AZD5847, a novel oxazolidinone with an MIC of 1 μg/mL, exhibits exposure-dependent killing kinetics against extracellular and intracellular *Mycobacterium tuberculosis*. Oral administration of AZD5847 to mice infected with *M. tuberculosis* H37Rv in a chronic-infection model resulted in a 1.0-log_{10} reduction in the lung CFU count after 4 weeks of treatment at a daily area under the concentration-time curve (AUC) of 105 to 158 μg·h/mL. The pharmacokinetic-pharmacodynamic parameter that best predicted success in an acute-infection model was an AUC for the free, unbound fraction of the drug/MIC ratio of ≥20. The percentage of time above the MIC in all of the efficacious regimens was 25% or greater.

**MATERIALS AND METHODS**

**Reagents.** Analytical-grade dimethyl sulfoxide was purchased from Sigma Life Science. High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from J. T. Baker, Philipsburg, NJ. Mass spectrometry-grade formic acid was purchased from Sigma-Aldrich Fluka. AZD5847 and AZD5847 DSP were synthesized at AstraZeneca.

**Microbial cultures.** *M. tuberculosis* H37Rv ATCC 27294, a strain susceptible to all of the standard anti-TB drugs, was used for all of the studies in this report. The inoculum used for all of the experiments was derived from a seed lot maintained at ~70°C that was prepared after a single round of broth amplification of bacilli isolated from infected mouse lungs. The inoculum was prepared as described earlier (12).

**Animals.** All of the experimental protocols involving animals and the use of animals were approved by the Institutional Animal Ethics Committee, registered with the Government of India (registration no. CPCSEA 1999/5). The BALB/c mice used for these studies were 6 to 8 weeks old with an average body weight of 30 to 40 g (Raj Biotech Laboratories, Pune, India). They were randomly assigned to cages and allowed to acclimatize for 2 weeks prior to experiments. Feed and water were given ad libitum.

**MIC and plasma protein binding.** The MIC of AZD5847 against *M. tuberculosis* strains was determined in Middlebrook 7H9 medium supplemented with 10% albumin-dextrose-catalase by previously described methods (11). Protein binding in 10% mouse, rat, dog, or human plasma was measured by equilibrium dialysis as reported earlier (13).

**PK in mice.** Blood samples from healthy and infected mice were collected under biosafety level 2 (BSL2) and BSL3 conditions, respectively. Blood samples from infected mice were processed in the BSL3 facility and brought outside for bioanalysis after plasma protein precipitation with acetonitrile, followed by 30 min of exposure of the extraction plate to UV light. For intravenous PK studies, a 5-mg/kg dose of AZD5847 DSP was administered as a solution containing 0.3% dextrose and 0.9% sodium chloride. For oral PK studies, AZD5847 was administered as a suspension...
Infected lungs were aseptically removed and homogenized in a final volume of 4186 ug of blood samples at 16,000 × g for 5 min. Three mice were used for each time point) postdosing were collected in Li-heparin-coated Microvette tubes (CB 300; Sarstedt, AG & Co., Nümbrecht, Germany). After centrifugation of blood samples at 16,000 × g for 5 min, 25 μL of plasma was transferred into 96-well, V-bottom, polypropylene microtiter plates for extraction with acetonitrile. Blood samples from infected mice during chronic-infection efficacy studies were collected after the 10th and 22nd doses. In the dose fractionation (DF) study, blood samples were collected from 8 out of 14 dose groups on day 18 in the 4-week study and 12 out of 13 dose groups on day 37 in the 8-week study. For the lung epithelial lining fluid (ELF) PK in healthy mice, bronchoalveolar lavage (BAL) was performed on healthy mice after the oral administration of AZD5847 at 250 mg/kg by previously described methods (14). Blood and BAL fluid samples were collected at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h after dosing. Urea estimation in BAL fluid and plasma samples collected at the respective time points was done to calculate the extent of dilution of ELF in BAL fluid samples.

Bioanalysis of plasma samples from PK studies. An API 3000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX) with an atmospheric pressure ionization source interface operated in the positive ion mode was used for multiple-reaction-monitoring liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. AZD5847 was analyzed on a C18 HPLC column (50 by 4.6 mm, 5 μm; Phenomenex) under isocratic conditions in a mobile phase containing acetonitrile and 0.1% formic acid in water (75:25, vol/vol) at 600 μL/min for a run time of 2.5 min. Mouse plasma samples collected after dosing of AZD5847 DSP by either the intravenous or the oral route were analyzed for AZD5847 and AZD5847 DSP. For simultaneous detection of AZD5847 and AZD5847 DSP, a Waters tandem quadrupole detector LC-MS/MS apparatus with an Acquity Ultra Performance Liquid Chromatography (UPLC) instrument was used. Chromatographic separation was achieved with an ethylene-bridged hybrid C18 Acquity UPLC column (50 by 2.1 mm, 1.7 μm; Waters) with an isocratic gradient of acetonitrile and 0.1% formic acid in water (4:6, vol/vol) at 250 μL/min for 3.0 min. The retention times of AZD5847 and AZD5847 DSP were 1.02 and 0.84 min, respectively.

Dose-response studies in the chronic-infection model. Mice were infected with 10^4.5 CFU of M. tuberculosis H37Rv per mouse via the inhalation route in an aerosol infection chamber as described earlier, and treatment was initiated after 3 days of infection. For DF studies, a cumulative dose of 500 mg/kg given over a period of 4 or 8 weeks was fractionated as shown in Table 1. The total dose over a period of 48 h was given once (every 48 h [q48h], twice [q24h], or four times [q12h] in 2 days.

Analysis of PK-PD data. Noncompartmental analysis of PK data was performed with the WinNonlin 5.2.1 software package (Pharsight Inc.). The colony counts obtained from plating were transformed to log_{10}(x + 1), where x equals the total number of viable tubercle bacilli calculated to be present in a given sample. In the DF study, estimates of the area under the concentration-time curve from 0 to 24 h (AUC_{0→24}) were derived from plasma samples of infected mice at day 18 or 37 of treatment. Log_{10} lung CFU counts were plotted against the dose, the AUC_{0→24}/MIC ratio, the percentage of time the free drug concentration stayed above the MIC (%F_%{T>MIC}), or the ratio of the maximum drug concentration in plasma (C_{max}) to the MIC. Nonlinear regression analysis to fit an inhibitory sigmoidal maximum-effect (E_{max}) model (variable slope) was performed with Phoenix WinNonlin 6.2.1 (Pharsight Inc.).

RESULTS

In vitro MIC and pharmacologic parameters of AZD5847. AZD5847 is active against extracellular, as well as intracellular, M. tuberculosis (11). Its MIC against M. tuberculosis H37Rv in broth is 1 μg/mL. Treatment of M. tuberculosis-infected murine bone marrow-derived macrophages with 16 μg/mL of AZD5847 for 10 days resulted in a 1-log_{10} reduction in the intracellular CFU count. The free or unbound fraction of AZD5847 in mouse, rat, dog, or human plasma was ~0.2.

PK of AZD5847 in mice. AZD5847 DSP could not be detected in plasma 5 min after intravenous or oral administration, suggesting rapid conversion of the prodrug to the parent molecule. Intravenous PK analysis after the administration of a bolus dose of 5 mg/kg showed that AZD5847 had a low clearance (4.7 ml/min/kg).

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<th>Total dose/wk (mg/kg)</th>
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and volume of distribution (0.5 liter/kg) in mice. The half-life of AZD5847 in mice was 1.3 h. A plot of the plasma AUC versus the dose after oral administration of AZD5847 or AZD5847 DSP to healthy mice is shown in Fig. 2. After oral administration of AZD5847, a linear increase in the drug’s AUC was observed up to 100 mg/kg. For AZD5847 DSP, a linear increase in the drug’s AUC was observed up to 900 mg/kg.

The AZD5847 PK profiles indicated biphasic elimination characteristics after the administration of large doses of AZD5847 DSP. The elimination half-life increased from 1.3 to 2 h at <100 mg/kg to 8 to 10 h at >200 mg/kg. The PK profiles of the groups orally administered AZD5847 DSP at 50 mg/kg q12h, 100 mg/kg q24h, 200 mg/kg q48h, or 400 mg/kg q48h during the 8-week DF study are shown in Fig.S1 in the supplemental material. The $C_{\text{max}}$ did not increase with the dose. However, the drug AUC increased linearly with the dose because of the longer elimination half-life when larger doses were given q48h. Changes in the elimination half-life when larger doses were given may be due to saturation of absorption of the parent diol after hydrolysis of the phosphate ester.

To confirm exposure at the site of infection, lung ELF samples from healthy mice were analyzed after the oral administration of AZD5847 at 250 mg/kg. ELF and total/free plasma PK profiles along with ELF PK parameters are shown in Fig.S2 and Table S1 in the supplemental material. The AUC in ELF was about twice as great as the AUC of the free drug in plasma. The acute-infection model, and the total duration of treatment was 4 or 8 weeks. The correlations between the major PK-PD indices ($C_{\text{max}}$/MIC ratio, %$T_{>\text{MIC}}$ and AUC$_{0-24}$/MIC ratio) are shown in Fig.S3 in the supplemental material. The AUCs estimated for various dose groups are shown in Fig. 3. The AUCs from time zero

In vivo efficacy of AZD5847 in the mouse model of chronic infection. The dose-response relationships of AZD5847 and AZD5847 DSP in the mouse model of chronic infection are shown in Fig. 3. A 1-log$_{10}$ reduction in the lung CFU count was achieved with a once-daily dose of 256 mg/kg given for 2 weeks or 128 mg/kg given for 4 weeks. A pharmacokinetic analysis of infected mice receiving the 128-mg/kg dose was performed at days 10 and 22 after treatment initiation. This analysis showed that the corresponding AUC$_{0-24}$ required for a 1.0-log$_{10}$ reduction in the chronic-infection model in 4 weeks was 105 to 158 $\mu$g h/ml. At a dose of 500 mg/kg given once daily for 2 weeks, AZD5847 DSP produced a 1.8-log$_{10}$ reduction in the lung CFU count.

DF studies for the determination of PK-PD indices correlating with bactericidal activity. DF studies were performed in the acute-infection model, and the total duration of treatment was 4 or 8 weeks. The correlations between the major PK-PD indices ($C_{\text{max}}$/MIC ratio, %$T_{>\text{MIC}}$ and AUC$_{0-24}$/MIC ratio) are shown in Fig.S3 in the supplemental material. The AUCs estimated for various dose groups are shown in Fig. 4. The AUCs from time zero
to time t (AUC$_{0-t}$) over the corresponding dosing intervals, estimated on days 18 and 37 after initiation of treatment, were constant to within 2-fold (Fig. 4A). A plot of the AUC$_{0-t}$ versus the dose indicated a linear relationship between the dose and exposure as measured by the AUC$_{0-t}$ (Fig. 4B).

The relationship between the dose, the AUC$_{0-24}$/MIC ratio, the %$_{T>MIC}$, or the C$_{max}$/MIC ratio and the mouse lung CFU count in the acute-infection model at the end of 8 weeks of treatment is shown in Fig. 5. The sigmoidal $E_{\text{max}}$ model fit to these data suggested that both the AUC$_{0-24}$/MIC ratio and the %$_{T>MIC}$ correlated with efficacy. Observed versus $E_{\text{max}}$ model-predicted lung CFU count plots and goodness-of-fit parameters indicated that the AUC$_{0-24}$/MIC ratio and the %$_{T>MIC}$ correlated with the lung CFU counts, whereas the C$_{max}$/MIC ratio did not (see Fig S4 and Table S2 in the supplemental material). Similar results were obtained in the 4-week treatment group (see Fig S5 in the supplemental material).

An AUC$_{0-24}$ of ~100 µg·h/ml was required to achieve a 1-log$_{10}$ CFU count reduction with respect to that of the late control in the acute-infection model. Increasing the total duration of treatment from 4 to 8 weeks resulted in an additional 1-log$_{10}$ reduction in the lung CFU counts (Fig. 5A).

**DISCUSSION**

AZD5847 is active against extracellular and intracellular M. tuberculosis, whereas AZD5847 DSP is completely inactive in vitro. Because AZD5847 DSP is a charged molecule, its inactivity may be due to the poor ability of the intact prodrug to enter macrophages or bacterial cells. However, AZD5847 DSP was efficacious in vivo. AZD5847 DSP is rapidly hydrolyzed by an alkaline phosphatase in plasma (15,16) to produce a pharmacologically active parent molecule, AZD5847, responsible for in vivo efficacy.

When AZD5847 was orally administered to mice as a parent diol, its level in plasma did not increase linearly with the dose beyond 100 mg/kg. This may be due to its limited aqueous solubility (40 µM). Oral administration of AZD5847 DSP with an aqueous solubility in the millimolar range achieved linearity over a wider dose range. Therefore, AZD5847 DSP was used instead of
AZD5847 in the DF study. As the hydrolysis of AZD5847 DSP to AZD5847 was very rapid, plasma AZD5847 concentrations were used for the PK-PD analysis.

AZD5847 and AZD5847 DSP were efficacious in the acute- and chronic-infection mouse models of TB. The significant whole-animal activity we observed in both the acute- and chronic-infection models indicates that AZD5847 was active against growing, as well as slowly replicating, M. tuberculosis in mouse lungs. Interestingly, we observed a 1-log, or greater reduction of lung CFU counts in the chronic-infection model, suggesting significant potency of AZD5847 against contained, stationary-growth bacilli in granuloma-like lesions.

To determine the pharmacologic parameter most closely predictive of bactericidal activity, we conducted a DF study with mice. The $E_{\text{max}}$ (delta log CFU count) in the chronic-infection model was limited to 1.8 log. We have used an acute-infection model to increase this window of efficacy from 1.8 to 4.0 log (7.5 log$_{10}$ CFU for no effect to 3.0 log$_{10}$ CFU for the $E_{\text{max}}$) for better differentiation among the various dose regimens used in the DF study. However, because of potential differences in susceptibility between actively growing and slowly replicating or nonreplicating M. tuberculosis, it is possible that the PK-PD indices determined in the acute and chronic TB infection models could be different. In this study, we found that both the %$T_{>\text{MIC}}$ and the AUC/MIC ratio showed a strong correlation with killing activity. Significant efficacy (a $>$1-log reduction of the initial inoculum) was observed with a %$T_{>\text{MIC}}$ of 25% or greater with all of the regimens used. This may be due to the longer elimination half-life when a large dose was given q48h. We could not achieve a %$T_{>\text{MIC}}$ of <21% even with the q48h regimen for the top two doses. We think that DF over an extended period like 72 or 96 h may break this correlation between the AUC/MIC ratio and the %$T_{>\text{MIC}}$. A DF study reported for PA824 also shows two different PK-PD indices, depending on the total duration of 48 versus 72 h used for the DF (17).

The in vitro efficacies of linezolid and PNU100480 in the mouse model of chronic infection have been reported earlier (18). Linezolid treatment gives a 0.75-log$_{10}$ CFU reduction at a dose of 100 mg/kg q24h (AUC$_{0-24}$, 250 μg · h/ml), whereas PNU100480 shows a 2.4-log$_{10}$ CFU count reduction at a dose of 25 mg/kg q12h (AUC$_{0-24}$, 40 μg · h/ml). Comparing equivalent exposure levels, the in vitro efficacy of AZD5847 in the mouse chronic TB model was superior to that of linezolid but inferior to that of PNU100480. While bactericidal efficacy is an important factor, other factors, such as toxic side effects, may limit the utility of some oxazolidinones such as linezolid, particularly in TB patients, who require prolonged treatment. AZD5847, being structurally different from linezolid, may have a better safety profile; potentially further improving its therapeutic utility.

Data on the most predictive PD parameter of linezolid versus M. tuberculosis are not available, but its efficacy against Staphylococcus aureus was better correlated with the AUC/MIC ratio than with the percentage of time above the MIC (19). In contrast, the percentage of time above the MIC was the PK-PD index that best predicted the M. tuberculosis-killing activity of PNU100480 (20). For AZD5847, our results show that an AUC/MIC ratio of >100 and a %$T_{>\text{MIC}}$ of >25% could be essential for its bactericidal activity.

In conclusion, the PK-PD analysis presented here further supports the continuation of in vitro and in vivo combination efficacy studies and clinical investigation of the safety, PK, and early bactericidal activity of AZD5847 in humans. Phase 1 studies of AZD5847 (21, 22) in healthy volunteers were completed in 2011, and a phase 2a study of patients with drug-susceptible TB is in progress.

REFERENCES


