

Dose Ranging Studies with the LeuRS Inhibitor GSK2251052 against *Escherichia coli* in a Mouse Thigh Suture Infection Model

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Abstract

GSK2251052 (formerly AN3365), a novel boron-containing leucyl-tRNA synthetase inhibitor with *in vitro* activity against *Pseudomonas aeruginosa* and multidrug-resistant *Enterobacteriaceae*, is currently being developed for the treatment of serious Gram-negative bacterial infections. Dose ranging studies were undertaken in mice to evaluate the efficacy of GSK2251052 against a range of *E. coli*, including isolates resistant to levofloxacin or ceftazidime. Mice were infected by insertion of a contaminated suture into the deep thigh musculature. Starting 1 h post infection, mice received twice daily oral doses of GSK2251052 (9.4 to 75 mg/kg) or sterile water. Baseline controls were sampled at 1 h, and all remaining mice sampled at 48 h post infection. Blood samples were taken from infected mice to determine exposure. The minimum inhibitory concentration of GSK2251052 for all five isolates was 0.5 µg/mL. Three were resistant to levofloxacin, one was resistant to ceftazidime and one was sensitive to both. GSK2251052 demonstrated a clear dose response against each isolate. At 75 mg/kg, bacterial numbers were reduced below or close to the limit of detection (1.7 log₁₀ CFU/thigh), with mean log₁₀ CFU reductions of >2 and ≥4 compared with baseline and end-of-study controls, respectively. At 37 mg/kg, reductions of 1-2 and 3-4 log₁₀ CFU, respectively, were obtained. Stasis or growth was observed at 9.4 and 18.75 mg/kg. C_{max} values obtained at 18.75, 37.5 and 75 mg/kg were 0.7, 1.6 and 5.9 µg/mL, respectively. The AUC₀₋₂₄ values at these doses were 3.4, 4.7 and 18.3 µg.h/mL, respectively. Dose dependent efficacy was obtained with GSK2251052 against five *E. coli*, including isolates resistant to levofloxacin or ceftazidime. The minimum oral dose for efficacy was 37.5 mg/kg, while at 75 mg/kg bacterial counts were reduced in most cases to the limit of detection. At these doses, associated daily AUC and C_{max} values were 9.4-36.6 µg.h/mL and 1.6-5.9 µg/mL, respectively. These data demonstrate that further investigation of GSK2251052 to treat clinical infections caused by *E. coli* is warranted.

Introduction

- GSK2251052 (formerly AN3365) is from a novel class of antibiotics that inhibit bacterial leucyl-tRNA synthetase (LeuRS). Through a novel mechanism, GSK2251052 forms a boron adduct with tRNA and inhibits the LeuRS enzyme, resulting in inhibition of bacterial protein synthesis.
- GSK2251052 is active *in vitro* against *P. aeruginosa* and *Enterobacteriaceae*, including isolates resistant to existing antibacterial agents, with MIC₉₀ values of 4 and 1 µg/ml, respectively.
- To demonstrate the potential for GSK2251052 to treat clinical infections caused by *E. coli*, a series of dose ranging efficacy studies were performed in non-neutropenic mice. Five isolates (GSK2251052 MIC 0.5 µg/ml) with varying susceptibilities to ceftazidime and levofloxacin were utilized.
- Pharmacokinetic studies were also conducted in infected mice to determine the exposure required for efficacy.

Methods

Bacterial isolates:

Five isolates of *E. coli* were selected for evaluation based on susceptibility profiles, previous validation of bacterial growth in the model and appropriate efficacy using positive and negative control antibacterials. All strains were grown overnight at 37°C in brain heart infusion broth using a shaker incubator. Lengths of sterile chromic gut suture (1 cm each) were added to a 1:50 dilution of the overnight broth culture and shaken at 37°C for approximately 0.5 h prior to infection. The isolates and corresponding minimum inhibitory concentrations (MICs) are shown in Table 1.

Table 1. Minimum inhibitory concentrations (µg/mL) for *E. coli* isolates

| Isolate | GSK2251052 | Ceftazidime | Levofloxacin |
|---------|------------|-------------|--------------|
| 298161 | 0.5 | 0.25 | 16 |
| 343659 | 0.5 | 0.25 | 16 |
| 1507576 | 0.5 | 32 | 0.03 |
| 1162222 | 0.5 | 0.25 | 16 |
| 1161434 | 0.5 | 0.125 | 0.03 |

Infection model:

Non-neutropenic male CD-1 mice (n=5 per group) were utilized. Under anesthesia, a small incision was made to expose the inner thigh muscle, and one contaminated suture was implanted into the deep musculature. Treatment was initiated at 1 h post infection, with subsequent administration of compound using a q12 dosing regimen for 2 days. GSK2251052 was administered via oral gavage at doses ranging from 9.4 to 75 mg/kg. Mice were euthanized at 1 h post infection (untreated baseline controls) or 48 h post infection. Thighs were removed aseptically and homogenized for bacterial enumeration.

Pharmacokinetics:

Exposure studies were performed separately in infected mice (n=3 per dose level) following the third dose in a twice daily dosing regimen. Serial samples were obtained by nicking a lateral tail vein with a microlancet and collecting blood into a heparinized capillary tube. Blood was immediately mixed with HPLC-grade water (10 µl blood + 10 µl water) and frozen at -80°C prior to analysis by LC/MS/MS. The area under the concentration-time curves (AUCs) from time 0 to 24 h for blood were calculated from the mean values by noncompartmental analysis using the trapezoidal rule. C_{max} was determined by using the highest concentration measured in each animal.

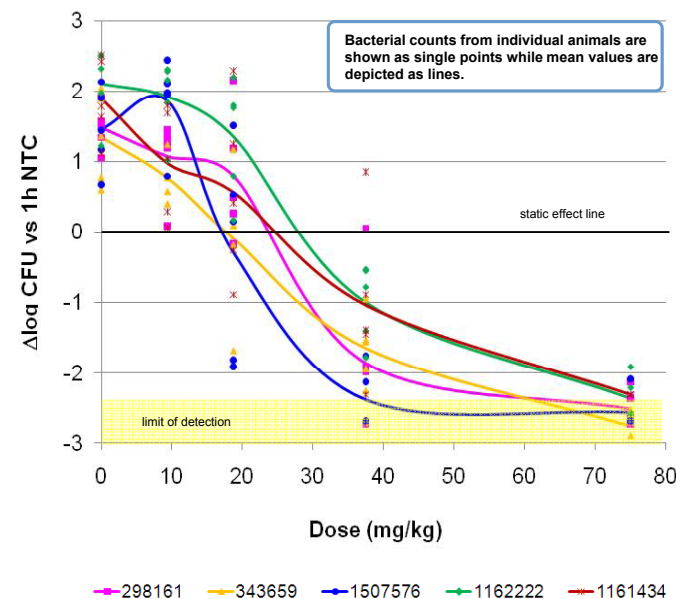
Results - Efficacy

As shown in Figure 1, all five *E. coli* isolates achieved good growth in the model, between 1 and 3 log₁₀ CFU increases compared with the 1h controls. The limit of detection for reduction in bacterial counts compared with 1h controls was between -2.3 and -3.0 Δlog₁₀ CFU, depending on the isolate tested.

GSK2251052 demonstrated substantial killing for all five isolates, with bacterial counts at or within 0.5 log₁₀ of the limit of detection in all animals at 75 mg/kg BID. A more variable effect was observed at 37.5 mg/kg BID, but mean bacterial reductions were at least 1 log₁₀ at that dose. Doses of 18.75 and 9.4 mg/kg BID were less effective, resulting in stasis or bacterial growth.

Protein binding values for GSK2251052 are very low in both mouse and human (<10%), so all exposure analyses are based on total drug concentrations. At 75 mg/kg BID (the maximal effective dose), the daily total AUC value was approximately 37 µg.h/ml and the C_{max} 5.9 µg/ml. At 37.5 mg/kg BID, the daily total AUC value was approximately 12 µg.h/ml and the C_{max} 1.6 µg/ml.

Figure 1. Dose response curves of GSK2251052 against 5 *E. coli* isolates in a non-neutropenic thigh infection model in mice



Results - Exposure

Exposure of GSK2251052 in mice infected in the thigh with *E. coli* (via a contaminated suture) is shown in Figure 2 and summarized in Table 2. Oral exposures demonstrated that slightly more than dose proportional concentrations were obtained at increasing doses. Variability was typical of that seen for early non-formulated preparations of compound.

Figure 2. Exposure of GSK2251052 in thigh-infected mice following a single oral dose (total concentrations)

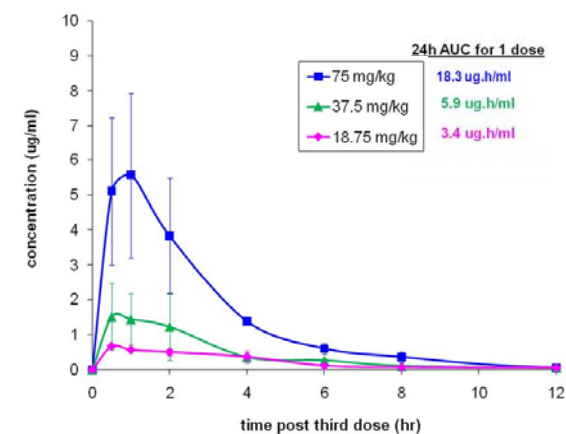


Table 2. Summary of GSK2251052 exposure results following a single oral dose to infected mice (total concentrations in µg/ml)

| | 75 mg/kg | 37.5 mg/kg | 18.75 mg/kg |
|--------------------------|------------|------------|-------------|
| C _{max} | 5.9 ± 2.4 | 1.6 ± 0.8 | 0.7 ± 0.03 |
| 24h AUC | 18.3 ± 5.3 | 5.9 ± 2.6 | 3.4 ± 0.5 |
| Daily AUC for BID dosing | 37 | 12 | 7 |

Conclusions

- GSK2251052 demonstrated excellent efficacy against 5 isolates of *E. coli* in a non-neutropenic mouse thigh infection model.
- Bacterial eradication was achieved with an oral dose of 75 mg/kg BID in the non-neutropenic mouse thigh infection model.
- Total daily AUC and C_{max} values associated with bacterial eradication in this model were approximately 37 µg.h/ml and 6 µg/ml, respectively.
- These data demonstrate that further investigation of GSK2251052 to treat clinical infections caused by *E. coli* is warranted.

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GSK2251052

