PHARMACOKINETICS, EXCRETION, AND METABOLISM OF [14C]CK-4021586 FOLLOWING SINGLE ORAL ADMINISTRATION TO RATS

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ABSTRACT

CK-4021586 (now known as *Ulacamten*) is a novel small-molecule cardiac myosin inhibitor designed to reduce hypercontractility associated with heart failure with preserved ejection fraction (HFpEF). This study determined the pharmacokinetics, distribution, metabolism, and excretion of [14C]CK-4021586 and characterized metabolites present in plasma and excreta in male rats following oral dose administration. Thus, the absorption, distribution, metabolism, and excretion (ADME) of radioactivity were determined after administration of a single oral dose of [14C]CK-4021586 (45 mg/kg, 200 μCi/kg) to male intact and bile duct-cannulated (BDC) Sprague Dawley (SD) rats. Blood was collected for pharmacokinetic analysis through 48 hours post-dose. Elimination of radioactivity in urine and feces was determined through 168 hours post-dose in intact SD rats. Excretion of radioactivity in bile, urine, and feces through 96 hours was determined in BDC rats. Samples were analyzed for total radioactivity by liquid scintillation counting. Metabolite profiling and identification in plasma, urine, bile, and feces was conducted using liquid chromatography (LC) radiometric and LC-tandem mass spectrometric (LC-MS/MS) analyses. Following oral administration of [14C]CK-4021586 to intact rats, the primary route of elimination of radioactivity was in the feces (63.3%), followed by urine (29.7%), with a 95.2% mean total recovery of radioactive dose. In BDC rats, hepatobiliary excretion was the major route of elimination of radioactivity with approximately 50.5% of the administered dose recovered in bile through 96 hours, followed by urine (40.1%), and with 95.6% total recovery observed. After a single oral dose of [14C]CK-4021586 in intact rats, C_{max} of mean plasma total radioactivity was 7.22 µg equiv./mL with median t_{max} at 1 hour post-dose. The mean $t_{1/2}$, area under the concentration—time curve (AUC)_{last}, and AUC_{inf} obs of plasma total radioactivity were 5.5-hour, 40.9 h·µg equiv./mL, and 42.8 h·µg equiv./mL, respectively. Quantitative whole-body autoradiography in Long Evans (LE) rats revealed wide distribution of drug-derived radioactivity throughout the body with quantifiable concentrations present in many tissues through 72 hours and the C_{max} of radioactivity in most tissues observed at 1 hour post-dose. Relatively high concentrations were observed in liver, eye uvea, kidney cortex, Harderian gland, and adrenal gland tissues while relatively low tissue concentrations were observed in spinal cord, brain, bone, and eye lens. [14C]CK-4021586 underwent extensive metabolism in rats where 23 metabolites were identified. Unchanged [14C]CK-4021586 was the major circulating component in AUC_{0-24h}-pooled plasma from intact rats and accounted for approximately 37.7% of the total radioactivity exposure. An N-deformylated metabolite (M1) and a methyl-group hydroxylated metabolite (M2) were the other major circulating components accounting for 36.3% and 9.9% of total radioactivity exposure. Low levels of unchanged [14C]CK-4021586 (1.2% of dose) were recovered in bile, while metabolite M15 (M2 oxidized to a carboxylic acid) was the major component in bile and accounted for 28% of dose and was the major component (45.8% of dose) in feces in intact SD rats. These results provide information for designing future [14C]CK-4021586 ADME studies in humans.

METHODS

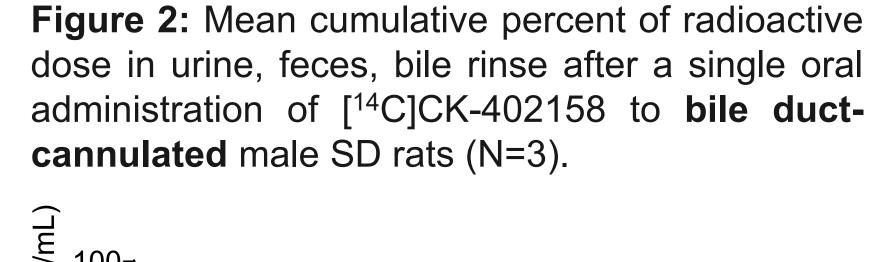
The in vivo ADME study was conducted at QPS, LLC, Newark, DE, USA. The absorption, distribution, metabolism, and excretion of radioactivity were determined after administration of a single oral dose (45 mg/kg, 200 μ Ci) of [14 C]CK-4021586 to male intact and BDC SD rats. Blood was collected for pharmacokinetic analysis at selected times through 48 hours post-dose. Elimination of radioactivity in urine, feces, after oral dosing to intact SD rats was determined through 168 hours post-dose. Excretion of radioactivity in bile, urine, and feces through 96 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-MS/MS analyses. LE animal carcasses were processed for QWBA analysis.

RESULTS

Table 1. Pharmacokinetic parameters for [¹⁴C]CK-4021586-derived radioactivity in the circulation after a single oral dose of 45 mg/kg, 200 μCi of [¹⁴C] CK-4021586. ND, not determined.

Study	Matrix	C _{max} (µg eq./g)	<i>t</i> _{max} (h)	<i>t</i> _{1/2} (h)	AUC _{last} (µg eq.*h/g)	AUC _{0-inf} (µg eq.*h/g)
Mass balance (Intact SD rat)	Plasma	7.22	1.0	5.5	40.9	42.8
QWBA	Blood	9.41	1.0	ND	63.9	ND
(LE rat)	Plasma	10.9	1.0	2.6	28.9	32.7

Figure 1: Mean cumulative percent of radioactive dose in urine, feces after a single oral administration of [14C]CK-4021586 to **intact** male SD rats (N=3).



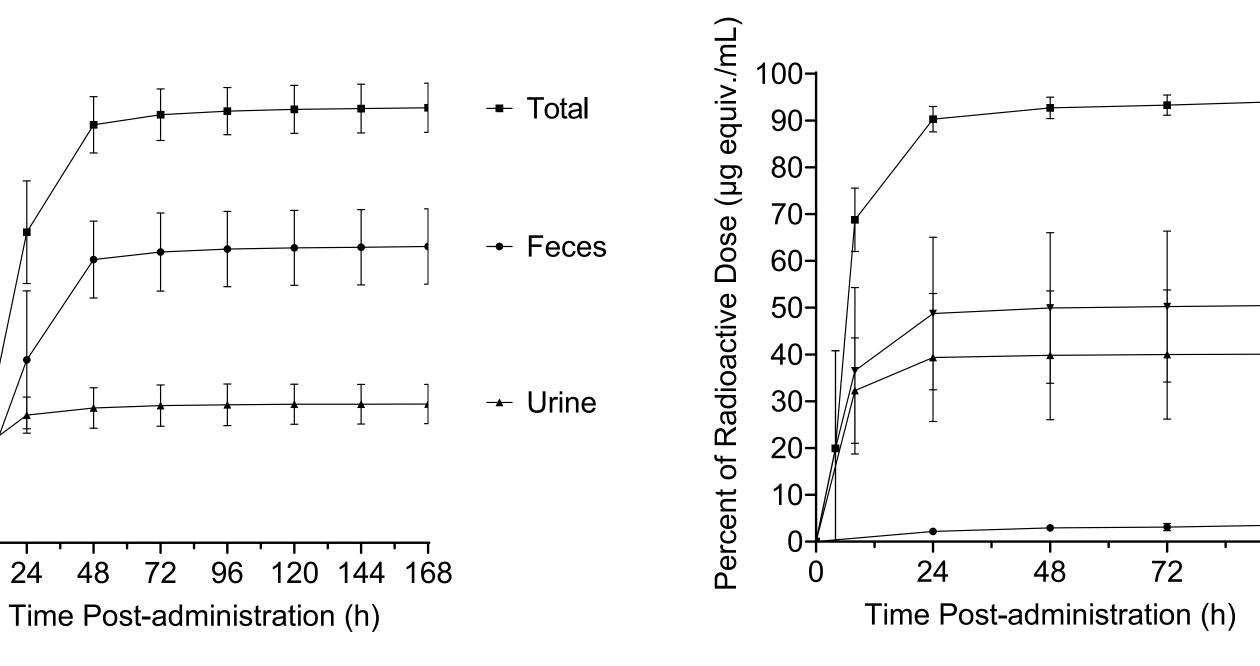


Figure 5. Radiochromatograms from the rat ADME study of [14C]CK-4021586 (45 mg/kg): (A) representative 0 to 24 h AUC-pooled plasma sample from intact rat, (B) 0 to 48 h pooled urine sample from intact rat, (C) 0 to 48 h pooled bile sample from BDC rat, and (D) 0 to 72 h pooled feces sample from intact rat.

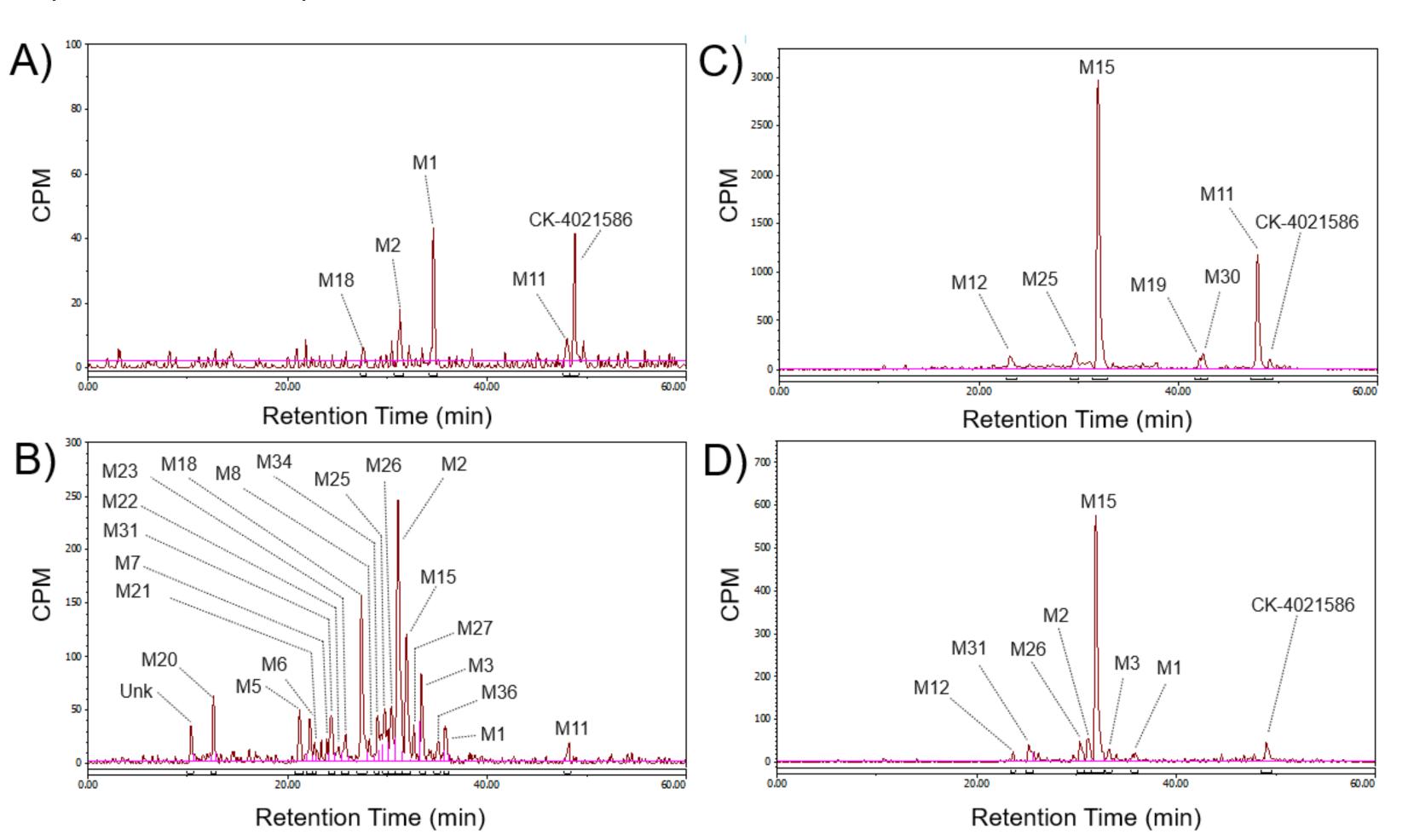


Figure 6. Chemical structures of 23 metabolites of [14C]CK-4021586 detected in rat plasma, urine, bile, and feces.

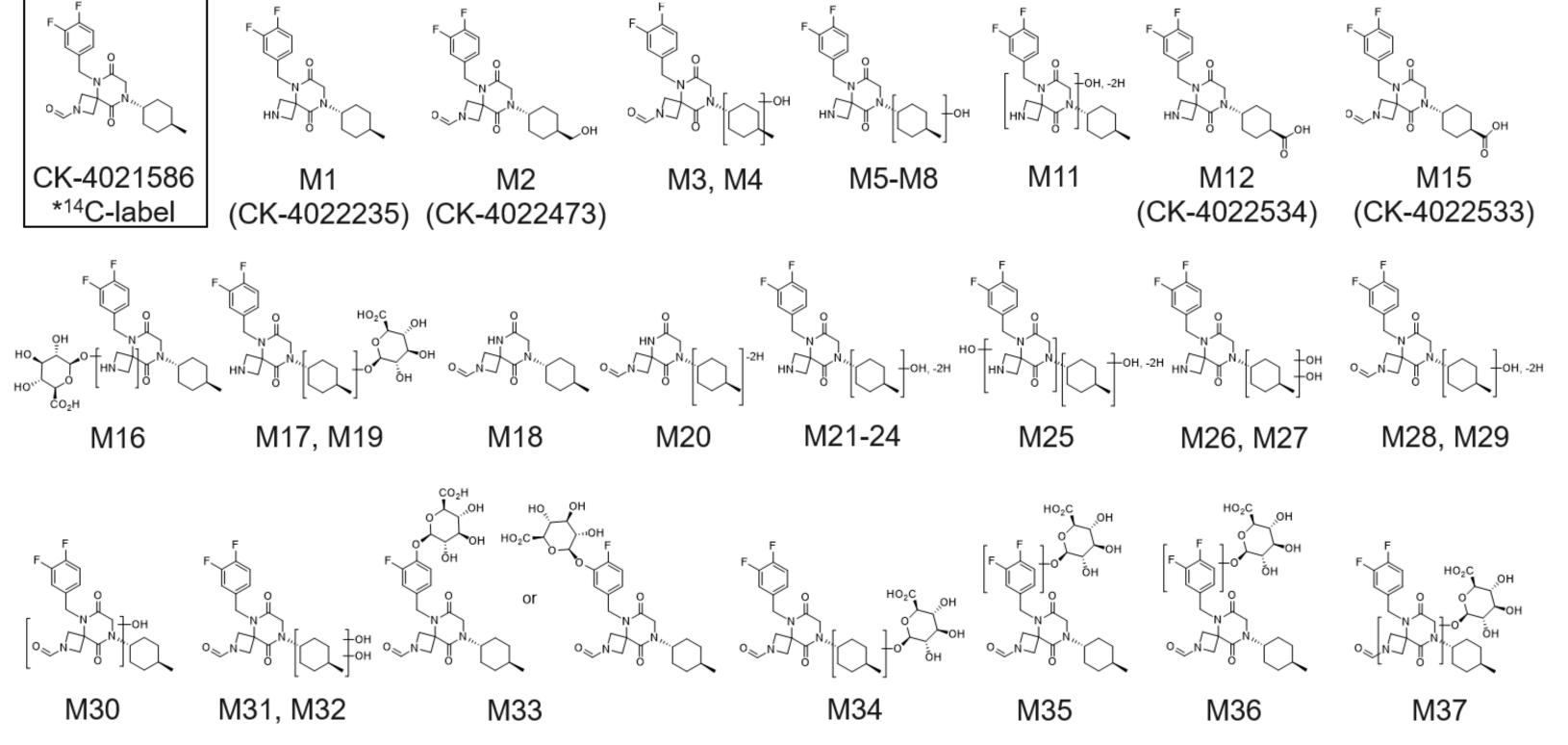


Figure 3. Selected autoradiography images (1 h post-dose) of tissue distribution in whole rat body after single-dose oral administration of 45 mg/kg, 200 μCi [¹⁴C]CK-4021586 to LE rats

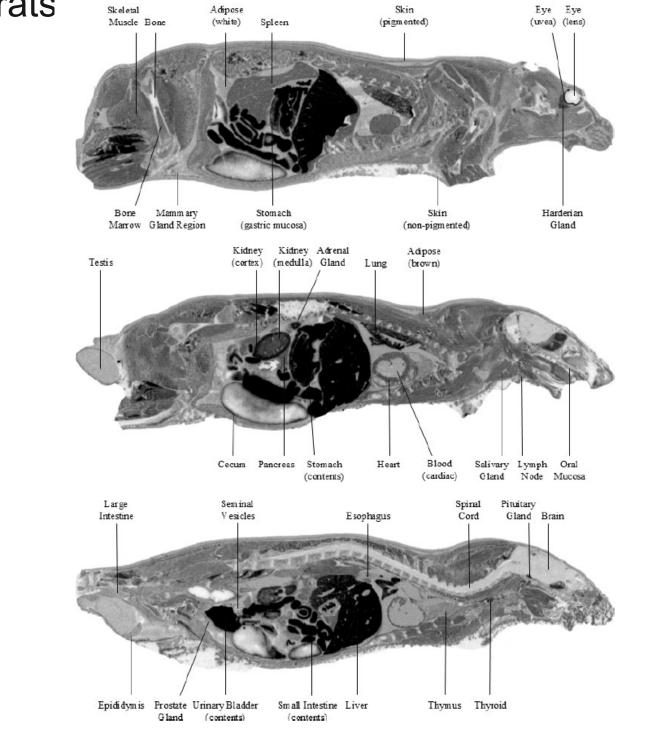


Figure 4. Distribution profile of total radioactivity (μ g equivalents per gram tissue) in various tissues after single-dose oral administration of 45 mg/kg, 200 μ Ci [14C]CK-4021586 to LE rats.

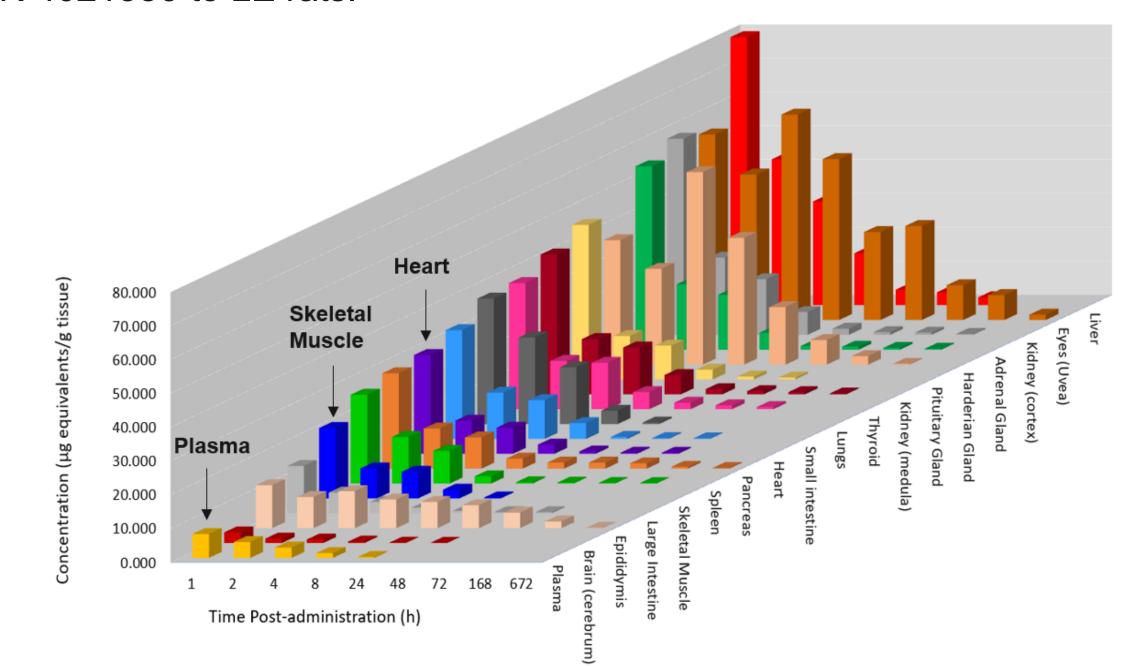


Table 2. Major Components Detected in Plasma and Excreta											
	Plasma (% AUC)		Urine (% Dose, Intact Rat)		Feces (% Dose, Intact Rat)		Bile (% Dose, BDC Rat)				
CK-586	37.7	M2	7.7	M15	45.8	M15	28.0				
M1 :	36.3	M15	4.5	_		M11	10.5				
M2	9.9	_		_	-	_	-				

CONCLUSIONS

- Radioactivity derived from [14C]CK-4021586 was rapidly excreted after oral administration.
- Plasma C_{max}, AUC_{0-inf}, and elimination half-life values for total radioactivity were **7.22 μg eq/mL**, **42.8 μg eq*h/g**, and **5.5 h**, respectively.
- After oral administration to intact rats, means of **29.7%** and **63.3%** of the administered radioactivity were excreted in urine and feces, respectively, by 168 hours.
- 50.5% of radioactive dose was eliminated in bile after oral dosing, indicating biliary excretion was the major route of elimination. Based on the radioactivity excreted in urine and bile, a minimum of approximately 90.6% of the orally administered dose was absorbed.
- [14C]CK-4021586-derived radioactivity was widely distributed to most tissues by 1 hour in LE male rats with highest distribution to liver, eye uvea, kidney cortex and Harderian and adrenal glands.
- Metabolite profiling and identification results indicated that [14C]CK-4021586 was eliminated in rats primarily *via* metabolism.
- Unchanged [¹⁴C]CK-4021586 was the major circulating component from intact rats and accounted for approximately 37.7% of the total radioactivity exposure, followed by metabolite M1 (36.3%) and metabolite M2 (9.9%).
- Metabolite M15 was the major component in bile (28% of dose).
- Metabolite M15 was the major component detected in feces from intact rats (45.8% of dose).



