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Review article

Anti-tuberculosis activity of -lactam antibiotics: prospects for the treatment of multi-drug-resistant tuberculosis

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Abstact

Tuberculosis (TB) is a global problem and the spread of multi-drug-resistant (MDR) TB, defined as resistance to at least isoniazid and rifampicin, is a formidable challenge for control programs. There are no widely accepted standard regimens for the treatment of MDR-TB. However, a considerable proportion of cases can be cured with a regimen containing both first-line and second-line drugs. In order to prevent the development of resistance to drugs already in use and also to try to develop a standard regimen, there is need to look for new drugs. This review is prepared to show results on the anti-TB activity of -lactam antibiotics. -Lactams are among the oldest drugs with little or no side effects. Both in vitro studies and clinical data indicate that -lactams have a promising activity for use in the management of MDR-TB. More studies are required to define the interaction of -lactams with other first-line and second-line drugs, and to clearly show the clinical usefulness in the management of MDR-TB.

Introduction

The World Health Organization (WHO) has declared that TB is a global emergency (1). A rapid detection and treatment of cases is currently the key strategy to control the spread of TB. However, the effectiveness of this control strategy is being limited because of the emergence and spread of drug-resistant TB, particularly MDR TB. MDR-TB, defined as resistance to at least isoniazid and rifampicin (2), is more commonly seen in cases with treatment failure (3) and its spread is enhanced in HIV-infected individuals or groups (4, 5).

The tubercle bacillus is unique in that it is intracellular and it has a capacity to live in dormancy. These unique characteristics make the clinical prediction from in vitro susceptibility results difficult (6). However, in vitro measures of anti-TB drug resistance has a considerable correlation with early bactericidal activity in smear-positive TB patients (7) and, therefore, can be used to screen drugs for the management of MDR-TB cases.

There are, today, no generally accepted standard regimens for the management of MDR-TB. There are, however, proposed ‘third-line' regimens for patients with different resistance pattern (8, 9, 10). Several studies have shown that it is possible to cure a significant proportion of MDR-TB cases if a combination of most effective second-line drugs and first-line drugs is used (11, 12, 13). It is expected that the cure rate decreases when the bacilli develop resistance to

more first-line or second-line drugs and this makes the search for new drugs crucially important. This review is prepared to show previously published findings on the anti-TB activity of -lactam antibiotics and to point out the prospects of these drugs in the treatment of MDR-TB.

-Lactams: classifications, mechanisms of action, mechanisms of resistance -Lactam antibiotics are among the oldest antibiotics for human use. They do not cause serious side effects, except for allergic reaction in some individuals (14). Based on their chemical structures, they are usually classified into four groups (i.e., penicillins, cepha-losporins, carbapenems, and monobactams). Each group could be further classified according to spectrum of antimicrobial activity (15). They act by binding to, and inhibiting, transpeptidase enzymes (also called penicillin binding proteins [PBPs]) which play a critical role in the cell wall synthesis (16, 17).

Bacteria develop resistance to these antibiotics by one or combinations of the following mechanisms (18): 1) production of -lactamases, 2) altering the affinity or concentration of target proteins (i.e., PBPs), and 3) decreasing the permeability. Myco-bacterium tuberculosis produces -lactamases. The -lactamase(s) of M. tuberculosis display both penicillinase and cephalosporinase activities (19, 20, 21). Based on the sequence of the encoding gene, the -lactamase from a reference strain of M. tuberculosis (H37Ra) was classified as a class A (22) which includes different subgroups of 2b -lactamases with different properties (23).

The permeability of mycobacterial cell wall to -lactams is lower than that of gram-negative organisms (e.g., Eschrichia coli) by three orders of magnitude (24, 25). A limited amount of -lactam that diffuses across the cell wall could easily be degraded by -lactamases (24, 26).

In vitro activity of -lactams on M. tuberculosis

The in vitro susceptibility of M. tuberculosis to -lactams was studied by different methods that use Lowenstein Jensen (LJ) media, Middlebrook agar or liquid media. Some of the published discrepancies in the minimum inhibitory concentration (MIC) of -lactams could be because of the methods used. Parmasivan et al. (27) showed that the combination of ampicillin and sulbactam has a

better anti-TB activity in Middlebrook 7H11 media than in LJ media. This could be because of the heat inactivation of these drugs during the preparation of LJ media. It was also shown that a significant reduction in the activity of -lactams and -lactamase inhibitors may occur even at 37oC (28). Therefore, rapid methods, such as the BACTEC radiometric system, may give a more reliable result (29).

Tables 1 and 2 show the results of studies that investigated the in vitro activity of -lactams on M. tuberculosis. The lowest MIC90 of amoxicillin, ampicillin, and ticarcillin on bacteria other than mycobacteria were 0.015 mg/L (for Streptococcus pneumoniae), 0.015 mg/L (for Streptococcus pyogenes), and 0.25 mg/L (for Haemophilus species), respectively (30). The relative in vitro resistance of M. tuberculosis to penicillins and cephalosporins, as shown in Tables 1 and 2, could be due to both cell wall permeability and -lactamase activity. Other -lactams, such as imipenem and meropenem, are also more active on non-mycobacterial species. The MIC90 of these drugs on the majority of M. tuberculosis strains tested was about 2 mg/L

(31, 32) whereas the lowest MIC90 of imipenem and meropenem on other bacteria were 0.007 mg/L (for Moraxella catarrhalis) and 0.03 mg/L (for Klebsiella pneumoniae), respectively (30).

-Lactamase inhibitors, such as clavulanic acid and sulbactam, are important to inhibit the enzymatic inactivation of -lactams. These combinations markedly decrease the MIC of -lactam antibiotics (33, 34, 35). Using logarithmic- and stationary-phase cultures, it was shown that one of these combinations (i.e., ampicillin-sulbacatam) at a concentration of 15 mg/L had bactericidal activity equivalent to 1 mg/L isoniazid or rifampicin on exponential phase cultures of M.tuberculosis but appreciable bactericidal activity was not seen in stationary phase cultures (36). This indicates that this combination can be useful particularly in the early phase of anti-TB treatment.

The ability of -lactams to penetrate the macrophages and act on the intracellular tubercle bacilli was also studied. Chambers et al (31) reported that 8 mg/L amoxicillin in combination with clavulanic acid or sulbactam significantly inhibited the multiplication of M. tuberculosis (H37Ra) in a mouse peritoneal macrophage cell line (J774 cells).

In vitro interaction between -lactams and first-line anti-TB drugs

Only few reports are available on the interaction between -lactams and first-line anti-TB drugs. Amoxicillin-clavulanate was shown to have a synergistic activity on MDR isolates of M. tuberculosis in vitro (37).

Similarly, a synergistic effect between cefepime and ethambutol was seen in 15 % MDR isolates of M. tuberculosis (38). The mechanism of interaction between -lactams and ethambutol was investigated using 3H-penicillin but no conclusive results were obtained on the effect of ethambutol on the binding to PBPs or the activity of -lactamase (37).
There are no reports on the interaction of -lactams with other first-line drugs on M. tuberculosis. However, studies on other bacteria showed that streptomycin and rifampicin act synergistically with -lactam drugs (39, 40, 41). It was reported that rifampicin and oxacillin had antagonistic interaction on Staphylococcus aureus (42). However, earlier report showed that the concentration of oxacillin affects the interaction with rifampicin on gram-positive bacteria and a synergistic interaction was obtained when the oxacillin/rifampicin ratio is low (40).

The use of -lactams in the treatment of MDR-TB Amoxicillin-clavulanate in combination with other drugs was used for the treatment of patients with MDR-TB (47, 48, 49). Only two patients received amoxiciilin-clavulanate (i.e., 500 mg/125 mg four times a day for one patient, and 1000 mg/250 mg four times a day for the second patient) in a study by Nadler et al. (48). Other drugs, such as streptomycin, ethionamide, cycloserine, and isoniazid, were co-administered for both patients and sputum conversion was achieved in both patients after one and three months of therapy. In a study by Yew et al. (49), five patients received amoxicillin-clavulanate (500 mg/250 mg three times a day for three patients, and 750 mg/375 mg twice a day for two patients) along with other drugs such as cycloserine (all patients), ethionamide (three patients), amikacin (for two patients), pyrazinamide and ethambutol (one patient). These patients were treated for 10 to 15 months and sputum cultures were consistently negative for two patients after one month therapy. MDR-TB patients in studies by Nadler et al.(48) and Yew et al. (49)

showed clinical improvement after treatment with regimens including a -lactam antibiotic. However, the evidence from these studies was not very convincing since the design did not allow logical interpretation of the value of addition of a -lactam antibiotic.

In a recent study by Chambers et al. (50), 31 patients were randomized into one of the three groups to receive a seven day oral regimen. Base line count of colony forming units (CFU) per ml of sputum was performed before administration of drug by culturing two overnight sputum samples. Ten patients were treated with amoxicillin-clavulanate (1000 mg/250 mg three times a day), ten other patients with ofloxacin (600 mg daily) and eleven patients with isoniazid (300 mg daily). The reduction in CFU was determined for the different groups by culturing a sputum sample collected daily. The mean (± SD) rate of reduction over two days was 0.34 ± 0.03 log10 CFU/ml for amoxicillin-clavulanate, 0.32 ± 0.05 and 0.60 ± 0.30 for ofloxacin and isoniazid, respectively. This shows that amoxicillin/ clavulanate has a good early bactericidal activity. The MIC of amoxicillin (with clavulanic acid) determined by the BACTEC method for strains isolated from patients in this study was 4-8 mg/L (50).

Conclusions

-Lactams are among the oldest antibiotics for human use. They are widely used because of their broad spectrum of activity with little or no side effect. In vitro studies demonstrated that -lactams do have anti-mycobacterial spectrum and combining them with ethambutol, which is one of the first-line anti-TB drugs, can increase the effect on M. tuberculosis. Moreover, the clinical data on these drugs indicated that -lactams have a promising prospect for use in the treatment of patients with MDR-TB. There is need for the further investigations of the interaction of these drugs with first-line anti-TB drugs and to conduct a more conclusive clinical trial on MDR-TB cases.

Tables

Table 1: **Summary of results on the *in vitro* activity of penicillins against *M. Tuberculosis***

Drug	Strains Tested (n)	MIC_{90} (mg/L)	Method Used	References
Amoxicillin	15	8	Broth dilution	34
Amoxicillin	11	>128	Agar dilution	23
Amoxicillin	35	>8	Broth dilution	35
Amoxicillin	6	16	Broth dilution[a]	31
Amoxicillin	24	>256	Agar dilution	43
Amoxicillin	13	>32	Broth dilution	44
Amoxicillin	6	>16	Broth dilution[a]	31
Benzyl penicillin	32	100	Broth dilution	20
Ticarcillin	21	>128	Agar dilution	45
Ticarcillin	28	>64	Broth dilution	35

Amoxicillin + Clavulanate	15	2	Broth dilution	34
Amoxicillin + Clavulanate	30	16	Agar dilution	23
Amoxicillin + Clavulanate	35	4	Broth dilution	35
Amoxicillin + Clavulanate	6	2	Broth dilution[a]	31
Amoxicillin + Clavulanate	24	16	Agar dilution	43
Amoxicillin + Clavulanate	13	4	Broth dilution	44
Ampicillin + Sulbactam	13	8	Broth dilution	44
Ampicillin + Sulbactam	5	2	Broth dilution[a]	31
Ampicillin + Sulbactam	92	128	LJ	27
Ampicillin + Sulbactam	92	64	Agar dilution	27
Ticarcillin + Clavulanate	21	32	Agar dilution	45
Ticarcillin + Clavulanate	28	32	Broth dilution	53

[a] BACTEC radiometric system

Table 2: **Summary of results on the *in virto* activity of cephaloporins against *M. tuberculosis***

Drugs	Test strains (n)	MIC_{90} (mg/L)	Method	References
Cefoperazone	6	>16	Broth dilution[a]	31
Ceforanide	65	50	Broth dilution[a] /Agar	46
Cefotaxme	24	128	Agar dilution	43
Cefoxitin	6	>16	Broth dilution[a]	31
Ceftriaxone	6	16	Broth dilution[a]	31
Cefriaxone	24	256	Agar dilution	43
Ceephaloridine	6	8	Broth dilution[a]	31
Cephalothin	32	10	Broth dilution	20
Cephalothin	6	>16	Broth dilution[a]	31
Cefoxitin + Clavulanate	4	8	Broth dilution[a]	31
Ceftriaxone + Clavulanate	6	4	Broth dilution[a]	31
Ceftriaxone + Clavulanate	24	16	Agar dilution	43

[a] BACTEC radiometric system

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