In vitro activity of AZD5847 against geographically diverse clinical isolates of Mycobacterium tuberculosis

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ABSTRACT

The minimum inhibitory concentration (MIC) of the novel anti-tuberculosis drug AZD5847 was determined against 146 clinical isolates from diverse geographical regions including Eastern Europe, North America, Africa and Asia using the automated BACTEC Mycobacterial Growth Indicator Tube (MGIT) 960 system. These isolates originated from specimens sources such as sputum, bronchial alveolar lavage, pleural fluid, abscess, lung biopsy and faeces. The overall MIC₉₀ was 1.0 mg/L (range 0.125 – 4 mg/L). The MIC of AZD5847 for isolates of *M. tuberculosis* was similar among drug sensitive strains, multi drug resistant (MDR) and extensively drug resistant (XDR) strains. The good *in vitro* activity of AZD5847 against *M. tuberculosis* and the lack of cross resistance make this agent a promising anti-TB drug candidate.
Tuberculosis (TB) caused by *Mycobacterium tuberculosis* continues to be a major global health problem with an estimated 8.6 million new cases and 1.3 million deaths reported in 2012 (1). The African and South-East Asian region contributed to about 57% of all new TB cases. Among all new cases, an estimated 450,000 people developed multi drug resistant (MDR) TB and an estimated 170,000 deaths from MDR-TB. This problem is further worsened by the high incidence of co-infection of TB patients with the human immunodeficiency virus (HIV). On average, an estimated 9% of MDR-TB strains resistant to a fluoroquinolone and an injectable second line drug (amikacin, kanamycin or capreomycin), have been reported as extensively drug resistant TB (XDR-TB) (1).

The standard regimen requires 6 months for full treatment of TB and up to two years for MDR-TB where less effective, more expensive and more toxic second line drugs have to be used. Therefore, there is an urgent need to develop drugs with a novel mechanism of action to curb the rapid spread of multidrug-resistant *M. tuberculosis* (MDR-TB) and extensively drug-resistant *M. tuberculosis* (XDR-TB) strains (2). Although, drug resistant TB cases have increased globally, there is a severe shortage in the availability of new drugs with novel mechanism of action to treat TB. Recently, Bedaquiline, a diarylquinoline targeting the ATP synthase has been approved to treat MDR-TB patients (3). Several candidates, such as SQ 109 (4), nitroimidazoles PA-824 (5), delamanid (6), sutezolid (7), gatifloxacin and moxifloxacin (8) are in various phases of clinical testing in TB patients with the aim of replacing the current four drug regimen with a novel drug combination.

AZD5847, an oxazolidinone has attractive antimycobacterial properties (9) and shown to be efficacious in murine models of TB (10). The aim of this study was to investigate the anti-TB activity of AZD5847 against a panel of well characterized clinical *M. tuberculosis* isolates, with different patterns of resistance to first and second line anti-TB drugs and isolated from different geographical locations. We determined the minimum inhibitory activity of AZD5847 against 146 clinical isolates of *Mycobacterium tuberculosis* (TB) using the BACTEC 960 MGIT method, which is an automated liquid culture based system for the drug susceptibility testing of TB (11).
A total of 146 clinical *M. tuberculosis* isolates from various geographical origins were selected from the National Strain Collection at the Public Health Agency of Sweden (former Swedish Institute for Communicable Disease Control, SMI) and P.D. Hinduja National Hospital and Medical Research Centre, Mumbai, India. The fully drug susceptible *M. tuberculosis* H37Rv strain (ATCC 25618) was used as the reference strain for this study. In the panel, a total of 73 isolates were determined as drug resistant (11 singly drug resistant (SDR), 48 multi drug resistant (MDR) and 14 extensively drug resistant (XDR) strains) along with 73 fully drug susceptible using reference techniques. All strains derived from different patients apart from four of the MDR strains that had been isolated from two patients at two separate occasions. All strains were stored at -70°C and sub-cultured on Lowenstein Jensen (LJ) medium prior to testing.

To determine the MIC of AZD5847 against the strains, the BACTEC 960 MGIT system (Becton Dickinson, Sparks, MD USA) was used with a test concentration range of 0.12 - 8 mg/L of AZD5847. Briefly, 800 μL of the OADC (Oleic Acid Albumin Dextrose Complex) enrichment was added to each MGIT culture tube. The AZD5847 compound was solubilised and diluted two-fold in dimethyl sulfoxide (DMSO) of which 100 μL was added to the corresponding MGIT culture tube. Bacterial suspensions were prepared by dispensing two to three 1-μl loops of bacteria from fresh LJ slopes in 3 ml of phosphate-buffered saline (PBS) and homogenized by sonication in an ultrasound water bath. The suspensions were allowed to sediment for 20 minutes and the upper phase was transferred to a new tube and allowed to sediment for another 15 minutes before adjusting to McFarland No. 0.5 and diluted 1:5 in PBS (A). Half a milliliter of A was used to inoculate the MGIT culture tubes, containing the drug, and an undiluted (drug-free) growth control. Additionally, a 1:100 diluted bacterial suspension was made from A to prepare the proportional growth control of the MGIT test. Before all tubes were placed into the BACTEC MGIT 960 instrument for analysis, they were shaken gently for homogenization. The assessment of drug susceptibility was determined automatically at the point the proportional growth control reached 400 growth units (GU). By the use of the automated BACTEC MGIT 960 system all strains were interpreted objectively and the MICs were determined. MIC was defined as the lowest drug concentration at...
which the GU was <100 at the point that the proportional growth control reached a GU of 400 as recommended by the manufacturer (12).

Our results demonstrated anti mycobacterial effect of AZD5847 on all tested strains, irrespective of their resistance to other first and second line drugs. Furthermore, there was only a very narrow variation in the MIC distribution for the strains (Table 1 & Figure 1). None of the strains had a MIC >4 mg/L. For the majority of strains there was a sharp cut off (usually GU=0) for the MIC. The median time to a drug resistance result was 7.19 days (5.18 - 12) for the fully susceptible strains and 7.10 days (5.20 - 11) for the drug resistant strains. Linezolid was tested in this study and the MIC range was found to be 0.125 - 4 mg/L among the various clinical isolates. The MIC for linezolid against H37Rv ATCC 25618 was 1 mg/L.

AZD5847 exhibits good activity versus *M. tuberculosis* (1.0 mg/L) via the inhibition of protein synthesis. The activity of AZD5847 is not impacted by resistance to other antimycobacterial agents (9). AZD5847 is efficacious in the acute and chronic murine aerosol infection models of TB (10). Taken together, this signifies the potential to use AZD5847 during the early bactericidal phase and the sterilization phase of treatment in humans.
Table 1. MIC distribution for the 146 *M. tuberculosis* strains categorized as drug susceptible or drug resistant.

<table>
<thead>
<tr>
<th>Species and Strains</th>
<th>Number of strains</th>
<th>MIC range (mg/L)</th>
<th>MIC$_{50}$ (mg/L)</th>
<th>MIC$_{90}$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em> (drug-sensitive)</td>
<td>73</td>
<td>0.125–4</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><em>M. tuberculosis</em> (SDR)</td>
<td>11</td>
<td>0.125–4</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><em>M. tuberculosis</em> (MDR)</td>
<td>48</td>
<td>0.5–2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><em>M. tuberculosis</em> (XDR)</td>
<td>14</td>
<td>0.5–4.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

MIC: Minimum Inhibitory Concentration

MIC$_{50}$: Minimum Inhibitory Concentration for 50% isolates

MIC$_{90}$: Minimum inhibitory concentration for 90% isolates

SDR: Singly drug resistant (singly resistant to isoniazid, rifampin, ethambutol, streptomycin, or ofloxacin);

MDR: Multidrug resistant (resistant to Isoniazid and rifampin);

XDR: Extensively drug resistant (MDR strain resistant to fluoroquinolone and an injectable drug such as amikacin, kanamycin or capreomycin).
Figure 1. Activity of AZD5847 across a panel of 146 *M. tuberculosis* strains categorized as drug susceptible or drug resistant.
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