Cross-Species Pharmacokinetics of CK-2017357, a Novel Activator of the Fast Skeletal Sarcomere

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Abstract

Purpose: CK-2017357 is a novel small molecule activator of the fast skeletal muscle troponin complex that has been developed to improve skeletal muscle function in disease states associated with muscular weakness or fatigue. To enable clinical development, the pharmacokinetics of CK-2017357 were characterized in mouse, rat, dog, and rabbit. Animal pharmacokinetics were compared to those of humans obtained from a multiple oral dose Phase I trial in healthy male subjects.

Methods: Pharmacokinetic studies of CK-2017357 were conducted in female mice, male/female rats, male/female dogs, and female rabbits as either single dose (IV and/or PO) or repeat dose (PO only). The multiple oral dose human pharmacokinetic study was conducted in healthy male subjects at two dose levels, 250 and 375 mg q.d. for 7 days. Plasma samples from these studies were extracted by protein precipitation and analyzed by LC/MS/MS. Non-compartmental pharmacokinetic parameters were determined using WinNonlin. Intrinsic clearance of CK-2017357 was also determined in liver microsomes of multiple species.

Results: In all animal species, CK-2017357 showed low systemic clearance, and low to moderate volume of distribution. The elimination kinetics appeared to be monophasic. Oral bioavailability was high in mice, rats, and dogs. In rats, there was a gender difference with females exhibiting a higher (~ 2-fold) systemic exposure than males following oral administration. This gender difference was not seen in dogs. In humans, the systemic exposure of CK-2017357 was high, and steady-state was achieved by day 7 with minimal drug accumulation following multiple daily oral doses. Dose normalized steady-state AUC24h was 43 ((hr*µg/mL)/(mg/kg/day)) in humans, and in animals ranged from 5 – 22. *In vitro* in liver microsomes, the intrinsic clearance of CK-2017357 was low in all species.

Conclusion: CK-2017357 demonstrated desirable pharmacokinetics in all animal species studied, and in male healthy subjects. These pharmacokinetic properties provided guidance for clinical development, and supported once daily oral dosing in patients.

Introduction

- CK-2017357 is a novel, selective small molecule activator of fast skeletal muscle
- CK-2017357 slows the rate of calcium release from the regulatory troponin complex of fast skeletal muscle and sensitizes the sarcomere to calcium
- Preclinically, CK-2017357 increases skeletal muscle force during sub-maximal neural stimulation and increases the time to fatigue
- In multiple animal species (mouse, rat, dog, rabbit, and monkey), CK-2017357 showed desirable pharmacokinetics (PK) with good oral bioavailability, and was predicted to exhibit good oral PK in humans
- Single dose and steady-state PK of CK-2017357 were characterized across multiple species following either IV or oral administration

Methods

***In vitro* studies:**

- Microsomal stability and intrinsic clearance of CK-2017357 (1 µM) were evaluated *in vitro* in liver microsomes of the rat, dog, monkey, and human.
- The *in vitro* protein binding of CK-2017357 in rat, monkey, dog, and human plasma was determined by equilibrium dialysis for 8-16 h at a target concentration of 5 µM.
- The association of CK-2017357 with blood cells was evaluated *in vitro* using blood from rats, dogs, and humans at concentrations of 0.5 and 5 µM.

***In vivo* studies:**

- Single dose PK studies with intensive blood sampling were conducted in female mice, male rats, and male dogs following both IV and PO administration. A radiolabeled mass balance/PK study was also conducted in male rats (both bile-duct intact and bile-duct cannulated). Single dose oral PK in humans was determined in a Phase I dose-escalation trial (CY4011) in healthy male subjects over a dose range of 20 – 2500 mg. Data from the 80 mg dose cohort are presented and used for cross-species comparison.
- Steady-state PK/TK of CK-2017357 were determined as a part of toxicology studies in male and female rats (28-day), male and female dogs (28-day), and female rabbits (13-day). For monkeys (cynos), TK parameters from a single dose MTD study were used since repeat dose studies were not conducted. Multiple oral dose human PK study was conducted in healthy male subjects at two dose levels, 250 and 375 mg q.d. for 7 days. Results from the 375 mg cohort are presented and used for cross-species comparison.
- Plasma samples from these *in vivo* studies were extracted by protein precipitation and analyzed using validated LC/MS/MS methods.
- Non-compartmental (NCA) methods were used to calculate descriptive PK/TK parameters after single and multiple doses (IV and/or PO).

Results

Table 1. Intrinsic clearance, protein binding, and blood-to-plasma partitioning of CK-2017357

Species	*In vitro* Hepatic Intrinsic Clearance, Cl_{int}, (mL/min/kg)	Protein binding (% bound)	*In vitro* blood-to-plasma ratio
Human	< 5.2	98.0%	0.59 – 0.61
Rat	19.8	94.6%	0.55 – 0.56
Dog	< 8.3	92.9%	0.61 – 0.71
Monkey	9.5	97.3%	---

- CK-2017357 exhibited low intrinsic clearance in all four species
- Protein binding was similar across-species
- CK-2017357 showed low partitioning in red blood cells

Table 2. Oral bioavailability of CK-2017357 in mice, rats, and dogs

Species	Dose (mg/kg)	Route of Administration	Sex	Time to Peak (hr)[c]	Oral Bioavailability (%)
Mouse	4 - 20	Gavage-Fasted	F	0.25 – 0.50	25 - 54
Rat	1 - 20	Gavage-Fasted	M	0.38 – 0.63	180 - 183
Rat	4.4 - 37 [a]	Gavage-Fasted	M	2.5 – 3.5	176 - 196
Rat[b]	20	Gavage-Fasted	M	1.7	126
BDC Rat[b]	20	Gavage-Fasted	M	1.7	62
Dog	1	Gavage-Fasted	M	0.58	93

[a] API-in-Capsule formulation
[b] [^{14}C]-CK-2017357 used in this study
[c] Mean value
BDC = Bile duct cannulated

- High oral bioavailability was observed in all animal species
- >100% oral bioavailability in rats was due to enterohepatic recirculation as data in BDC rats suggested

Table 3. Comparative pharmacokinetics of CK-2017357 following a single IV or oral dose

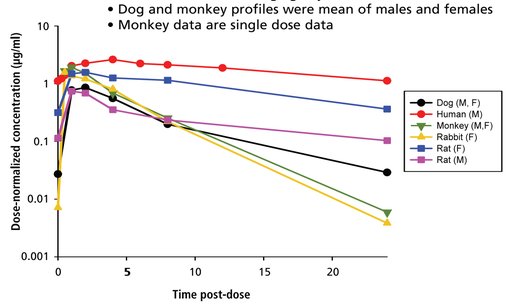
Species (Gender)	Dose (mg/kg)	Route	C_{max} (µg/mL)	t_{max} (hr)	AUC_{0-t} (hr*µg/mL)	CL (L/hr/kg)	V_{ss} (L/kg)	$t_{1/2}$ (hr)	Dose Normalized AUC
Mouse (F)	4	IV	15.5	-	21.0	0.20	0.43	3.0	5.25
	4	PO	3.7	0.25	4.98	-	-	2.7	1.25
	20	PO	13.2	0.50	48.3	-	-	4.1	2.42
Rat (M)	1	IV	3.3	-	4.10	0.29	1.2	3.9	4.10
	1	PO	2.3	0.38	6.44	-	-	3.5	6.44
	20	PO	25.1	0.63	124	-	-	7.7	6.20
Rat (M)	4.4[a]	PO	3.7	2.5	29.2	-	-	4.6	6.64
	37[a]	PO	29.7	3.5	218	-	-	5.9	5.89
Rat (M)[b]	5	IV	10.2	-	38.9	0.14	0.70	4.4	7.78
	20	PO	18.6	1.7	189.8	-	-	5.5	9.50
	20[c]	PO	17.2	1.7	96.6	-	-	1.9	4.83
Dog (M)	1	IV	2.0	-	4.03	0.26	1.7	8.2	4.03
	1	PO	0.85	0.58	3.80	-	-	8.5	3.80
Human (M)	1.14[a]	PO	1.40	5.7	29.4	-	-	13.3	25.7

[a] API-in-Capsule formulation
[b] [^{14}C]-CK-2017357 ADME study in rats
[c] BDC rats

Across species, CK-2017357 showed high systemic exposure with a low clearance and low to moderate volume of distribution

Figure 1. Dose-normalized CK-2017357 steady-state plasma concentration – time profiles in multiple species

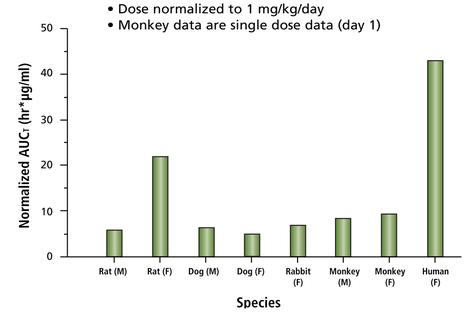
- Dose normalized to 1 mg/kg/day
- Dog and monkey profiles were mean of males and females
- Monkey data are single dose data



- CK-2017357 showed a higher clearance in dogs, monkeys, and rabbits than in rats and humans
- A gender difference in PK/TK was observed in rats, but not in dogs and monkeys

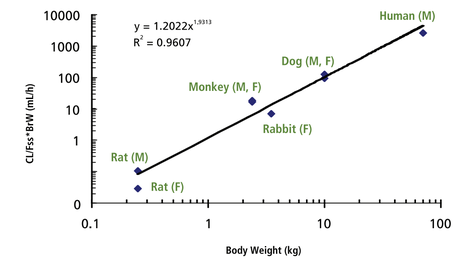
Figure 2. Dose-normalized CK-2017357 steady-state exposure (AUC) across species

- Dose normalized to 1 mg/kg/day
- Monkey data are single dose data (day 1)

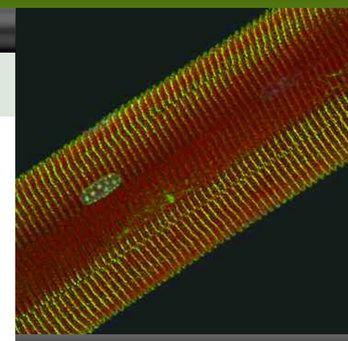


- Female rats showed the highest exposure across animal species, and were the closest to humans
- A gender difference in exposure was observed in rats, but not in dogs and monkeys

Figure 3. Allometric relationship of steady-state apparent clearance (CL/Fss) across species following oral administration



Good correlation observed with brain weight (BrW) correction



Conclusions

1. *In vitro*, CK-2017357 exhibited low intrinsic clearance in liver microsomes of multiple species, and low partitioning to red blood cells.
2. *In vivo*, CK-2017357 showed low systemic clearance, and low to moderate volume of distribution across species. The elimination kinetics appeared to be monophasic. Oral bioavailability was high in mice, rats, and dogs. In rats, there was a gender difference with females exhibiting a higher (~2-fold) systemic exposure than males following oral administration. This gender difference was not seen in dogs and monkeys.
3. In healthy male subjects following single or multiple oral doses, CK-2017357 showed high systemic exposure, higher than any animal species based on dose-normalized AUC (dose normalized to 1mg/kg/day). The terminal $t_{1/2}$ was 13.3 hr at a dose of 80 mg.
4. Allometric scaling of steady-state apparent total drug clearance of animals with brain weight correction predicted the human clearance.
5. CK-2017357 demonstrated desirable pharmacokinetics in all animal species studied, and in male healthy subjects. These pharmacokinetic properties provided guidance for clinical development, and supported once daily oral dosing in patients.

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