

Poster#P84

17th North American Regional ISSX
Atlanta, Georgia
October 16-20, 2011

Metabolic Fate and Disposition of GSK2251052, a Novel Boron-Based Antimicrobial against Gram-Negative Bacteria, in Sprague Dawley Rats and Cynomolgus Monkeys

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Abstract

GSK2251052 (formerly AN3665), a novel boron-containing leucyl-tRNA synthetase inhibitor, is currently being developed for the treatment of serious Gram-negative bacterial infections. Distribution, metabolism, and excretion of GSK2251052 were investigated following a single intravenous administration of ¹⁴C-GSK2251052 to male and female Sprague Dawley rats, and male Long Evans rats, at 50 mg/kg (200 µCi/kg), and male and female cynomolgus monkeys at 25 mg/kg (150 µCi/kg). QWBAs in rats showed radioactivity was widely and rapidly distributed to most tissues with noticeable retention in pigmented ocular tissues by 35 d, and the majority of radioactivity was eliminated from most tissues by 7 d. Preferential distribution of radioactivity into the cellular component of blood was observed with blood to plasma ratio of 1.3 at 24 h. The overall mean recoveries of radioactivity in male and female rats and monkeys were 95% and 82%, respectively. Most of the ¹⁴C-GSK2251052-derived radioactivity was excreted within 48 h, with urinary excretion as the primary route of elimination, representing 67.5% and 51.7% of the total dose in rats and monkeys by 7 d, respectively. Metabolism of GSK2251052 was moderate in rats and extensive in monkeys. Significant quantities of an oxidative metabolite (M3), whereby the hydroxypropyl moiety of GSK2251052 was oxidized to a propanoic acid moiety, were present in monkey plasma. Differences in urinary excretion profiles were demonstrated, with GSK2251052 as the major component excreted in rat urine, accounting for 40% of the radioactivity dose, in comparison to less than 10% of the dose present in urine from monkeys. Metabolite M3 was the major component excreted in urine from monkeys. No gender difference on metabolic profile and excretion of GSK2251052 was observed in rats and monkeys. The species differences in the metabolic profiles suggest renal clearance of GSK2251052 predominates in rats, while the metabolic clearance is substantial in monkeys.

Introduction

GSK2251052 (formerly AN3665) currently in clinical development for the treatment of serious Gram-negative bacterial infections, is a novel boron-based antimicrobial, which specifically targets bacterial leucyl-tRNA synthetase (LeuRS), an essential enzyme in protein synthesis.

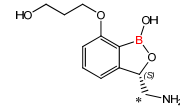
The objectives of the study:

- To investigate the pharmacokinetics, distribution, metabolism, and excretion of GSK2251052 following a single intravenous administration of ¹⁴C-GSK2251052 in male and female Sprague Dawley rats and male Long Evans rats, at 50 mg/kg (200 µCi/kg)
- To investigate the pharmacokinetics, metabolism, and excretion of GSK2251052 following a single intravenous administration of ¹⁴C-GSK2251052 in male and female cynomolgus monkeys at 25 mg/kg (150 µCi/kg).

Materials and Methods

Test Article: ¹⁴C-GSK2251052 (GSK2251052; formerly AN3665), MW=239.12 (free base), synthesized in GE Healthcare, UK

Radiochemical Purity: The radiochemical purity of ¹⁴C-GSK2251052 was over 99% prior to dosing as determined by HPLC analysis. The specific activity was 58 µCi/nmol.



Materials and Methods (Cont'd)

Dose Formulation:

-Rat: 50 mg/kg in sterile water. The specific activity of ¹⁴C-GSK2251052 in the IV dose formulation was calculated to be 3.75 µCi/mg.
-Monkey: 25 mg/kg in sterile water. The specific activity of ¹⁴C-GSK2251052 in the IV dose formulation was calculated to be 5.81 µCi/mg.

Animals:

-Rat: Thirty-eight male and 38 female intact Sprague Dawley rats [HLa(SD/CVJ)], 3 male and 3 female bile duct-cannulated (BDC) Sprague Dawley rats [HLa(SD/CVJ)], bile duct-cannulated by the supplier, and 7 male intact Long Evans rats [HLa(LE/CVJ)] were received from Hilltop Lab Animals, Inc. At dosing, the animals weighed 178 to 335 g and were approximately 6 to 11.5 weeks of age.

-Monkey: Three male and three female drug naïve Cynomolgus monkeys were received from Covance Research Products Inc., Denver, Pennsylvania. At dosing, the animals weighed 2.0 to 2.1 kg and were approximately 33 months of age.

Experimental Design and Procedures:

Species	Number of Animals		IV Dose (mg/kg)	Samples Collection and Assessments
	Male	Female		
Rat	3	3	50 (200 µCi/kg)	Urine, Feces, and Carcass for mass balance and metabolic profiling; 24 h interval through 168 h postdose
	2	30	50 (200 µCi/kg)	Blood and plasma for PK and Metabolic; profiling at 0.083, 0.33, 1, 2, 4, 8, 24, 48, 72, and 120 h postdose Carcasses for QWBA at 0.25, 1, 4, 24, and 72 hours postdose
	3	5	50 (200 µCi/kg)	Bile, Urine, Feces, and Carcass for mass balance through 96 h postdose
	4	3	3	50 (200 µCi/kg)
Monkey	3	3	25 (150 µCi/kg)	Blood, plasma, urine, and feces; for PK, mass balance and Metabolic profiling through 168 h postdose Blood: 0, 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, and 168 h postdose Urine: 0, 8, 24, and 24 h interval through 168 h postdose Feces: 24 h interval

Analytical Methods:

Conventional techniques of liquid scintillation counting were used to measure concentrations of total radioactivity in blood, plasma, urine, feces, and bile. All feces samples were combusted in a Model 307 Sample Oxidizer (Packard Instrument Company) and the resulting ¹⁴CO₂ was trapped in a mixture of Perma Fluor and Carbo-Sorb. Distribution of radioactivity was assessed using quantitative whole-body autoradiography in rats. Metabolite profiles in plasma and excreta were determined by using gradient HPLC with online radioactive detectors, while structural characterization was performed by using liquid chromatography with mass spectrometry (LC-MS) techniques.

Data Analysis:

Pharmacokinetic parameters were calculated by using WinNonlin Professional Edition, Version 5.2 (Pharsight Corporation).

Results

Pharmacokinetics:

Maximum concentrations (C_{max}) of total radioactivity in plasma and blood were observed at the first time point (5 min) after intravenous administration to rat and monkeys. Plasma excretions (AUC_{0-∞}) of total circulating radioactivity were similar between male and female rats and between male and female monkeys. However, AUC_{0-∞} values of total circulating radioactivity were greater in blood than plasma in both species. Similarly, terminal elimination half-lives (t_{1/2}) of total radioactivity in blood in rat and monkey were greater than that observed in plasma. Systemic clearance (CL) of total radioactivity in blood and plasma was greater in rats compared with that observed in monkeys as expected. A summary of the mean PK parameters of total radioactivity in plasma and blood is shown in Table 1.

Tissue Distribution:

Quantitative whole-body autoradiography (QWBA) data in rats indicate ¹⁴C-GSK2251052-derived radioactivity was widely and rapidly distributed to most tissues, and was independent of strain or sex. In male and female cynomolgus Sprague Dawley rats and in male pigmented Long Evans rats, maximum concentrations of radioactivity occurred in most tissues by 1 h post-dose. Excluding excreta, tissue concentrations of radioactivity declined to unquantifiable levels in most tissues by 72 h post-dose, independent of strain or sex. In male pigmented rats, quantifiable concentrations of radioactivity were observed in the uveal tract and pigmented skin at 72 h post-dose, and noticeable radioactivity was observed in pigmented skin at 35 d post-dose. These data suggest that ¹⁴C-GSK2251052-derived radioactivity has a potential affinity for pigmented tissues containing melanin. The representative whole-body autoradiographs are presented in Figure 1.

Excretion and Mass Balance

-Rat: The overall mean recoveries of radioactivity in male and female Sprague Dawley (SD) rats were 94.8 and 95.3% for bile duct-intact and bile duct-cannulated (BDC) rats, respectively, after a single intravenous (IV) 50-mg/kg dose of ¹⁴C-GSK2251052 (Table 2). No sex-dependent differences in excretion were observed. The majority of radioactivity was recovered within the first 24 h after dose administration for intact and BDC rats. The high percentage of radioactivity present in the urine of intact and BDC SD rats after IV administration suggests urinary excretion is the primary route of elimination of ¹⁴C-GSK2251052-derived radioactivity, while the biliary excretion is a secondary route of elimination. The cumulative recoveries in intact or BDC rats are presented in Figure 2.

-Monkey: The overall mean recoveries of radioactivity in male and female cynomolgus monkeys were 76.3 and 86.9%, respectively, through 7 d post-dose after a single IV administration of ¹⁴C-GSK2251052 (Table 2). The overall recovery was relatively incomplete; however, it met the historical recovery values in monkeys studies conducted in Covance. No sex-dependent differences in excretion were observed. The majority of ¹⁴C-GSK2251052-derived radioactivity was excreted within the first 48 h after dose administration. The high percentage of radioactivity present in the urine of monkeys after IV administration suggests urinary excretion is the primary route of elimination. The high percentage of radioactivity present in the urine of intact and BDC rats after IV administration suggests urinary excretion is the primary route of elimination. The cumulative recoveries are presented in Figure 3.

In Vivo Biotransformation of GSK2251052

¹⁴C-GSK2251052 was moderately metabolized in the rat and extensively metabolized in the monkey. GSK2251052 was the major circulating component in plasma from rat and monkey at 2 h post-dose. The metabolite M3 was also an abundant circulating metabolite in plasma in monkeys. ¹⁴C-GSK2251052 was the major radioactive component excreted in rat urine and accounted for 45.9% (0-24 h) of the dose. In contrast, urinary excretion of ¹⁴C-GSK2251052 was minor in monkey and accounted for only 6.6% (0-48 h) of the dose whereas M3, the acid analog of GSK2251052, was the major component excreted in urine and accounted for 21.7%. The significant differences in urinary excretion profiles of GSK2251052 are substantially different between rats and monkeys. In rats, urinary clearance predominates, whereas in the monkey, metabolic clearance is likely significant. The proposed metabolic pathways of GSK2251052 are shown in Figure 4.

Results (Cont'd)

Table 1. Mean pharmacokinetic parameters for radioactivity in blood and plasma after a single IV administration of ¹⁴C-GSK2251052 to rats (50 mg/kg) and monkeys (25 mg/kg).

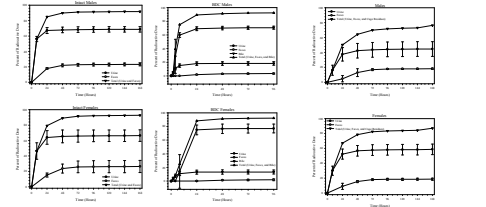
Species (Sex)	C _{max} (ng eq/kg)	t _{1/2} (h)	AUC _{0-∞} (ng eq/h)		Cl (ml/h/kg)
			Blood	Plasma	
Rat (Male)	76.1	127	108	115	435
Rat (Female)	69	125	125	139	361
Monkey (Male)	539	729	98.6	103	247
Monkey (Female)	54.4	69.5	107	112	235
Rat (Male)	52.2	27.2	79.9	81.3	615
Rat (Female)	52.7	53.5	94.4	95.8	522
Monkey (Male)	30.2	42.7	86.4	87.8	292
Monkey (Female)	31.6	32.0	92.8	94	268

ng eq/kg = Equivalents ¹⁴C-GSK2251052

Table 2. Summary of mean total percentages of radioactive dose recovered in urine, feces, and bile samples collected from rats and monkeys after a single intravenous administration of ¹⁴C-GSK2251052 (Rat, 50 mg/kg and Monkey, 25 mg/kg)

Matrix	Mean Percent of Radioactive Dose					
	Rat		BDC Male		Monkey	
	Intact Male	Intact Female	BDC Male	BDC Female	Male	Female
Urine	68.6 ± 3.5	66.4 ± 7.5	70.6 ± 2.2	77.0 ± 6.4	44.6 ± 9.9	58.7 ± 7.1
Feces	23.2 ± 2.0	26.3 ± 7.9	3.45 ± 0.84	1.88 ± 1.29	18.1 ± 0.9	18.7 ± 1.4
Bile	NA	NA	18.3 ± 2.6	13.3 ± 3.0	NA	NA
Total	93.9 ± 1.3	95.7 ± 2.5	95.2 ± 3.4	95.4 ± 4.5	76.3 ± 8.4	86.9 ± 3.1

Figure 2. Mean cumulative percent of radioactive dose in urine and feces after a single IV dose of ¹⁴C-GSK2251052 to male and female intact or BDC rats (50 mg/kg)



Conclusion

A single IV dose of ¹⁴C-GSK2251052 was administered to Sprague Dawley rats, male Long Evans rats, and cynomolgus monkeys, the species used for safety assessment, to investigate the disposition and metabolic fate of GSK2251052.

- > ¹⁴C-GSK2251052 was widely and rapidly distributed to most tissues. The majority of radioactivity was eliminated from most tissues by 7 d, while noticeable retention in melanin-containing tissues was observed in Long Evans rats.
- > Partitioning to blood cells was observed.
- > Significant quantities of metabolite M3 were present in monkey plasma.
- > Excretion of ¹⁴C-GSK2251052-derived radioactivity was rapid with urinary excretion as the primary route of elimination, and fecal/biliary excretion as the secondary routes in both species, without gender difference.
- > GSK2251052 was the major component excreted in rat urine with only small amounts present in urine from monkeys, while metabolite M3 was the major component excreted in urine from monkeys.
- > The species differences in metabolic profiles suggest renal clearance of GSK2251052 predominates in rats, while metabolic clearance is significant in monkeys.

The insights of the distribution, metabolism and excretion of GSK2251052 in rats and monkeys aid in investigation of disposition and metabolic fate of GSK2251052 in human for clinical development.

Figure 4. Proposed metabolic pathways of GSK2251052 in rats and monkey

