In Vitro Activity of AZD5847 against Geographically Diverse Clinical Isolates of Mycobacterium tuberculosis

Jim Werngren,* Maria Wijkander,a Nasrin Perskvist,b V. Balasubramanian,c* Vasan K. Sambandamurthy,c Camilla Rodrigues,c*d Sven Hoffnera

The Public Health Agency of Sweden, Department of Microbiology, Unit of Highly Pathogenic Bacteria, Solna, Sweden; Karolinska University Hospital, Laboratory Medicine, Department of Pathology, Huddinge, Sweden; Infection Innovative Medicines Unit, AstraZeneca R&D, Bangalore, India; P.D. Hinduja National Hospital and Medical Research Centre, Mumbai, India.

The MIC of the novel antituberculosis (anti-TB) 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 specimen sources such as sputum, bronchial alveolar lavage fluid, pleural fluid, abscess material, lung biopsies, and feces. The overall MIC50 was 1.0 mg/liter (range, 0.125 to 4 mg/liter). The MICs of AZD5847 for isolates of Mycobacterium tuberculosis were similar among drug-sensitive strains, multidrug-resistant (MDR) strains, 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.

**TABLE 1**

<table>
<thead>
<tr>
<th>Strain categorya</th>
<th>No. of strains</th>
<th>MIC range (mg/liter)</th>
<th>MIC50 (mg/liter)</th>
<th>MIC90 (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug sensitive</td>
<td>73</td>
<td>0.125–4</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>SDR</td>
<td>11</td>
<td>0.125–4</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MDR</td>
<td>48</td>
<td>0.5–2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>XDR</td>
<td>14</td>
<td>0.5–4.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a 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).

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Address correspondence to Jim Werngren, jim.werngren@folkhalsomyndigheten.se, or Camilla Rodrigues, dr_crodrigues@hindujahospital.com.

* Present address: V. Balasubramanian, Cellworks Research India Ltd., Bangalore, India.

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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 southeast Asian regions contributed to about 57% of all new TB cases. Among all new cases, an estimated 450,000 people developed multidrug-resistant TB (MDR-TB) and an estimated 170,000 died from MDR-TB. This problem is further worsened by the high incidence of coinfection of TB patients with human immunodeficiency virus (HIV). On average, an estimated 9% of MDR-TB strains resistant to a fluoroquinolone and an injectable second-line drug (aminocin, kanamycin, or capreomycin) have been reported as extensively drug-resistant TB (XDR-TB) strains (1).

The standard regimen requires 6 months for full treatment of TB and up to 2 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 MDR-TB and XDR-TB strains (2). Although the numbers of drug-resistant TB cases have increased globally, there is a severe shortage in the availability of new drugs with a novel mechanism of action to treat TB. Recently, bedaquiline, a diarylquinoline targeting the ATP synthase, was approved for treatment of MDR-TB patients (3). Several candidates, such as SQ 109 (4) and the nitroimidazoles PA-824 (5), delamanid (6), sutezolid (7), and 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 has been shown to be efficacious in murine models of TB (V. Balasubramanian, S. Solapure, R. K. Shandil, S. Gaonkar, K. N. Kumar, J. Reddy, A. Deshpande, S. Bharath, N. Kumar, L. Wright, D. Melnick, and S. Butler, submitted for publication). The aim of this study was to investigate the anti-TB activity of AZD5847 against 146 clinical isolates of Mycobacterium tuberculosis (TB) using the Bactec 960 Mycobacterial Growth Indicator Tube (MGIT) method, which is an automated liquid culture-based system for the drug susceptibility testing of TB (10).

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. Fully drug-susceptible M. tuberculosis strain H37Rv...
which the GU value was strains were interpreted objectively and the MICs were determined automatically at the point at which they were shaken gently for homogenization. The assessment of bacterial suspensions was prepared by dispensing two to three loops of bacteria from fresh LJ slopes into 3 ml of phosphate-buffered saline (PBS), and the reaction mixture was homogenized by sonication in an ultrasound water bath. The suspensions were allowed to sediment for 20 min, and the upper phase was transferred to a new tube and allowed to sediment for another 15 min before adjustment to a McFarland standard of 0.5 and dilution (1:5) in PBS (mixture A). Half a milliliter of mixture 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 mixture 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 at which the proportional-growth control reached 400 growth units (GU). By the use of the automated Bactec MGIT 960 system, all drug susceptibility was determined automatically at the point at which the proportional-growth control reached a GU of 0.125 to 4 mg/liter. The MIC for linezolid against H37Rv ATCC 25618 was 1 mg/liter. AZD5847 exhibits good activity against M. tuberculosis (1.0 mg/liter) 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 (Balasubramanian et al., submitted). Taken together, this signifies the potential to use AZD5847 during the early bactericidal phase and the sterilization phase of treatment in humans.

FIG 1 Activity of AZD5847 across a panel of 146 M. tuberculosis strains categorized as drug susceptible or drug resistant.

(A TCC 25618) was used as the reference strain for this study. In the panel, a total of 73 isolates were identified as drug resistant (11 singly drug resistant [SDR], 48 as multidrug resistant [MDR], and 14 as extensively drug resistant [XDR] strains) along with 73 isolates identified as 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 on two separate occasions. All strains were stored at -70°C and subcultured on Lowenstein Jensen (LJ) medium prior to testing.

To determine the MIC of AZD5847 against the strains, a Bactec 960 MGIT system (Becton, Dickinson, Sparks, MD) was used with a test concentration range of 0.12 to 8 mg/liter of AZD5847. Briefly, 800 μl of an oleic acid-albumin-dextrose-catalase (OADC) enrichment was added to each MGIT culture tube. The AZD5847 compound was solubilized and diluted 2-fold in dimethyl sulfoxide (DMSO), and a 1:100-μl volume 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 into 3 ml of phosphate-buffered saline (PBS), and the reaction mixture was homogenized by sonication in an ultrasonic water bath. The suspensions were allowed to sediment for 20 min, and the upper phase was transferred to a new tube and allowed to sediment for another 15 min before adjustment to a McFarland standard of 0.5 and dilution (1:5) in PBS (mixture A). Half a milliliter of mixture 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 mixture 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 at which 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. The MIC was defined as the lowest drug concentration at which the GU value was <100 at the point that the proportional-growth control reached a GU of 400 as recommended by the manufacturer (11).

Our results demonstrated an antimycobacterial 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 range for the strains (Table 1 and Fig. 1). None of the strains had a MIC > 4 mg/liter. For the majority of strains, there was a sharp cutoff (usually GU = 0) for the MIC. The median times to a drug resistance result were 7.19 days (5.18 to 12) for the fully susceptible strains and 7.10 days (5.20 to 11) for the drug-resistant strains. Linezolid was tested in this study, and the MIC range among the various clinical isolates was found to be 0.125 to 4 mg/liter. The MIC for linezolid against H37Rv ATCC 25618 was 1 mg/liter.

REFERENCES