

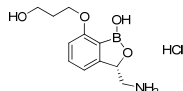
Allometric Scaling for Prediction of Human Intravenous Pharmacokinetics of GSK2251052, A Novel Boron-Based Antimicrobial against Gram-negative Bacteria

Liu L*, Zane LT, Wu A, Bu W, Alley MRK, Hernandez V, Sexton H, Fan XQ, Feng L, Baker S, Maples K, and Heyman I

Introduction

GSK2251052 (formerly AN3365), a novel boron-containing leucyl-tRNA synthetase inhibitor, is currently being developed for the treatment of hospital-acquired Gram-negative bacterial infections. This study demonstrated the pharmacokinetic (PK) properties and evaluated the interspecies scaling on PK profiles from four mammalian preclinical species, including mouse, rat, monkey, and dog, to project human PK profile for first-in-human study (FIH).

Figure 1. Structure of GSK2251052, a novel boron-containing antibiotics



Objective

- To define PK profiles of GSK2251052 in mice, rats, monkeys, and dogs
- To predict human PK profile with allometric scaling

Methods

1) *In vivo* Pharmacokinetic Studies

- Mice (female CD-1, 12 mice/group) received dose at 30 mg/kg by IV injection via tail vein
- Rats (male Sprague-Dawley, 3 rats/group) were dosed at 10 mg/kg by IV injection via femoral vein cannula
- Monkeys (male Cynomolgus, 3 animals/group) received dose at 10 mg/kg by IV injection
- Dogs (male Beagle, 3 animals/group) were dosed at 10 mg/kg by IV injection via saphenous vein
- GSK2251052 (HCl salt) was formulated in sterile water or isotonic saline as IV formulation.
- Venous blood was collected, and K₂EDTA as anticoagulant was used for plasma

Methods (cont'd)

2) Bioanalysis

The plasma samples were analyzed for concentrations of GSK2251052 by LC/MS/MS at Anacor. The extraction method involved protein precipitation (PPT) of plasma with cold methanol containing a structural analog as internal standard (IS), conducted on a Strata 96-well PPT plate. The methanol extract was mixed with 0.1% acetic acid in water and injected onto a reversed-phase HPLC column (Waters Atlantis T3 column maintained at 30° C). Detection of GSK2251052 and internal standard was by hybrid tandem/linear trap mass spectrometry (4000 QTrap, AB/Sciex) using positive electrospray ionization in the MRM (multiple reaction monitoring) mode. The limit of quantitation (LOQ) was 2-8 ng/mL in plasma from various species.

3) Pharmacokinetic Analysis

Plasma concentration-time profiles following IV administration were analyzed with two-compartmental analysis using WinNonlin Pro version 5.2, and the bi-exponential decline was defined by the equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

A, B, α and β are macro constants. Secondary PK parameters, including plasma clearance (CLp), volume of distribution at central compartment (Vc) and at steady state (Vss), and mean residence time (MRT), were also computed.

4) Allometric Scaling

For interspecies scaling, the transformed PK parameters obtained from each species with compartmental analysis were plotted against its body weight (kilograms) on a log-log scale. Linear least-squares regression analysis was performed on these plots to fit relationships to the equation:

$$Y = aW^b$$

(Y---PK parameters, a---allometric coefficient, b---allometric exponent, and W---body weight in kg)

5) Confirmation of Human PK Projection

The projected time-concentration profile was compared to the actual mean time-concentration profile from six male volunteers following 1-h IV infusion at 200 mg (SAD, Cohort-1). The PK results of the clinical trial will be presented in a separate poster by Zane LT et. al (P 1521).

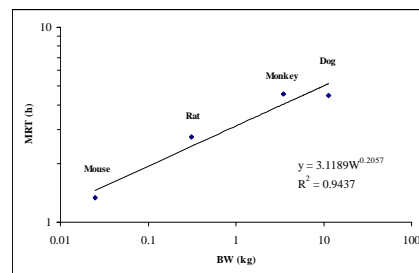
Results

Pharmacokinetic profiles were defined following IV administration in mice, rats, monkeys and dogs (Table 1). Applying the interspecies scaling on PK parameters from mouse, rat, monkey and dog, the allometric equations were demonstrated as CLp (mL/h) = 1153.6W^{0.74} (r²=0.999), Vc (mL) = 595.8W^{0.87} (r²=0.990), Vss (mL) = 3637W^{0.95} (r²=0.997), MRT (h) = 3.12W^{0.21} (r²=0.944) (Figures 2 and 3). The predicted values of CLp, Vc, Vss, and MRT for a 70-kg human were, 26.7 L/h, 23.7 L, 204 L, and 7.5 h (Table 2), respectively, which was approximately 50% deviated from actual human PK parameters (CL=18 L/h, T-1/2=10.9 h) reported in recent clinical trial FIH study following 1-h IV infusion at 200 mg (Figure 4).

Table 1. Pharmacokinetic Parameters Following IV Administration in Mouse, Rat, Monkey, and Dog

Parameters	Unit	Mouse	Rat	Monkey	Dog
Body Weight	kg	0.025	0.314	3.5	11.4
Dose	mg/kg	30	10	10	10
A	µg/mL	36.07	21.44	16.14	51.52
B	µg/mL	1.219	0.863	1.418	2.163
α	1/h	5.663	7.752	4.359	9.954
β	1/h	0.311	0.234	0.158	0.172
CL	mL/h/kg	2914	1686	786	621
MRT	h	1.337	2.746	4.558	4.455
Vc	mL/kg	805	921	570	348
Vss	mL/kg	3895	4689	3557	2860

Figure 2. Allometric Scaling Plot for Mean Residence Time (MRT)



Results (cont'd)

Figure 3. Allometric Scaling Plots for Clearance (CLp), Volume at Central Compartment (Vc) and Volume at Steady-State (Vss)

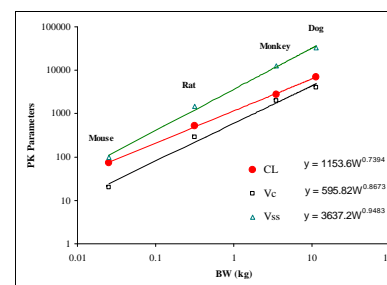


Table 2. Allometric Equations and Predicted Human PK Parameters for GSK2251052 Using Parameters from Mouse, Rat, Monkey, and Dog

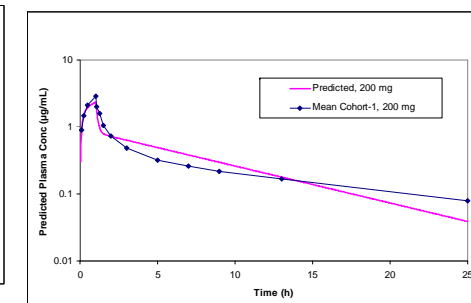
PK Parameters	Allometric Equations	Prediction in 70-kg Human Subject
A (µg/mL)	2.2655W ^{0.1772}	4.810
B (µg/mL)	0.1099W ^{0.2638}	0.337
α (1/h)	6.681W ^{0.0389}	7.88
β (1/h)	0.2043W ^{0.1088}	0.129
CL (mL/h)	1153.6W ^{0.7394}	26.7 x 10 ³
MRT (h)	3.1189W ^{0.2057}	7.5
Vc (mL)	595.82W ^{0.8673}	23.7 x 10 ³
Vss (mL)	3637.2W ^{0.9483}	204 x 10 ³

REFERENCES

- Rock FL, Mao W, Yaremchuk A, Tukalo M, Crepin T, Zhou H, Zhang YK, Hernandez V, Akama T, Baker SJ, Plattner JJ, Shapiro L, Martinis SA, Benkovic SJ, Cusack S, Alley MRK An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site. *Science*, 316:1759-1761.
- V. Hernandez, T. Akama, M.R.K. Alley, S. Baker, W. Mao, F. Rock, Y.K. Zhang, Y. Zhang, V. Zhou, T. Crepin, S. Cusack, A. Palencia, J. Nieman, M. Aruguila, M. Baek, C. Diaper, C. Ha, M. Karanam, X. Lu, R. Mohammadi, K. Sawant, R. Sharma, R. Singh, R. Subedi, J. Plattner. Discovery and Mechanism of Action of AN3365: A Novel Boron-containing Antibacterial Agent in Clinical Development for Gram-negative Infections. Poster F1-1637, 50th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 12-15, 2010, Boston, MA (available at www.anacor.com/conference_poster.php)
- W. Bu, XQ, Fan, P. Huston, H. Sexton, P. Torres, A. Wu, I. Heyman, and L. Liu, High Throughput LC/MS/MS Determination of AN3365, A Novel Boron-Containing Compound in Various Biological Matrices to Support Non-GLP, AAPS, November 14-18, 2010, New Orleans, LA (available at www.anacor.com/conference_poster.php)

Results (cont'd)

Figure 4. Comparison of Projected Time-Concentration Profile and Actual Mean Time-Concentration Profile in Human Subjects Following 1-h IV Infusion at 200 mg



Conclusions

- The pharmacokinetic profiles of GSK2251052 in mouse, rat, monkey, dog were defined.
- Human pharmacokinetic profile of GSK2251052 was successfully projected from four mammalian preclinical species with allometric Interspecies scaling
- For the first time, the applicability of allometric scaling has been demonstrated to predict the pharmacokinetics of a novel boron-based antimicrobial in human.
- Allometric scaling on macro constants of PK parameters also allows the simulation of concentration-time profiles following various dose regimens in human to assist clinical trial FIH study design.