Activity of Amoxicillin/Clavulanate in Patients with Tuberculosis

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Some β-lactam antibiotics are active in vitro against *Mycobacterium tuberculosis*. There are anecdotal reports of successful treatment of tuberculosis caused by multiple-drug-resistant strains of *M. tuberculosis* with regimens that included amoxicillin/clavulanate. Reduction of *M. tuberculosis* in the sputum of patients with pulmonary tuberculosis during administration of amoxicillin/clavulanate was measured by a quantitative culture method to determine the activity in vivo. Patients were randomized to receive isoniazid, ofloxacin, or amoxicillin/clavulanate for 7 days. Isoniazid was the most effective agent, reducing *M. tuberculosis* after 2 days at a mean rate (± standard deviation) of 0.60 ± 0.30 $\log_{10}$ cfu/mL per day, compared with 0.32 ± 0.05 and 0.34 ± 0.03 for ofloxacin and amoxicillin/clavulanate, respectively. The early bactericidal activity of amoxicillin/clavulanate was comparable to that reported for antituberculous agents other than isoniazid. Further studies of β-lactam antibiotics with in vitro activity against *M. tuberculosis* are warranted to define their role in treatment of tuberculosis.

Resurgence of tuberculosis and outbreaks caused by multiple-drug-resistant (MDR) strains of *Mycobacterium tuberculosis* are reminders that new drugs are still needed [1]. β-lactam antibiotics have been largely overlooked as antituberculous agents. Mycobacterial cell wall [2] has been regarded as an insurmountable barrier to drug penetration. Mycobacterial β-lactamases hydrolyze many penicillins and cephalosporins [3–6]. Early work demonstrating that inhibition of *M. tuberculosis* β-lactamase improved the activity of benzylpenicillin against *M. tuberculosis* [7, 8] went largely unnoticed.

With the advent of potent β-lactamase inhibitors, such as sulbactam and clavulanate, and carbapenems (e.g., imipenem and meropenem), which are not substrate for *M. tuberculosis* β-lactamases, the activity of β-lactam drugs against *M. tuberculosis* in vitro was rediscovered [9–11]. We have shown that in vitro susceptibility to β-lactam antibiotics is consistent with their biochemical properties: they bind target penicillin-binding proteins with high affinity, they penetrate mycobacterial cell wall, and β-lactamase inactivation can be circumvented [12].

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Clinical experience with β-lactam antibiotics for tuberculosis has been minimal. A total of seven patients with tuberculosis caused by MDR strains and treated with the β-lactam antibiotic–inhibitor combination amoxicillin/clavulanate have been reported, and four seemed to respond [13, 14]. The necessity of using combinations of several drugs in the treatment regimen precludes assessment of the individual activity of amoxicillin/clavulanate. We examined whether amoxicillin/clavulanate was active in patients by measuring its effect on recovery of *M. tuberculosis* cfu from sputum of patients with smear-positive pulmonary tuberculosis.

Methods

Determination of Early Bactericidal Activity in Patients with Tuberculosis

These studies were conducted in the General Clinical Research Center of the University of California San Francisco at San Francisco General Hospital (San Francisco) and at Hacettepe University and Atatürk Chest Disease Hospital (Ankara, Turkey). Study protocols were approved by the institutional review boards of the participating institutions. Patients at least 18 years of age with pulmonary tuberculosis and a sputum smear positive for acid-fast bacilli were eligible for enrollment in the study.

Exclusionary criteria were as follows: (1) advanced HIV disease with an AIDS-defining opportunistic infection other than tuberculosis; (2) body weight <75% of ideal; (3) significant hemoptysis, i.e., >25 mL/d; (4) pregnancy or lactation; (5) significant respiratory impairment; (6) evidence of disseminated tuberculosis or tuberculous meningitis; (7) presence of

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Informed consent was obtained from the patients, and guidelines for human experimentation of the U.S. Department of Health and Human Services and those of the authors' institutions were followed in the conduct of this research.

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serious underlying medical illness, such as liver failure, renal failure, or decompensated heart failure; (8) allergy or contraindication for use of study drug; (9) treatment for tuberculosis during the preceding 3 months; and (10) lack of informed consent.

Patients were randomized in an unblinded fashion to one of the three oral regimens, administered for 7 days: (1) amoxicillin/clavulanate, 1,000 mg/250 mg three times a day; (2) ofloxacin, 600 mg once daily; or (3) isoniazid, 300 mg once daily. Two 12-hour, overnight sputum samples were collected for quantitative culture before administration of drug to establish baseline counts. Therapy was initiated and sputum was collected daily for the next 7 days. At the conclusion of the study period, standard isoniazid/rifampin-based four-drug combination regimens were administered to patients, all of whom successfully completed therapy.

Quantitative Culture of Sputum Specimens

A 2-mL portion of the sputum specimen was decontaminated by the N-acetyl-L-cysteine (NALC)/NaOH method [15]. A separate 2-mL portion was treated with either 2 mL of 0.5% NALC or 4 mL of 6.5 mM dithiothreitol (DTT) in phosphate buffer [16]. Samples were processed and quantitatively cultured as described [16, 17] onto both nonselective Middlebrook 7H10 agar (for NaOH-decontaminated samples) and Mitchison's selective Middlebrook 7H11 agar (for NaOH-treated and NALC- or DTT-treated samples).

Colonies were counted after 6 weeks, and the results were expressed as $\log_{10}$ cfu per mL of sputum. Differences in baseline counts among patients were adjusted for by subtracting the average of the two pretreatment days from the value for each treatment day. The mean elimination rate was calculated by linear regression. Differences among groups were analyzed for statistical significance by analysis of variance with the Bonferoni correction for multiple comparisons.

In Vitro Susceptibility Studies

Drug susceptibility was determined by either the agar dilution method [18] or the broth dilution method with a TB System (Becton Dickinson Diagnostic Instrumentation Systems, Sparks, MD) [19, 20]. Amoxicillin/clavulanate was tested by the BACTEC method (Becton Dickinson) at amoxicillin concentrations of 2, 4, and 8 μg/mL plus a fixed concentration of clavulanate at 8 μg/mL.

Results

Forty-five patients were enrolled at the two study sites: 18 patients at San Francisco General Hospital and 27 at the site in Turkey. Fourteen patients were not evaluable: 9 had insufficient growth on culture, 3 were too ill to risk experimental therapy and dropped out of the study, and 2 had nontuberculous mycobacteria isolated. Ten patients were treated with amoxicillin/clavulanate, 10 with ofloxacin, and 11 with isoniazid. Mitchison's agar method was $\sim 0.5-1$ $\log_{10}$ cfu/mL more sensitive (data shown below were calculated from these results) than the NaOH method [20], but elimination rates were virtually identical.

The MICs of isoniazid were ≤ 0.2 μg/mL for isolates from patients who were administered this drug. MICs of isolates from patients given ofloxacin were ≤ 2 μg/mL. MICs of amoxicillin/clavulanate were 4–8 μg/mL.

All three regimens reduced *M. tuberculosis* in sputum. Isoniazid was the most effective agent, reducing cfu over 2 days at a mean ($\pm$SD) rate of 0.60 ± 0.30 $\log_{10}$ cfu/mL per day, vs. 0.32 ± 0.05 and 0.34 ± 0.03 for ofloxacin and amoxicillin/clavulanate, respectively (figure 1 and table 1) ($P < .001$ for isoniazid vs. amoxicillin/clavulanate; $P = .023$ for isoniazid vs. ofloxacin; $P > .1$ for amoxicillin/clavulanate vs. ofloxacin). The rate over 7 days of isoniazid treatment was -0.27 $\log_{10}$ cfu/mL per day, similar to published values of -0.19 to -0.25

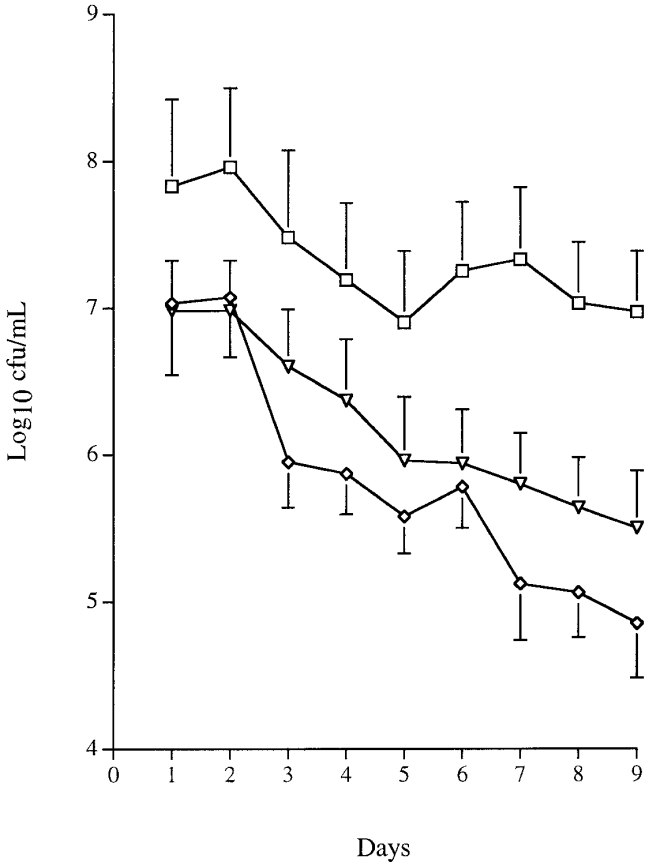

Figure 1. Results of quantitative sputum cultures in patients with pulmonary tuberculosis who received isoniazid (300 mg orally once daily; ◇), ofloxacin (600 mg orally once daily; ▽), or amoxicillin/clavulanate (1,000 mg/250 mg orally three times daily; □) for 7 days. Data are mean ± SE for each of the 2 pretreatment baseline days and each of the 7 treatment days.

Table 1. Mean (± SD) change in $\log_{10}$ cfu of *Mycobacterium tuberculosis* per mL per day in sputa of patients treated with a single agent for 7 days.

Agent	First 24 h	0–2 d	2–7 d	0–7 d*
Amoxicillin/clavulanate (n = 10)	−0.39 ± 0.32	−0.34 ± 0.03	−0.02 ± 0.04	−0.10 ± 0.03
Ofloxacin (n = 10)	−0.41 ± 0.59	−0.32 ± 0.05	−0.16 ± 0.02	−0.20 ± 0.02
Isoniazid (n = 11)	−1.12 ± 1.06	−0.60 ± 0.30	−0.21 ± 0.04	−0.27 ± 0.04

* The square of the correlation coefficient, r^2, for linear regression was 0.517 (P = .04) for amoxicillin/clavulanate, 0.941 ($P < .001$) for ofloxacin, and 0.842 (P = .001) for isoniazid.

$\log_{10}$ cfu/mL per day [16, 21]. Rates ranging from −0.09 to −0.25 $\log_{10}$ cfu/mL per day during the first 2 days of treatment and −0.10 to −0.18 $\log_{10}$ cfu/mL per day during a 2-week treatment period have been reported for rifampin, ethambutol, pyrazinamide, and streptomycin [16].

Thus, elimination rates of −0.34 and −0.10 $\log_{10}$ cfu/mL per day for amoxicillin/clavulanate for the first 2 days of treatment and days 0–7, respectively, compare favorably to those for conventional antituberculous agents other than isoniazid. The magnitude of reduction in mycobacteria over the 7-day treatment was linearly related to the number of organisms present in pretreatment sputum specimens for patients receiving isoniazid (P = .02) or amoxicillin/clavulanate (P = .03) but not ofloxacin.

The reduction in mycobacterial burden with amoxicillin/clavulanate was a direct drug effect. Means and standard deviations for the two pretreatment days were virtually identical (7.83 ± 1.86 [n = 10] and 7.96 ± 1.69), with a reduction in counts occurring only after drug was administered. There was a dose response: in dose-ranging experiments three patients who were treated for 7 days at an amoxicillin/clavulanate dosage of 500 mg/125 mg three times a day had no reduction in cfu (0.00 ± 0.03 $\log_{10}$ cfu/mL per day).

Ampicillin/sulbactam, another β-lactamase-inhibitor combination that has in vitro activity against *M. tuberculosis,* was evaluated in separate studies. Six patients were given ampicillin/sulbactam at a dosage of 440 mg/300 mg orally twice daily for 7 days. The overall rate was −0.16 ± 0.06 $\log_{10}$ cfu/mL per day (r^2 = 0.93; $P < .001$), similar to that observed for amoxicillin/clavulanate.

Discussion

Our results are the first to demonstrate that β-lactam antibiotics have activity against *M. tuberculosis* in patients with tuberculosis. Amoxicillin/clavulanate was tested for three reasons: (1) biochemical and susceptibility data predicted activity; (2) anecdotal clinical data, although inconclusive, suggested that it was effective; and (3) it can be administered orally. Early bactericidal activity was assessed by a quantitative culture method originally devised by Mitchison [16] to rank-order the relative efficacy of antituberculous drugs. We reasoned that this approach would be useful in demonstrating the activity of agents of unknown efficacy, e.g., β-lactam antibiotics; after a relationship between in vitro activity and in vivo efficacy was established for a prototype, it should be generalizable to other agents exhibiting similar activity in vitro. Results with ampicillin/sulbactam confirmed this.

Amoxicillin/clavulanate had early bactericidal activity against *M. tuberculosis* in patients that was similar to that observed for ofloxacin and other agents except isoniazid [16]. However, little reduction in counts occurred after the third day of amoxicillin/clavulanate therapy, for unclear reasons. The phenomenon may be somewhat independent of the drug used because for many agents greatest activity has occurred early during therapy [16]. The effect also could be due to the fact that β-lactam antibiotics are active only against dividing cells and that they do not penetrate into mammalian cells particularly well, such that there is preferential elimination of extracellular organisms, with a plateau due to persistence of intracellular organisms.

This may account for the observation that those patients with more cfu in sputum before treatment had a greater overall reduction in mycobacterial burden over the 7 days of amoxicillin/clavulanate treatment. Patients with smear-positive tuberculosis have a large number of extracellular organisms, and as the burden of organisms increases, correspondingly more may be accessible to drug. A phenotypic change (e.g., increased β-lactamase production) in cells surviving after the first few days could also account for the plateau in activity. Further studies are needed to characterize this phenomenon and its therapeutic implications.

Our results lend support to the position advanced by Mitchison [22, 23] that bactericidal endpoints and rates during the early stages of therapy (i.e., sterilization of sputum cultures at 2 months or reductions in organism burden during the first few days of drug treatment) in a small number of patients can provide extremely useful information about drug efficacy, short of the ''gold standard'' of relapse-free survival. Although efficacy studies utilizing early bactericidal activity will never replace definitive, long-term studies (for example, pyrazinamide, which is a good sterilizing agent, has relatively poor early bactericidal activity), this approach is underutilized as a relatively rapid and efficient means of evaluating new candidates for treatment of tuberculosis.

That amoxicillin/clavulanate reduced the burden of *M. tuberculosis* in the sputum of patients with pulmonary tuberculosis tells us that β-lactam antibiotics have activity. Additional studies of β-lactam drugs in definitive therapy for tuberculosis caused by drug-susceptible and MDR strains of *M. tuberculosis* are warranted to define their role in therapy.

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