Meropenem-clavulanic acid has high \textit{in vitro} activity against multidrug-resistant \textit{Mycobacterium tuberculosis}

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Abstract

We investigated the activity of meropenem-clavulanic acid (MEM-CLA) against 68 *M. tuberculosis* isolates. We included predominantly multi- and extensively drug-resistant tuberculosis isolates (MDR/XDR-TB), since the activity of MEM-CLA for resistant isolates has not been studied extensively previously. Using Middlebrook 7H10 medium, all but four isolates showed a MIC distribution of 0.125-2 mg/L of MEM-CLA, below the non-species related breakpoint for MEM of 2 mg/L defined by EUCAST. MEM-CLA is a potential treatment option for MDR/XDR-TB.
Multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) is unrelentingly increasing worldwide. As MDR/XDR-TB is notoriously difficult to treat, already approved drugs, such as trimethoprim-sulfamethoxazole, are being investigated as treatment options (1). The activity of penicillin against *Mycobacterium tuberculosis* was investigated already in the 1940s (2), but β-lactams were deemed ineffective. However, it was later shown that the β-lactamase BlaC causes the hydrolysis of β-lactam antibiotics (3-6). This hydrolysis can be inhibited by the β-lactamase-inhibitor clavulanic acid (CLA), which irreversibly inactivates BlaC (6, 7). Meropenem (MEM) is a β-lactam antibiotic of the carbapenem group. Even though MEM is a relatively poor substrate for BlaC (8), MEM on its own shows conflicting evidence regarding antituberculous activity (9-12). Hence, the combination meropenem-clavulanic acid (MEM-CLA) is an interesting treatment alternative for drug-resistant TB, but there is a lack of both in vitro and in vivo studies for this combination. The aim of this study was to investigate the *in vitro* effect of MEM-CLA against *M. tuberculosis*, predominantly MDR/XDR-TB isolates.

Using Middlebrook 7H10, 94 *M. tuberculosis* isolates were studied. The isolates consisted of clinical isolates and isolates submitted to the Public Health Agency of Sweden for proficiency drug susceptibility testing, with all isolates being globally sourced. A total of 68 isolates showed sufficient growth to be studied further and they were categorized into three resistance groups and consisted of 36 MDR-TB, 13 XDR-TB and 19 with mixed resistance patterns (non-MDR/XDR-TB). H37Rv (ATCC 27294) was used as control. Middlebrook 7H10 agar (BD AB, Stockholm, Sweden) enriched with OADC (10% oleic acid/albumin/dextrose/catalase) and 5% glycerol was prepared in 14 cm Petri dishes, each dish containing 60 ml of agar. A stock solution was prepared by diluting MEM with water, and then applied in serial two-step dilutions, reaching a final antibiotic concentration range of 0.002-512 mg/L of MEM. CLA was added to all dishes at a concentration of 64 mg/L, in
order to ensure a sufficient concentration of the β-lactamase inhibitor throughout the whole experiment. Due to the short half-life of CLA in solid media (1.4 days) (13), the concentration of CLA after the first week of our experiment, was expected to be around 2 mg/L, similar to the concentrations of CLA seen in serum after a dose of 500 mg/125 mg of amoxicillin-clavulanic acid (AMX-CLA) (14).

The MICs of the 68 M. tuberculosis isolates were determined by inoculating bacterial suspensions onto the Middlebrook 7H10 medium using a 96-stick replicator, as previously described (15). The mycobacterial cultures were incubated at 37°C and growth was evaluated after three weeks. The MIC was defined as the lowest concentration with less growth than the 1:100 diluted control. Sterile water was used as a negative control (15).

The MIC distribution of MEM-CLA (expressed as the concentration of MEM) was 0.125-32 mg/L (Figure 1). All but four isolates had MICs of ≤ 2 mg/L of MEM-CLA, which is the non-species related susceptibility breakpoint of MEM defined by EUCAST (16). The MIC90 and the MIC of the control strain H37Rv were both 1 mg/L.

Overall, we observed low MICs of MEM-CLA against M. tuberculosis in vitro, even for highly drug-resistant isolates. The majority of the isolates followed a Gaussian wild-type distribution, with MICs below or equal to the EUCAST non-species related susceptibility breakpoint of MEM of 2 mg/L (16). Four isolates (two MDR-TB and two XDR-TB isolates) had very high MIC levels (16 and 32 mg/L), a drug concentration unlikely to be achieved in serum even with high-dose regimens (17). The four M. tuberculosis isolates with very high MICs (16 and 32 mg/L) of MEM-CLA were already highly resistant to other pharmacologically unrelated anti-TB drugs, which could be due to a generally lower permeability, as previously suggested for highly resistant M. tuberculosis isolates (13).
β-lactam antibiotics have little intrinsic activity against *M. tuberculosis* (18-21), but are effective *in vitro* when combined with a β-lactamase inhibitor (18, 20-23). The combination of β-lactam antibiotics and β-lactamase inhibitors has shown anti-TB effect in humans (24, 25), rodents (26), within macrophages (10) as well as against non-replicating *M. tuberculosis* *in vitro* (8). MEM has poor intrinsic activity (12), but is bactericidal *in vitro* when combined with CLA (8, 27). The *in vitro* effect of MEM-CLA has been investigated previously for H37Rv (10, 26) and 13 isolates of *M. tuberculosis*, all XDR-TB (8). All isolates were susceptible to ≤1mg/L of MEM-CLA, thus in line with our tentative breakpoint of 2 mg/L. However, the concentration of the β-lactamase inhibitor seems important. When Gonzalo *et al* used 2.5 mg/L of CLA, they found a higher MIC of ≤3 mg/L of MEM-CLA for 28 mainly drug-resistant *M. tuberculosis* isolates. On the other hand, a synergistic effect was seen when AMX-CLA and MEM were combined, resulting in only 3/28 *M. tuberculosis* isolates with higher MICs than 1.25 mg/L (11). CLA is presently only available in combination with AMX (i.e. Augmentin®), necessitating the use of MEM in combination with AMX-CLA for drug-resistant TB.

Clinically, MEM in combination with AMX-CLA has been used in 44 drug-resistant cases (32 MDR-TB, 12 XDR) (27-29). However, the successful drug-regimens also contained moxifloxacin and linezolid, thus making the attributable effect of combined MEM and AMX-CLA difficult to assess.

A dose of 1g MEM thrice daily ensures a coverage of 40% of the dosing interval with free serum concentrations exceeding the MIC of 2 mg/L (*T>MIC ≥40%*), which is regarded as an appropriate pharmacodynamic target for gram-positive and gram-negative bacteria. The need of frequent dosing of β-lactam antibiotics has not been evaluated systematically for *M. tuberculosis*. Nevertheless, when AMX-CLA was divided into three daily doses, the early bactericidal activity (EBA) was similar to the EBA of fluoroquinolones (1 000 mg/250mg
AMX-CLA three times daily) (24), but the drug combination showed no effect on the EBA when given as a single high dose (3,000 mg/750 mg AMX-CLA once daily) (30). The drawback of MEM-CLA is that it requires an intravenous access. The advantages are tolerable side effects (31), low plasma protein binding (32) and good lung penetration (33).

Our study was limited by the absence of wild-type *M. tuberculosis* isolates, the use of only one method of MIC-determination (Middlebrook 7H10) and a high exclusion rate of isolates due to poor growth (27%). Perhaps the use of Middlebrook 7H11 media, considered better for the growth of drug-resistant strains (34), would have resulted in improved growth and a lower exclusion rate. However, most of the excluded isolates displayed a higher degree of susceptibility to CLA alone in a concurrent experiment (data not shown). This raises the question whether the high concentration of CLA contributed to the poor growth of the excluded isolates. Previous studies have shown no (19, 21) or very little (13) intrinsic activity of CLA, although the concentrations tested (16 mg/L and 19.9 mg/L respectively) were lower than the concentration used in our study and liquid media was used. Nevertheless, the excluded isolates were distributed among all three of our resistance groups and the exclusion rate is unlikely to compromise the validity of our results.

In conclusion, our study is unique because of the high number of MDR/XDR-TB isolates investigated showing clinically achievable MICs of MEM-CLA. MEM-CLA is a potential treatment option for selected cases of multi-drug resistant tuberculosis.
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Figure 1. The MIC-distribution of meropenem-clavulanic acid for the *M. tuberculosis* isolates (n=68) tested using Middlebrook 7H10 medium, shown by bars shaded as follows: non-MDR/XDR-TB (gray); XDR-TB (black) and MDR-TB (hatched). The MIC distribution shows the concentration of meropenem, as the concentration of clavulanic acid was fixed uniformly at 64 mg/L.