Bactericidal action of oral ampicillin/sulbactam against *Mycobacterium leprae*  
B. Randhawa, E. B. Harris and K. Prabhakaran*

Gillis W. Long Hansen’s Disease Center at Louisiana State University, US Public Health Service, PO Box 25072, Baton Rouge, LA 70894-5072, USA

We reported previously that an injectable form of ampicillin/sulbactam, Unasyn, was bactericidal to *Mycobacterium leprae* multiplying in mouse foot pads. In this study, we examined the effect of an orally active form of ampicillin/sulbactam, Sultamicillin, on the growth of *M. leprae* in mice. Three concentrations of the drug, mixed with the feed, were administered from the start until the mice were killed at 6 months; 0.01% of the drug inhibited bacterial growth by 54%, 0.10% by 74% and 0.20% by 93%. To test whether oral ampicillin/sulbactam was bactericidal, 0.50% of the drug, mixed with the feed, was administered to experimentally infected mice for 3 months during the logarithmic phase of bacterial growth, and then discontinued; multiplication of the bacilli was monitored monthly for the next 8 months. The results showed that orally active ampicillin/sulbactam is bactericidal to *M. leprae*.

**Introduction**

When β-lactam antibiotics became commercially available, they were tried against leprosy but found to be ineffective. The reason for this was not evident until we demonstrated derepression of a possibly chromosome-encoded β-lactamase in *Mycobacterium leprae*.¹ β-Lactamases hydrolyse penicillins and cephalosporins, inactivating them. Inhibitors of β-lactamase can be used to suppress the inactivation of the β-lactam by a wide range of β-lactamases. Of two β-lactamase inhibitors we tested, sulbactam inhibited the enzyme produced by *M. leprae* better than clavulanate.¹ β-Lactam/β-lactamase-inhibitor combinations are now widely employed to treat diseases caused by β-lactamase-producing organisms. The commercially available injectable form of ampicillin/sulbactam (2:1, w/w, Unasyn (Pfizer, Groton, CT, USA)) prevented multiplication of *M. leprae* in mice.² In a subsequent study, we showed the drug to be bactericidal to *M. leprae* in the murine model.³

Sultamicillin, a highly stable, orally active double ester of ampicillin/sulbactam (1.5:1, w/w), is available from Pfizer Central Research, as a tosylate salt. In the present study we tested the effect of the compound on the growth of *M. leprae* in mice, by administering the drug by the ‘continuous’ method for 6 months, and by the ‘kinetic’ method where the drug is given for a short period of time and then discontinued.⁴ The results showed that oral ampicillin/sulbactam was bactericidal to *M. leprae*. (The terms ‘continuous’ and ‘kinetic’ in drug trials against *M. leprae* in mouse foot pads were introduced by Shepard⁴ in 1965, and have been generally used since then.)

**Materials and methods**

**Bacteria and animals**

*M. leprae* harvested from foot pads of BALB/c or *nu/nu* mice was used in the experiments. The bacteria, originally derived from a patient, have been maintained by serial passages in mouse foot pads. In one experiment, *M. leprae* from *nu/nu* mice was inoculated (1 × 10⁴/0.03 mL/foot pad) into both hind foot pads of 20 BALB/c mice (10 control, 10 experimental). In another experiment, the bacteria (harvested from BALB/c foot pads) were inoculated into the foot pads of 20 ‘normal’ littermates of beige (C57BL/6J bg/bg) mice (10 control, 10 experimental). These mice were used only because of their availability at the time. The strain of mice or the source of inoculum apparently made little difference in these experiments. The bacteria multiply to about 1 × 10⁶/foot pad in approximately 6 months; if the animals are maintained for longer periods, the number of bacteria/foot pad might increase for about 3 or 4 months, and then decline. To evaluate the bactericidal action of ampicillin/sulbactam, 60 BALB/c mice were inoculated...
with *M. leprae* derived from foot pads of BALB/c mice (30 control, 30 experimental).

**Treatment**

Sultamicillin tosylate dihydrate (purity: 73.3% as ampicillin/sulbactam) was generously supplied by Pfizer Central Research. In the ‘continuous’ method of drug administration, three concentrations of the drug were tested: 0.01% and 0.10% in littermates of *bg/bg* mice and 0.20% in BALB/c mice. Because there was no previous experience in using oral ampicillin/sulbactam against *M. leprae*, the concentrations used were chosen arbitrarily; the results showed that they worked. The drug was mixed with ground mouse chow, fresh mixtures being prepared once a week. Animals in the control groups received regular ground mouse chow. The feed was changed in the cages twice a week. Water and feed were provided *ad libitum*. The animals were killed by CO₂ asphyxiation at approximately 6 months, when growth of *M. leprae* was detected in the untreated controls, by periodic monitoring. The bacteria in the foot pads of six mice in each group were counted by the method of Shepard & McRae. For bacterial enumeration, the hind foot pad tissues of each mouse were pooled before homogenizing. The results are presented as means (± S.D.). Significance of the difference between each control and experimental group was determined by Student’s unpaired t-test. A *P* value of <0.05 was considered significant.

In the ‘kinetic’ experiment to determine whether the drug was bactericidal, it was mixed with the feed at a concentration of 0.50%. Treatment was started 2 months after inoculation of the mice with *M. leprae*, when the bacteria were in the logarithmic phase of growth. Drug administration was stopped after 3 months. Three mice from the treated and the control groups were killed every month for the next 8 months, and the acid-fast bacteria in the foot pads of individual mice were enumerated. Mean values of the three counts for each month are given in the results. The animals showed no adverse effects such as emaciation or loss of hair.

**Results and discussion**

Normal multiplication of *M. leprae* was observed in the foot pads of the mice in the control groups. Since the inoculum of *M. leprae* is not derived from an exponentially growing bacterial culture, the number of viable organisms present in the inoculum may vary, and as such the rate of multiplication of the bacilli in different experiments may not always be the same.

As seen in Figure 1, ‘continuous’ administration of oral ampicillin/sulbactam suppressed growth of the bacteria in both strains of mice used. For the three different drug concentrations used, 0.01% resulted in a growth inhibition of 54% (*P* < 0.005); 0.10% in inhibition of 74% (*P* < 0.0005); 0.20% in inhibition of 93% (*P* < 0.0005). In the ‘kinetic’ method, where drug administration was discontinued after 3 months of treatment, multiplication of the bacteria, compared with the controls, continued to be inhibited by the drug (Figure 2). The results indicated a bactericidal effect of the drug on the bacteria. According to this procedure, if the bacteria in the treated group do not grow to the level of the controls in 3 months after discontinuation of treatment, the drug is considered bactericidal.

![Figure 1. Effect of oral ampicillin/sulbactam on growth of *M. leprae* in mouse foot pads, by the ‘continuous’ method. (A) Littermates of *bg/bg* mice; (B) BALB/c mice. Concentration of drug used: 0.01%, 0.10% and 0.20% in the feed.](image1)

![Figure 2. Suppression of the growth of *M. leprae* in the foot pads of BALB/c mice by oral ampicillin/sulbactam, by the ‘kinetic’ method. Concentration of drug used: 0.50% in the feed. ■, control; □, experimental.](image2)
Oral ampicillin/sublactam kills *M. leprae*

The results of this study are consistent with our previous reports on an intramuscular trial of ampicillin/sublactam, by the ‘continuous’ method, and by the ‘kinetic’ method. Another β-lactam/β-lactamase-inhibitor, co-amoxiclav (amoxicillin/clavulanate) has been reported to be effective against *M. leprae* in mice, and against multidrug-resistant tuberculosis in two patients (500 mg every 6 h up to 4 weeks). Early bactericidal action of co-amoxiclav against *Mycobacterium tuberculosis* in 48 tuberculosis patients was reported recently (1.25 g three times a day for 7 days).

Multidrug treatment of leprosy has been adopted since 1980 for the ‘elimination’ of the disease as a public health problem by the year 2000. However, the ability of bacteria to develop multidrug resistance has been known for over three decades. Multidrug resistance of *M. leprae* has been reported before, and sporadic reports of relapses after multidrug treatment of leprosy continue to appear, although on a small scale. Multidrug-resistant tuberculosis has become a major problem in many developing countries, and in Russia, in recent years. Mycobacterial β-lactamase is not a metallo-enzyme (metallo-enzymes are highly resistant to inhibitors), and inhibitor-resistant β-lactamases have not been reported in mycobacteria. We have shown earlier that injectable ampicillin/sublactam suppressed the growth of several species of cultivable mycobacteria in vitro, and that the drug was bactericidal to *M. tuberculosis* H37Rv. Results of the present study demonstrate that oral ampicillin/sublactam is bactericidal to *M. leprae*, as was reported before for injectable ampicillin/sublactam, in similar studies.

Sublactam inhibits chromosomal β-lactamase more effectively than clavulanate; in our studies by the BACTEC method, the MIC of sublactam for *M. tuberculosis* H37Rv was 9.38 mg/L, while that of clavulanate was 31.25 mg/L, indicating that sublactam is more active than clavulanate in the system. Orally administered ampicillin/sublactam is readily absorbed, and hydrolysed to release ampicillin in the body. The compound would probably be useful as a rational antimicrobial agent to treat mycobacterial infections resistant to other drugs.

Acknowledgements

The study was supported in part by Pfizer Pharmaceuticals, Pfizer Inc. We thank Pfizer Central Research for providing Sultamicillin. Part of the data was presented at the Thirty-Seventh Interscience Conference on Antimicrobial Agents and Chemotherapy, 28 September–1 October 1997, Toronto, Canada (Abstract C7).

References


Received 3 November 1998; returned 26 February 1999; revised 8 March 1999; accepted 29 March 1999

281