%)	1.0
Positive margins	16	13 (81%)	12/12 (100%)	1/4 (25%)	< 0.01

* Number of treatment successes/number of treatment episodes.
† Comparison of antibiotics versus no antibiotics, determined by 2-tailed Fisher's exact test. ◆

All eight episodes treated with rifampicin (150–300 mg twice daily) and ciprofloxacin (250–500 mg twice daily) had successful outcomes. This included two episodes where previous combined medical and surgical therapy had failed, six episodes involving major surgery, six episodes with positive margins, and one episode complicated by osteomyelitis. All these episodes were treated for 90 days, except the one in the patient with osteomyelitis, which was treated for 180 days. Only one episode required a 50% dosage reduction of both rifampicin (from 300 mg to 150 mg twice daily) and ciprofloxacin (from 500 mg to 250 mg twice daily) because of persistent nausea, and no episodes required cessation of antibiotic therapy because of side effects.

For adjunctive antibiotic therapy associated with treatment success, the duration of use was 3 months or less for 18 of 21 episodes (1 month for two episodes; 2 months for four episodes; 3 months for 12 episodes). The number of antibiotic medications combined was two for 13 episodes, three for six episodes, one for one episode, and four for one episode. The adjunctive antibiotic regimens associated with failure are shown in Box 4, and failure occurred in two of 20 episodes in patients who completed up to 3 months of therapy.

The association with treatment outcomes in surgically treated lesions, and complication rates, for antibiotics used in treating BU can be seen in Box 1.

Antibiotics were given before surgery in six episodes (mean, 13 days before surgery; range, 4–28 days). None of the patients involved had relapses compared with five of the 20 patients who received antibiotics after surgery ($P = 0.30$).

Amikacin (15 mg/kg daily) was associated with treatment success in only three of the seven surgically treated episodes in which it was used; this rate was significantly lower than all other episodes treated with both surgery and antibiotics ($P = 0.01$). Amikacin was used for a median of 14 days (range, 5–21 days), and caused clinical ototoxicity requiring cessation of therapy in three of eight episodes (in patients aged 58, 77 and 88 years) after treatment durations of 12, 14 and 18 days.

The high overall complication rate with the antibiotic clarithromycin (9 of 15 episodes; Box 1) is notable, with patients reporting nausea, metallic taste and rash.

DISCUSSION

Until recently, the recommended treatment for *M. ulcerans* lesions has been wide surgical excision.[6,13] In our series, wide surgical excision resulted in significant morbidity, with 60% requiring either a vascularised flap or a skin graft. Despite this, treatment failure rates with surgery alone were high, affecting more than one in every four episodes, and recurrence rates were significantly associated with the presence of positive margins ($P < 0.01$). These high failure rates would not have been improved significantly by more aggressive initial local surgery, as almost 70% of recurrences observed in our study were more than 3 cm from the original lesion.

However, we were able to demonstrate that adjunctive antibiotic therapy significantly improved the rates of treatment success overall for first treatment episodes (Box 3; $P = 0.03$), if there were positive histological margins ($P < 0.01$), or if major surgery was required for the first treatment episode ($P < 0.01$). Thus we believe that, in these circumstances, antibiotics in addition to surgical excision may be effective in reducing both local and distant recurrences of BU.

The most useful antibiotic regimen appeared to be the combination of rifampicin and ciprofloxacin, both in terms of effectiveness (100% treatment success) and tolerability (no patients ceased treatment). This was despite the fact that it was generally used for the most difficult cases. Rifampicin has often been used as adjunctive treatment for BU based on in-vitro studies showing excellent sensitivity,[10] and mouse models showing some effectiveness.[17] There has also recently been some evidence that early human *M. ulcerans* lesions can be rendered culture-negative when patients are treated with rifampicin combined with streptomycin for a minimum of 4 weeks.[14] *M. ulcerans* has also been shown to be highly susceptible to ciprofloxacin in vitro.[10] Although there are no other previous reports of ciprofloxacin use in *M. ulcerans* infection, the combination of its good oral bioavailability and known excellent penetration into bone and tissues may explain its effectiveness. Finally, the combination of these drugs may have increased their effectiveness.[18]

In contrast, amikacin was associated with a significantly reduced likelihood of treatment success, and significant toxicity. Previous studies have shown amikacin has good effectiveness against *M. ulcerans* in vivo, and that combinations of rifampicin and amikacin have good effectiveness against

4 Adjunctive antibiotic regimens used in patients who experienced at least one episode of treatment failure

	Patient	Regimen and duration	Time to failure
Episode with failure	1	R C E (25 days) Am (5 days)	During treatment
	1	R (80 days) Am (18 days)	During treatment
	1	R (45 days)	During treatment
	2	R E (90 days) Am (21 days)	14 days after treatment
	3	R Az (90 days) Am (14 days)	180 days after treatment
Episode with cure	1	R (180 days) Cp (180 days)	
	2	R (90 days) Cp (90 days)	
	3	R C E (109 days) Am (12 days)	

R = rifampicin; C = clarithromycin; E = ethambutol; Am = amikacin; Az = azithromycin; Cp = ciprofloxacin. ◆

M. ulcerans in mice.[17,19] In addition, there is evidence in observational human studies that rifampicin combined with streptomycin is effective in selected patients with BU in Africa.[14] However, previous experience with the drug clofazimine has shown that antibiotics may not be effective against *M. ulcerans* in vivo, despite the microorganism showing in-vitro sensitivity.[20] Our clinical experience does not support the use of amikacin in the treatment of *M. ulcerans* disease, especially in elderly patients, for reasons of both effectiveness and toxicity.

The exact duration of adjunctive antibiotic treatment or the number of drugs required to achieve cure could not be determined from our study. However, our results suggest that regimens of 2 or 3 drugs given for up to 3 months were associated with only a 10% chance of recurrence.

We acknowledge that this study has major limitations. It is a relatively small case series, is retrospective, and treatment was not randomised. In addition, because of small numbers, multivariate analysis of potentially confounding factors such as age, sex and comorbid illnesses could not be performed. However, there are few studies of drug treatment in BU, so despite our study's weaknesses, we feel the findings contribute significant information on the treatment of *M. ulcerans* infection. A further limitation was that our population was mainly elderly, and our experience limited to an endemic area in non-tropical south-eastern Australia. Therefore our results may not be generalisable to populations in tropical sub-Saharan Africa, where the greatest burden of disease is present mainly in young children. We thus recommend that larger randomised controlled studies be performed to validate our findings.

Conclusions

Adjunctive antibiotic therapy may be effective in reducing recurrences in those who require major surgery for treatment of their first *M. ulcerans* lesion, or who have positive histological margins. The combination of rifampicin and ciprofloxacin seems promising, and has the potential to provide an easily accessible, relatively well tolerated and inexpensive oral antibiotic treatment that can result in a significant reduction of the morbidity and cost associated with the treatment of *M. ulceran* infection.

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COMPETING INTERESTS

None identified.

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