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PHARMACOKINETICS, EXCRETION, AND METABOLISM OF [^{14}C]*AFICAMTEN* FOLLOWING SINGLE ORAL DOSE ADMINISTRATION TO RATS

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PURPOSE

Aficamten is a next-in-class, small-molecule, selective cardiac myosin inhibitor in Phase 3 development as a potential treatment for hypertrophic cardiomyopathy.

The purpose of this study was to determine the pharmacokinetics, distribution, metabolism, and excretion of [^{14}C]*aficamten* and to characterize the metabolites present in plasma, urine, bile, and feces in male rats following single oral dose administration. Tissue distribution was investigated by quantitative whole-body autoradiography (QWBA) in Long Evans (LE) rats.

OBJECTIVES

- To determine the pharmacokinetics, distribution, metabolism, and excretion of [^{14}C]*aficamten* in rat.
- To determine the metabolic route of metabolite M18 formation *in vivo* leading to its detection in rat feces.

METHODS

The in vivo ADME study was conducted at **Labcorp, Early Development Laboratories**, Inc., Madison, WI. The absorption, distribution, metabolism, and excretion of radioactivity were determined after administration of a single oral dose (8 mg/kg, 100 µCi) of [^{14}C]*aficamten* to male intact and bile duct-cannulated (BDC) Sprague Dawley (SD) rats. Blood was collected for pharmacokinetic analysis at selected times through 72 hours post-dose. Elimination of radioactivity in urine, feces, and expired air after oral dosing to intact SD rats was determined through 168 hours post-dose. Excretion of radioactivity in bile, urine, and feces through 168 hours was determined in BDC rats after oral administration. Samples were processed and analyzed for total radioactivity by liquid scintillation counting. Profiling and identification of metabolites in plasma, urine, and feces were conducted by liquid chromatography (LC)-radiometric and LC-tandem mass spectrometric (LC-MS/MS) analyses. LE animal carcasses were processed for QWBA analysis.

Bile from *aficamten*-dosed (8 mg/kg, 0-24 h) SD rats (n=3) and rat intestine and colon from one naïve SD male rat were obtained at **Cytokinetics**. Rat intestinal contents (RI) were obtained by mixing opened intestine and colon sections with 100 mL of 0.01 M Tris-HCl buffer pH 7.4 containing 0.15 M KCl under nitrogen atmosphere. Rat bile (0-24 h, 50 µL) was mixed with 2 mL of RI for 0-20 h for assessment of M18 formation as determined by LC-MS/MS analysis and confirmed with authentic synthetic standard (M18, CK-4017583).

Confirmation of the identity of metabolite M5 (at **Cytokinetics**, M1 glucuronide) was obtained by treating 50 µL of *aficamten*-dosed rat bile (0-24 h) with 1 mL 0.1 M potassium phosphate buffer (pH 7.4, 37°C) containing 1 mg/mL of β-glucuronidase enzyme (from *Escherichia coli*; 20,000 units/mg protein) with LC-MS/MS analysis for the degradation M5 glucuronide and formation of the M1 product.

RESULTS

Table 1. Pharmacokinetic parameters for [^{14}C]*aficamten*-derived radioactivity in the circulation.

Study	Matrix	C_{max} (ng Eq/g)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng Eq·h/g)	$AUC_{0\text{-}inf}$ (ng Eq·h/g)	$t_{1/2}$ (h)
Mass balance (group 2)	Blood	5330	4.0	72	108000	108000	6.85
	Plasma	9430	4.0	72	179000	179000	5.81
QWBA (group 4, LE rat)	Blood	6290	2.0	48	89780	90772	7.40
	Plasma	11300	2.0	48	170609	172261	7.07

Figure 1: Figure 1: Mean cumulative percent of radioactive dose in urine, feces, and total after a single oral administration of [^{14}C]*aficamten* to intact male SD rats (Group 1).

Total includes urine, feces, cage rinse, cage wash, cage wipe, and carcass.

Figure 2: Mean cumulative percent of radioactive dose in urine, feces, bile, and total after a single oral administration of [^{14}C]*aficamten* to bile duct-cannulated male SD rats (Group 5).

Figure 3. Selected autoradiography images (2 h post-dose) of tissue distribution in whole rat body after single-dose oral administration of 8 mg/kg [^{14}C]*aficamten* to LE rats.

Figure 4. Tissue concentration-time profiles for pigmented LE rats. The concentration of radioactivity in tissues were expressed as the nanogram equivalent (ng Eq) per gram of sample. One rat was used per timepoint.

Figure 5. Radiochromatograms from the rat ADME study of [^{14}C]*aficamten* (8 mg/kg): (A) representative 0.25 to 48 h pooled plasma sample from intact rat (group 2), (B) 0 to 48 h pooled urine sample from BDC rat (group 5), (C) 0 to 8 h pooled bile sample from BDC rat (group 5), and (D) 0 to 24 h pooled feces sample from intact rat (group 1).

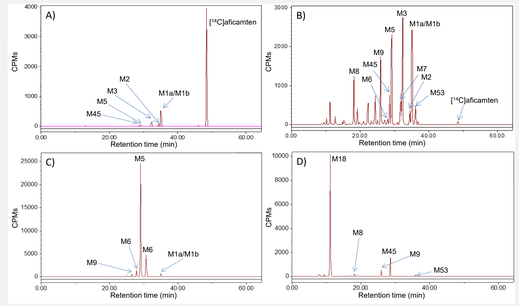


Figure 6. Metabolism of *aficamten* by P-450-mediated oxidation, UGT-mediated conjugation, reductive ring-opening in the gastrointestinal tract leading to oxidized metabolites M1 and M3, glucuronide M5, and the reduced ring-opened metabolite M18, respectively.

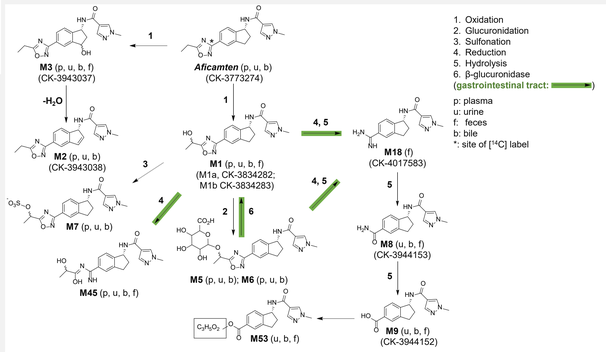


Table 2. Radiochromatography and LC-MS/MS metabolite profiling and identification and summary of protonated molecular ions and characteristic product ions for *aficamten* and identified metabolites. (Metabolite is found in matrix designated with "X")

Analyte Designation	Retention time (min)	Proposed Identification	Protonated molecular ion, MH^+ (m/z)	Structurally informative fragment ions (m/z)	Matrix			
					Plasma	Urine	Bile	Feces
M18	11.09	CK-4017583	284	267, 159, 142, 126, 117, 109, 83				X
M8	18.03-18.37	CK-3944153	285	160, 143, 126, 117, 109, 83		X	X	X
M9	25.65-26.17	CK-3944152	286	161, 143, 126, 117, 109, 83		X	X	X
M6	27.73-28.08	CK-3834282 or CK-3834283-glucuronide	530	405, 354, 229, 157, 126	X	X	X	
M45	28.60-28.77	Oxy-oxadiazole-ring opened-CK-3773274	357	268, 232, 143, 126, 109, 83	X	X	X	X
M5	28.95-29.29	CK-3834282 or CK-3834283-glucuronide	530	405, 354, 229, 157, 126, 109	X	X	X	
M7	30.51-31.89	CK-3834282 and/or CK-3834283-sulfate	434	309, 229, 157, 126, 109, 83	X	X	X	
M3	32.24-32.93	CK-3943037	354	336, 280, 229, 211, 173, 155, 126, 109, 83	X	X	X	X
M2	34.32-34.49	CK-3943038	336	229, 157, 126, 109, 83	X	X	X	
M1a/M1b	35.01-35.19	CK-3834282/ CK-3834283	354	229, 157, 126, 109, 83	X	X	X	X
M53	35.88-36.23	CK-3944152-adduct	358	268, 233, 161, 143, 126, 109, 83		X	X	X
Aficamten	48.36-48.53	CK-3773274	338	213, 157, 126, 109, 83	X	X	X	

Table 3: *Aficamten* and metabolites percent of radioactive dose in urine, feces, and bile after a single oral administration of [^{14}C]*aficamten* to intact and BDC male Sprague Dawley rats (ND, not detected).

[^{14}C]Aficamten Rat Mass Balance Metabolites (% of Radioactive Dose, 8 mg/kg PO)						
Analyte	Intact (Group 1)		Bile duct-cannulated (BDC, Group 5)			Intact (Group 2)
	Urine 0-48 h	Feces 0-72 h	Urine 0-48 h	Feces 0-72 h	Bile 0-72 h	Plasma (AUC pool, % of LC/Rad run)
Aficamten	0.0421	ND	0.0367	ND	0.125	80.11
M1	1.00	ND	1.37	0.0169	1.80	11.67
M5	1.30	ND	1.18	ND	**35.2**	0.95
M18	ND	**35.3**	ND	7.12	ND	ND
M45	0.443	7.24	0.318	3.72	0.0179	0.11

Figure 7: Complete degradation of M5 (M1-glucuronide) to M1 after treatment of rat bile with β-glucuronidase in buffer (pH 7.4, 37°C).

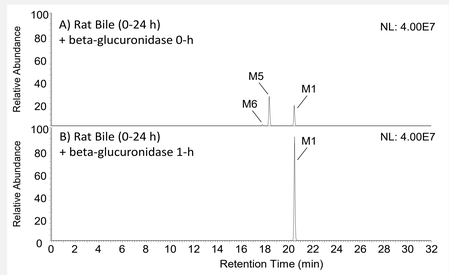
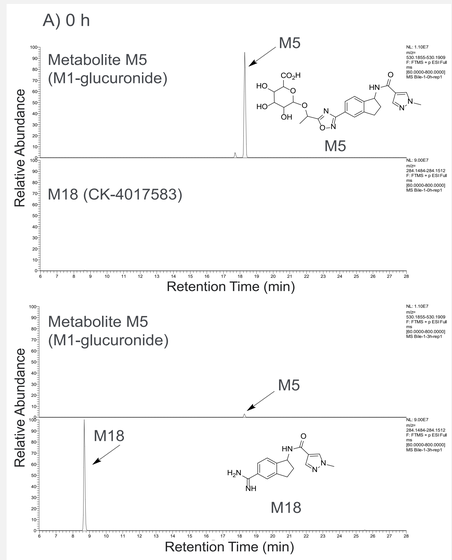


Figure 8: Treatment of *aficamten*-dosed rat bile (8 mg/kg, 0-24 h, 50 µL) with rat intestinal contents (2 mL, 37°C, nitrogen atmosphere, dark) leads to the complete degradation of metabolite M5 to metabolite M18.



CONCLUSIONS

- Plasma C_{max}, $AUC_{0\text{-}inf}$, and elimination half-life values for total radioactivity were 9340 ng Eq/mL, 179000 ng Eq·h/g, and 5.81 h, respectively (group 2, Table 1).
- Radioactivity derived from **[^{14}C]*aficamten*** was rapidly excreted after oral administration (Table 1).
- After oral administration to intact rats, means of **8.27** and **90.7%** of the administered radioactivity were excreted in urine and feces, respectively, by 168 hours (Fig 1).
- **51.7%** of radioactive dose was eliminated in bile after oral dosing, indicating that biliary excretion was the major route of elimination. Based on the radioactivity excreted in urine and bile, a minimum of approximately **60%** of the orally administered dose was absorbed (Fig. 2).
- **[^{14}C]*Aficamten***-derived radioactivity was widely distributed to most tissues by 0.5 to 1 hour in LE male rats with highest distribution to myocardium tissue (Fig. 3, Fig. 4).
- Metabolite profiling and identification results indicated that **[^{14}C]*aficamten*** was eliminated in rats primarily *via* metabolism (Fig.5, Fig. 6, Table 2).
- Unchanged **[^{14}C]*aficamten*** was the major circulating component from intact rats and accounted for approximately **80%** of the total radioactivity exposure (Figure 5A, Table 3).
- Metabolite **M5** was the major component in bile (**35.2% of dose,** Fig. 5C, Table 3).
- Metabolite **M18** was the major component detected in feces from intact rats (**35.3% dose,** Fig. 5D, Table 3).
- Incubation of aficamten-dosed rat bile with naïve rat intestinal contents led to the complete degradation of metabolite **M5** (M1-glucuronide) and to the corresponding formation of metabolite **M18** (Fig. 8) after 20 h of incubation.
- From these results, evidence was obtained that metabolite **M18** detected in rat feces *in vivo* was formed from the metabolism of ***aficamten*** to hydroxylated metabolite (**M1**) followed by conjugation leading to the glucuronide metabolite (**M5**) in rat liver, then biliary excretion of **M5** into rat gastrointestinal tract with subsequent reduction-type metabolism leading to metabolite **M18** (Fig. 6).

DISCLOSURES AND ACKNOWLEDGMENTS

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RS, DX, MPG, and BPM: Employees of and hold stock in Cytokinetics, Incorporated.