Minimal inhibitory concentrations of sulbactam/ampicillin against drug sensitive and drug resistant isolates of *Mycobacterium tuberculosis*

C. N. Paramasivan*, P. Venkataraman and D. Herbert

Tuberculosis Research Centre, Indian Council of Medical Research, Spur Tank Road, Chetput, Madras 600 031, India and Department of Bacteriology, Tuberculosis Research Centre, Indian Council of Medical Research, Spur Tank Road, Chetput, Madras, 600 031, India (*Reprint address)

Key words: *Mycobacterium tuberculosis,* sulbactam/ampicillin, *in vitro* antimicrobial testing, minimal inhibitory concentration

Abstract

A total of 92 isolates of *Mycobacteriurn tuberculosis* consisting of equal numbers of sensitive and resistant strains was tested for their susceptibility to sulbactam and ampicillin (in the ratio of 1:2) on Lowenstein-Jensen (LJ) and 7H11 agar media. The geometric mean MIC was 63.97 µg/ml for the drug sensitive strains and 65.92 µg/ml for the resistant strains, and the overall mean was 65.01 µg/ml. The high MIC on LJ medium could be attributed to the higher protein content which resulted in greater binding of sulbactam/ampicillin. On the other hand, the geometric mean MIC on 7H11 medium was 26.73 µg/ml for sensitive strains and 23.82 µg/ml for resistant strains; the overall mean being 25.23 µg/ml. Although these MICs of sulbactam-ampicillin are higher than those reported earlier, they can be easily achieved in serum. Further studies on experimental tuberculosis and in humans will be needed to prove the efficacy of sulbactam/ampicillin in the treatment of patients with multidrug resistant tuberculosis.

Introduction

Multidrug-resistant tuberculosis (MDR-TB) is rapidly emerging as a major problem in the treatment of tuberculosis both in the developed countries and in the developing countries (Riley, 1993; Nagpaul, 1994). The prognosis for chemotherapy in tuberculosis patients with *Mycobacterium tuberculosis* resistant to rifampicin and isoniazid is poor (Hong Kong Chest Services/British Medical Research Council, 1992; Goble *et al.*, 1993; Rema Mathew *et al.*, 1993). In these patients administration of effective treatment with regimens containing at least two or three drugs to which the organisms are sensitive is essential for curing the disease and blocking the transmission (Goble *et al.*, 1993).

A large number of new drugs has been introduced in recent years for the treatment of bacterial, parasitic, fungal and viral diseases. In contrast, very few major new antituberculosis agents have become available since rifampicin was introduced in the early 1970s (O'Brien and Snider, 1985). The current TB epidemic associated with HIV infection and the occurrence of MDR-TB have led to the need for rapid development of new antituberculosis drugs and it has become

necessary to screen new drugs for antituberculosis activity (Mitchison *et al.*, 1988; Parenti, 1989). Hence, systematic evaluation of new antituberculosis drugs is being undertaken at this Centre (Selvakumar *et al.*, 1992; Venkataraman *et al.*, 1992a,b,c; Herbert *et al.*, 1997; Kamala *et al.*, 1997).

Even though the ß-lactam drugs are ineffective against mycobacteria which are ß-lactamase producing organisms, combinations of ß-lactam antibiotics with ß-lactamase inhibitors have been shown to have activity against these organisms (Casal *et al.*, 1987; Sorg and Cynamon, 1987; Parenti, 1989; Nadler *et al*, 1991). Among these, sulbactam/ampicillin (SA) has been shown to have a minimal inhibitory concentration (MIC) of 8 µg/ml against *M. tuberculosis* isolates (Sorg and Cynamon, 1987).

The present study was carried out to determine the MICs of SA for drug sensitive and drug resistant *M. tuberculosis* isolates from southern Indian patients both on Lowenstein-Jensen (LJ) and Middlebrooks 7H11 media to see whether the high protein content of LJ medium would have any effect on the MICs.

Materials and methods

Bacterial strains

A total number of 46 *Mycobacterium tuberculosis* isolates from tuberculosis patients admitted to various clinical trials at the Tuberculosis Research Centre (TRC), Madras, which were sensitive to streptomycin (S), isoniazid (H) and rifampicin (R) and 46 isolates which were SHR/HR resistant were used in the study.

Drug containing medium

Pure powders of sulbactam and ampicillin (kindly provided by Unichem Laboratories Ltd, India) were dissolved in sterile distilled water and added to LJ medium before inspissation of the slopes and sterile 7H11 medium containing oleic acid-albumin-dextrose complex (OADC) enrichment before pouring the plates to get final concentrations of 1, 2, 4, 8, 16, 32, 64 and 128 µg/ml ampicillin with a sulbactam:ampicillin ratio of 1:2.

Sensitivity testing

From 2- to 3-week-old cultures of each *M. tuberculosis* strain on LJ, standard bacterial suspensions of 4 mg/ml were prepared in bijoux bottles with sterile distilled water and beads and shaken for uniform distribution. One loopful each of the suspension was inoculated onto two drug-free LJ slopes and one drug containing-LJ slope of each concentration with a 3 mm diameter loop, and 10 µl each was inoculated onto duplicate sectors of drug-free and drug-containing 7H11 plates

with a micropipette. The inoculated media, of 7H11 plates in polythene bags, were incubated at 37°C and read at the end of 4 weeks. The lowest concentration of the drug which inhibited growth to less than twenty colonies compared with at least numerous discrete colonies (++ growth) on drug-free medium was taken as the minimal inhibitory concentration (MIC).

Results

The frequency distribution of MICs of SA on LJ medium is presented in Table 1. The MICs ranged from 8 to >128 µg/ml. For the majority of the strains, SA had high MICs on LJ medium. Of the 92 strains tested on LJ medium, the MIC was >64 µg/ml for 66 (71.7%) and the overall geometric mean MIC (GM) was 65.01 µg/ml. The GM was 63.97 µg/ml for the 46 drug sensitive strains and 65.92 µg/ml for the 46 drug resistant strains. The difference in GM between the drug sensitive and the drug resistant strains was not significant ($p > 0.5$).

The frequency distribution of MICs of SA on 7H11 medium is shown in Table 2. The MICs of SA were lower on 7H11 compared with that on LJ media and ranged from 8 to 64 µg/ml. The overall GM for the 64 strains tested on 7H11 was 25.23 µg/ml which was significantly lower than that on LJ medium ($p < 0.001$). For none of the strains tested, the MIC was >64 µg/ml and for 47 of the 64 strains (73.4%) the MIC was $\leq$ 32 µg/ml. The GM was 26.73 µg/ml for the 31 drug sensitive strains and 23.82 µg/ml for the 33 drug resistant strains. Again, this difference in GM of drug sensitive and drug resistant strains was not significant ($p > 0.5$).

Table 1 Frequency distribution for MIC of sulbactam/ampicillin in µg/ml of ampicillin against SHR sensitive and SHR/HR resistant isolates of *M. tuberculosis* on LJ medium

M. tuberculosis isolates	MIC (µg/ml of ampicillin) of SA on LJ: 8	16	32	64	128	>128	Total	GM
SHR sensitive	2	1	8	20	14	1	46	63.97
SHR/HR sensitive	0	2	13	14	15	2	46	65.92
Total	2	3	21	34	29	3	92	65.01

Table 2 Frequency distribution for MIC of sulbactam/ampicillin in µg/ml of ampicillin against SHR sensitive and SHR/HR resistant isolates of *M. tuberculosis* on 7H11 medium

M. tuberculosis isolates	MIC (µg/ml of ampicillin) of SA on 7H11: 8	16	32	64	128	Total	GM
HR sensitive	5	8	8	10	0	31	26.73
SHR/HR resistant	6	9	11	7	0	33	23.82
Total	11	17	19	17	0	64	25.23

Discussion

Abraham et al. (1941) observed that *M.* tuberculosis was not inhibited *in vitro* by high concentrations of penicillin. The penicillinase activity of *M. tuberculosis* was subsequently identified (Hand and Bains, 1949; Soltys, 1952). Later, this penicillinase was shown to be a ß-lactamase in studies on the effect of ß-lactamase susceptible and resistant antibiotics on the *in vitro* growth of *M. tuberculosis* (Kasik, 1964). It was also shown that a combination of ß-lactamase stable oxacillin with penicillin was more effective than either drug alone in inhibiting mycobacterial growth and that this penicillin-sparing effect was due to ß-lactamase inhibition (Kasik *et al,* 1967). This activity of penicillin+ lactamase inhibitor combination on *M. tuberculosis* was also demonstrated in a murine model of tuberculosis (Kasik *et al.,* 1966).

Clavulanic acid and sulbactam are specific inhibitors of ß-lactamase which are currently used clinically to protect ß-lactamase susceptible antibiotics (Bush, 1988). Of these two, clavulanic acid is a natural product while sulbactam was developed later (Brown *et al.,* 1976; English *et al.,* 1978). Augmentin is an oral preparation of clavulanic acid in combination with amoxicillin. Timentin is a parental preparation of clavulanic acid with ticarcillin while Sulbacin is a parental preparation of sulbactam with ampicillin. Ticarcillin in combination with clavulanic acid has been studied *in vitro* against various mycobacteria (Casal *et al.,* 1989). All the *M. tuberculosis, M. bovis* and *M. africanurn* strains were susceptible at 32 µg/rnl or less of ticarcillin in combination with 5 µg/ml of clavulanic acid. The MIC of amoxycillin/clavulanic acid against *M. tuberculosis* has been reported

to be 4.4 µg/ml and that of sulbactam/ampicillin to be 8.8 µg/ml (Sorg and Cynamon, 1987) In mouse foot-pad, while amoxycillin clavulanic acid combination had no effect on the growth of *M. leprae*, the same concentration of SA was found to suppress their multiplication (Prabhakaran *et al.,* 1992).

In the present study, the mean MICs of SA against *the M. tuberculosis* were significantly lower when tested on 7H11 medium compared with LJ medium. This was probably due to the higher protein content and in turn higher binding of sulbactam and ampicillin in LJ medium. Protein binding in serum has been reported to be 28% for ampicillin and 38% for sulbactam (Wise, 1986). In connection with the results on 7H11 medium, the overall mean MIC of SA for all the *M. tuberculosis* strains tested was 25.23 µg/ml, with no significant difference between the GM of SHR/HR resistant and SHR sensitive strains indicating that cross resistance does not exist between SA and SHR.

The MICs of SA in the present work were higher than the MIC of 8 µg/ml reported earlier by Sorg and Cynamon (1987). However, even these higher levels can be easily achieved in serum because peak serum concentrations of >90 µg/ml of ampicillin have been reported after intravenous dosage regimen of 2 g of ampicillin and 1 g of sulbactam (Foulds *et al.,* 1983), and from 5l-82 µg/ml and > 15 µg/ml of ampicillin, respectively, after intravenous and intramuscular administration of 1 g of ampicillin and 0.5 g of sulbactam (Foulds, 1986; Ripa *et al.,* 1990). The other favourable features of SA are that useful concentrations of SA can be achieved in pus, sputum and middle ear fluid. Moreover, penetration of SA into CSF is increased in patients with bacterial meningitis (Rodriguez *et al.,* 1986; Foulds, 1986) and this combination has been remarkably free of major side effects (Lees *et al.,* 1986).

Our investigation shows that SA could be useful in the treatment of tuberculosis, particularly in drug resistant patients. It has already been shown that the multiplication of *M. leprae* in foot pads of experimentally infected mice is suppressed by intramuscular administration of SA and that SA also inhibits the growth of drug resistant *M. leprae* which could grow in the presence of rifampicin or dapsone (Prabhakaran *et al.,* 1992). It has been shown that on log-phase cultures of *M. tuberculosis* in 7H9 liquid medium SA has bactericidal action similar to that of rifampicin and isoniazid (Herbert *et al.,* 1997). Further studies are required on the activity of SA on *M. tuberculosis* in mouse models and in tuberculosis patients.

Acknowledgement

Sulbactam and ampicillin were kindly provided by Unichem Laboratories Ltd, India.

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Accepted 3 February 1997

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