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ORIGINAL ARTICLE

In–vitro susceptibility of Mycobacterium tuberculosis to amoxicillin–clavulanate

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ABSTRACT

Background: The evidence of rapid spread of multidrug resistant Mycobacterium tuberculosis (MDR–TB) strains especially in high risk communities, persuade clinicians to find new drugs for this problem. β– lactams with few or no side effects has been reviewed as an alternative drugs for this issue. This study carried out to determine the effectiveness of amoxicillin–clavulanate in a group of Iranian patients.

Materials and methods: Amoxicillin–clavulanate was studied in five different minimal inhibitory concentrations (32-512μg/ml) on 90 clinical strains of Mycobacterium tuberculosis (50 strains were sensitive and 40 were resistant to first-line anti-TB drugs).

Results: All strains were found resistant to amoxicillin–clavulanate in concentration of 32μg/ml. Only in concentration of 64μg/ml, sensitive strains (to first-line anti-TB drugs) were significantly more susceptible to amoxicillin–clavulanate than resistant strains. Five different MICs showed a non-significant difference in susceptibility to amoxicillin–clavulanate between strains with various resistance patterns to first-line anti-TB drugs. However, in 29 strains MIC were above 512μg/ml.

Conclusion: Although amoxicillin–clavulante might be a suitable candidate as a second-line anti-TB drug, further clinical trials are required to draw a firm conclusion.

Keywords: *Mycobacterium tuberculosis, Multidrug resistant, Amoxicillin-clavulanate*.
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INTRODUCTION

The world health organization (WHO) has declared that tuberculosis is a global emergency (1). Prompt detection and treatment of cases are currently the key strategies to control spread of tuberculosis, however, the effectiveness of these control strategies are being limited because of the emergence and spread of drug resistant tuberculosis, particularly MDR-TB (Multidrug resistant tuberculosis) (2). MDR-TB, defined as resistance to at least isoniazid and rifampicin (3), is particularly seen in high risk groups including homeless subjects, drug abusers, alcoholic and HIV-infected patients. This unpleasant situation outlines the urgent need for developing new agents to confront this resistance (4).

Mycobacterium tuberculosis is unique in that it is an intracellular bacterium that is capable of living in dormant state, thus, the clinical prediction based on in-vitro susceptibility results is relatively difficult (5). However, in-vitro measuring of anti-

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TB drug resistance has a considerable correlation with early bactericidal activity in smear positive tuberculosis patients (6) and therefore, can be used to screen drugs for the management of MDR-TB cases.

Mycobacterial cell wall has been regarded as an insurmountable barrier to drug penetration. Mycobacterial β-lactamase hydrolyze most of penicillines and cephalosporins (7). Early studies demonstrated that inhibition of Mycobacterium tuberculosis β-lactamase could improve the activity of benzyl penicillin against M. tuberculosis (8,9). With the advent of potent β-lactamase inhibitors, such as sulbactams, clavulanate and carbapenems (i.e., imipenem, meropenem), which are not substrate for M. tuberculosis β-lactamase, the in-vitro activity of β-lactam drugs against M. tuberculosis was rediscovered (10-12). Clinical experience with β-lactam antibiotics for tuberculosis are scant; however, few surveys have addressed the effectiveness of amoxicillin-clavulanate against M. tuberculosis (13-15). Therefore, further studies are required to evaluate the effectiveness of this regimen on M. tuberculosis strains, based on which, amoxicillin-clavulanate could be safely prescribed as the second-line anti-TB combination therapy, especially in MDR-TB and in critical patients with first-line anti-TB drug-induced hepatitis. On the other hand, amoxicillin-clavulanate is extraordinary prescribed in our country, especially for respiratory infections, thus, it seems favorable to evaluate the susceptibility of clinical strains of M. tuberculosis to this regimen.

PATIENTS and METHODS

This prospective study was conducted in the laboratory of Tuberculosis and Pulmonary Diseases Research Center and Infectious Diseases and Tropical Medicine Research Center affiliated to Tabriz University of Medical Sciences, during June 2003 to August 2004.

The study samples included 90 Mycobacterium tuberculosis strains isolated from the patients with pulmonary tuberculosis (41 males and 49 females). Of these, 50 strains were revealed to be sensitive to first-line anti-TB drugs (isoniazid, rifampicin, streptomycin, and ethambutol), while the remaining 40 strains were resistant to these drugs.

The strains were cultured on Lowenstein-Jensen medium, then in–vitro susceptibility of amoxicillin-clavulanate was tested by double dilutions (from 32μg/ml to 512μg/ml) with disc diffusion technique. Drug resistance was reported if more than 1% bacterial growth was observed on antibiotic-enriched media as compared with antibiotic-free media. Middlebrook 7H9 agar medium with disc diffusion mode was used as the control medium for some strains. Determination of susceptibility was achieved by proportional method.

Data were analyzed with chi-square test. Descriptive findings were reported as percentage. The level of significance was considered as $p<0.05$ for all tests. SSPS software (version 11.5, SPSS Inc., USA) was used for all analysis.

RESULTS

In total, 90 strains of M. tuberculosis with various susceptibility patterns to first-line anti-TB drugs (table 1) were included. Surprisingly, all of the strains were found resistant to amoxicillin–clavulanate in MIC 32μg/ml; however, in MIC 64μg/ml, 51 strains (29 resistant and 22 sensitive strains to first-line anti-TB drugs) showed resistance to amoxicillin-clavulanate. In this group, resistant strains (to first-line anti-TB drugs) demonstrated a significantly higher resistance to amoxicillin-clavulanate when compared with sensitive strains.

In MIC 128μg/ml, 34 strains (15 resistant and 19 sensitive strains to first-line anti-TB drugs) were revealed resistant to amoxicillin-clavulanate.

Furthermore, 31 strains (15 resistant and 16 sensitive strains to first-line anti-TB drugs) were resistant to amoxicillin–clavulanate in MIC 256μg/ml and, finally, in MIC 512μg/ml 29 strains (15 resistant and 14 sensitive strains to first-line anti-TB drugs) were found resistant to amoxicillin–clavulanate (table 2).

Table 1. *Susceptibility pattern of 90 strains of M. tuberculosis to first-line anti-TB drugs according to the sex (M: male, F: female)*

Susceptibility pattern	Number of strains
Sensitive to first-line anti-TB drugs	50(24 M, 26 F)
Resistant to first-line anti-TB drugs	40(17 M, 23 F)
Resistant to one of the first-line anti-TB drugs	26(11 M, 15 F)
Resistant to two of the first-line anti-TB drugs	7(3 M, 4 F)
Resistant to three of the first-line anti-TB drugs	6(3 M, 3 F)
Resistant to all of the first-line anti-TB drugs	1 (F)
Resistant to *isoniazid*	20(8 M, 12 F)
Resistant to *rifampicin*	9(3 M, 6 F)
Resistant to *streptomycin*	28(12 M, 16 F)
Resistant to *ethambutol*	5(3 M, 2F)

Table 2. *Number of resistant strains to amoxicillin-clavulanate at different MICs*

Susceptible pattern to first-line anti-TB drugs	Minimal inhibitory concentration (μg/ml)				
	32	64	128	256	512
Sensitive	50	22	19	16	14
Resistant	40	29	15	15	15

Despite differences in the resistance rates of first-line anti-TB resistant strains to amoxicillin-clavulanate in MICs 128, 256, and 512μg/ml, they did not reach a statistically significant level (p=0.6, 0.4, and 0.2, respectively). Similarly, in strains with different pattern of resistance to first-line anti-TB drugs, the susceptibility rate to amoxicillin-clavulanate did not differ significantly in MICs 64, 128, 256 and 512μg/ml (p=0.5, 0.3, 0.2, and 0.2, respectively). Meanwhile, there was non-significant differences in susceptibility to amoxicillin-clavulanate between isolated strains of both sexes (p=0.9).

DISCUSSION

β-lactams have been prescribed as antibiotic for human use during the past decades. They are widely used because of their broad spectrum of activity with few side effects. In-vitro studies demonstrated that β-lactams have anti-mycobacterial activity; however, they have a synergetic effect when combined with ethambutol (15). Moreover, the clinical data indicated that β-lactams have a promising prospect for use in treatment of patients with MDR-TB (15).

Our results demonstrate that amoxicillin-clavulanate is an effective combination against M. tuberculosis isolated from patients with pulmonary tuberculosis. Amoxicillin-clavulanate was tested for three reasons:

- Biochemical and susceptibility data predicted activity;
- Anecdotal clinical data, although inconclusive, suggested that it was effective;
- Drug 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 anti-TB drugs. Amoxicillin-clavulanate has an early bactericidal activity against M. tuberculosis similar to what observed for ofloxacin and other agents except for isoniazid. However, following the third day of treatment, a reduction was occurred in count, for unclear reason. This phenomenon may be somewhat independent of the drug type. In addition, β-lactams are active only against dividing microorganisms, thus, they do not penetrate well in mammalian cells so that there is preferential elimination of extra-cellular organisms, with a plateau, due to persistence of intracellular organisms (16).

Clavulanate failed to show antibacterial activity against M. tuberculosis due to the lack of affinity for penicillin binding proteins (PBPs) (17), but it is a powerful inhibitor of its β-lactamase (18). Indeed, results were not simply due to an additive effect unrelated to β-lactamase inhibition, since tested β-lactamase bound to M. tuberculosis PBPs at therapeutically achievable concentration (18), while they are also reinforced by the fact that clavulanate did not reduce aztreonam MICs at the assayed concentrations (18).

β-lactam antibiotics could be efficiently prescribed for MDR tuberculosis. Chambers et al evaluated positively not only the factors that determine the susceptibility of M. tuberculosis strains to β-lactam antibiotics (PBPs and permeability) but also the macrophage penetration (18). Their study revealed that amoxicillin-clavulanate in the MIC of 408μg/ml in BACTEC medium had the best bactericidal activity. Similarly, in our experience which was achieved in Lowenstein-Jensen (L-J) medium, the MIC of the most resistant strain was above 256μg/ml. In another study, it was shown that ampicillin-sulbactam has higher MIC in L-J media in comparison to 7H11 agar media, which can be due to inactivation of this and other antibiotics in the chemical heat production of L-J media (19). Therefore, new methods such as BACTEC radiometric method may have promising results and can be more reliable (19).

Nakagawa and his colleagues found no correlation between β-lactam activity and susceptibility pattern of strains to first-line anti-TB drugs (20). In another study from Texas University in U.S., sensitive and MDR-TB strains were examined with multiple antibiotics and a susceptibility pattern was determined (21). However, no correlation was found between sensitivity to β-lactams and sensitivity or resistance of strains to first-line anti-TB drugs (21). This was in agreement with ours. We could not find significant correlation between sensitivity to amoxicillin–clavulanate in doses of 125, 256, and 512μg/ml and susceptibility pattern of strains to first-line anti-TB drugs, however, such correlation was significant in doses of 64μg/ml.

A relationship between in-vitro activity and in-vivo efficacy of β-lactams has been established by prior investigators and their findings for ampicillin-sulbactam confirm this fact (16). Furthermore, the amoxicillin–clavulanate combination has been successfully prescribed for MDR-TB patients, particularly when the combination was administered with second-line anti-TB drugs (22). Amoxicillin–clavulanate was shown to have a synergistic activity on MDR isolates of M. tuberculosis in-vitro condition (23). Only few reports are available on the interaction between β-lactams and first-line anti-TB drugs. Although in-vitro susceptibility studies must be conducted as the initial step to take primary decision about clinical efficacy, clinical trials, especially combination therapy, should be performed as well.

In summary, amoxicillin–clavulanate was effective against sensitive and resistant strains. There was no significant difference in susceptibility pattern of these two strains to amoxicillin–clavulanate except for doses of 64μg/ml in Lowenstein–Jensen medium. In order to clarify the future role of β-lactams for TB management, surveying additional strains and evaluating the activities of β-lactams in combination with other anti-mycobactericidal drugs could be of great help.

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