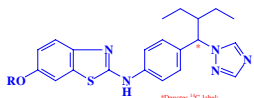


Mass Balance, Metabolism, and Excretion of Rambazole, a Novel Retinoic Acid Metabolism-Blocking Agent (RAMBA) in Mice, Rats, and Dogs

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Introduction



Rambazole ((R)-N-[4-(2-ethyl-1-(1H-1,2,4-triazole-1-yl)butyl)phenyl]-2-benzothiazolamine), a novel enantiomerically pure retinoic acid metabolism-blocking agent (RAMBA), is currently in clinical investigation for oral and topical treatment of keratinisation disorders such as psoriasis and acne. The anticipated daily dose in humans is between 0.01 and 0.04 mg/kg.

Mouse, rat, and dog were the animal species used for the safety evaluation of Rambazole. Similar metabolite profiles in these species to those in humans would support the use of these species as appropriate animal species for the safety evaluation of Rambazole. In the present studies, the disposition of [¹⁴C]Rambazole was examined in mice, rats, and dogs after oral administration to provide information on the absorption, metabolism and excretion of Rambazole.

Experimental

High-Performance Liquid chromatography (HPLC):

LC System: Waters 2695 Separations Module
 Analytical Column: C18 column, 4.6 x 150 mm, 3 μm
 Flow Rate: 1.0 mL/min
 Mobile Phase A: 2% HCOOH in H₂O (pH 3.2)
 Mobile Phase B: CH₃CN
 Gradient:

(min)	(mL/min)	A (%)	B (%)
0	0.7	100	0
3	0.7	100	0
28	0.7	75	25
48	0.7	65	35
78	0.7	30	70
83	0.7	0	100
88	0.7	0	100
90	0.7	100	0
105	0.7	100	0

Mass Spectrometry:

MS System: Finnigan LCQTM Mass Spectrometers
 Ionization Mode: Positive ESI
 Ion Spray (IS): 4.5 kV
 Temperature: 240°C
 Sheath Gas Flow: 80 units
 Auxiliary Gas Flow: 20 units
 Collision Gas: Helium
 Collision Energy: 1–25 eV

Study Design

Male and Female CD-1 mice (19–29g, n = 3/ex/timepoint for blood sampling, n=3/ex for mass balance), Sprague Dawley rats (0.205–0.237 kg, n = 3/ex/timepoint for blood sampling, n=3/ex for mass balance), and Beagle dogs (7–12 kg, n=3) were given a single oral dose of [¹⁴C]Rambazole in 20% hydroxyl propyl β-cyclodextrin at 5 mg/kg. Blood samples were collected at various timepoints after dosing and plasma prepared. Urine and feces were collected for 2, 7 and 8 days (mouse, rat, dog).

Radioactivity in various matrices was measured by liquid scintillation counting (LSC). Select plasma, urine, and fecal samples were subject to metabolic radioprofiling and characterization. Metabolite radioprofiling was accomplished using HPLC with fraction collection followed by solid scintillation counting (Packard TopCount). Radioactivity peaks were integrated and the percent distribution of individual metabolites in each sample was determined. Metabolite characterization and identification were accomplished by LCMS (Finnigan MAT LCQ in positive or negative ESI mode) in conjunction with an appropriate radioactive monitor (RAM). The mass data of M4 was compared with the reference standard, R143191.

For all species, plasma, urine, and fecal samples were pooled across animals and analyzed. Plasma was analyzed at several time-points out to 24 hours. Urine was analyzed over one time-interval (0–24 hour for mouse, 0–48 hour for rat, 0–72 hour for dog). Feces was analyzed over 2–3 time-intervals (0–24 and 24–48 hour for mouse and rat, 0–24, 24–48, and 48–72 hour for male dogs, and 24–48, 48–72, and 72–96 hour for female dogs). Plasma PK parameters for [¹⁴C]Rambazole radioactivity were determined from the mean (mouse and rat) or individual (dog) plasma concentration versus time data. PK Parameter values were determined by non-compartmental methods using WinNonlinTM.

Table 1. Mean Pharmacokinetic Parameters of [¹⁴C]Rambazole Equivalents in Plasma

Species (n=3)	Sex	C _{max} (ng eqv/L)	T _{max} (hr)	t _{1/2} (hr)	AUC _{0-∞} (hr·ng eqv/L)	AUC ₀₋₂₄ (hr·ng eqv/L)
Mouse (0–48 h)	Male	2633	3.0	7.6	10215	10276
	Female	1839	1.0	12.4	6632	6767
Rat (0–48 h)	Male	1130	2.0	17.4	6720	6960
	Female	838	4.0	14.8	7670	7810
(0–168 h)	Male	2533	0.67	55.7	19555	19970
	Female	2719	0.67	49.0	21388	21902

Concentrations are ng equivalents [¹⁴C]Rambazole/g

Table 2. Percent of Dose Recovered in Excreta

Species (Interval)	Sex	% in Urine	% in Feces	% in Cage Wash	Total % Recovered
Mouse (0–48 h)	Male	4.3	92.7	4.4	91.4
	Female	3.0	91.6	6.8	95.4
Rat (0–168 h)	Male	6.3	77.5	4.01	95.2
	Female	10.1	77.5	3.88	95.2
Dog (0–192 h)	Male	4.1	88.7	0.8	93.6
	Female	2.9	89.0	0.5	92.4

1 Pharmacokinetics of Radioactivity: PK parameters for [¹⁴C]Rambazole are given in Table 1.

Excretion of Radioactivity: In the mouse, rat, and dog, over 90% recovery of the radioactive dose was achieved after oral dosing (Table 2). The radioactive dose excreted in feces ranged from 78–89% and 78–92% in male and female animals, respectively.

2 Metabolite Profiles: Rambazole was extensively metabolized with the majority of metabolites excreted in the feces. In addition to unchanged drug, 17, 26, and 19 radioactive components were observed in plasma, urine, and feces from mouse, rat, and dog, respectively.

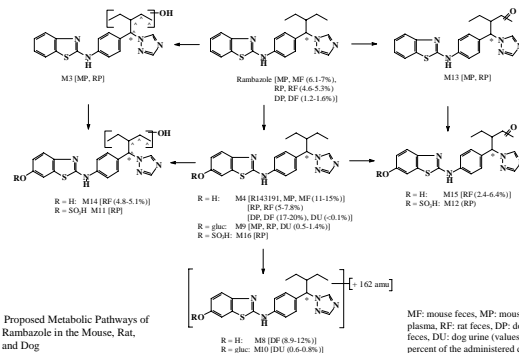


Fig. 1. Proposed Metabolic Pathways of Rambazole in the Mouse, Rat, and Dog

Results and Discussion

Unchanged [¹⁴C]Rambazole, M3, M4 (R143191), M9, and M13 were the prominent radioactive components in mouse plasma. Rat had the greatest number of circulating metabolites in plasma. In addition to the metabolites observed in mouse, M11, M12, and M16 were observed in rat plasma. In the dog, only unchanged [¹⁴C]Rambazole and M4 (R143191) were characterized.

Unchanged [¹⁴C]Rambazole and M4 (R143191) were the prominent metabolites in mouse feces, accounting for 6.11 and 10.56% of the dose in male mouse feces and 7.04 and 15.16% of the dose in female mouse feces. Unchanged [¹⁴C]Rambazole, M4 (R143191), M14, and M15 were the major metabolites in rat feces, and accounted for 5.34, 4.95, 5.05 and 6.42% of the dose in male rat feces and 4.60, 7.76, 4.82 and 2.38% of the dose in female rat feces. M8 and M4 (R143191) were the major metabolites in dog feces, and accounted for 11.73 and 19.88% of the dose in male dog feces and 8.86 and 17.01% of the dose in female dog feces.

No unchanged [¹⁴C]Rambazole was detected in mouse urine. Unchanged [¹⁴C]Rambazole and M4 (R143191) were observed as minor radio-components in rat urine, accounting for 0.07–1.90% of the dose. Two minor metabolites, M9 and M10, were identified in dog urine, accounting for 0.45–1.34% of the dose.

3 Metabolite Characterization/Identification Table 3 lists the metabolites characterized/identified by LC/MS/MS. [¹⁴C]Rambazole was metabolized to M4 (R143191) via oxidation of the triazolophenyl ring, and to M3 and M13 via oxidation of the side chain. Dioxidation on both the triazolophenyl ring and the side chain yielded M14 and M15. Conjugation of M4 with a glucuronoyl or sulfate moiety resulted in M9 and M16, and conjugation of M14 and M15 with a sulfate moiety yielded M11 and M12, respectively. Another metabolite route found only in dogs was the addition of a 162 amu moiety (most likely a monosaccharide) to M4 or M9 to give M8 and M10. The metabolic pathways of [¹⁴C]Rambazole in the mouse, rat, and dog are proposed in Figure 1.

Conclusion

Fecal excretion of [¹⁴C]Rambazole-related radioactivity predominated in mouse, rat, and dog, indicating that biliary excretion and/or gastrointestinal secretion was the major elimination route for Rambazole-derived radioactivity.

Rambazole was extensively metabolized. In addition to [¹⁴C]Rambazole, 4 metabolites (M3, M4, M9 and M13), 9 metabolites (M3, M4, M9, M11, M12, M13, M14, M15 and M16), and 4 metabolites (M8, M9, M10 and M11) were characterized or identified by LC/MS in plasma, urine and/or feces from mouse, rat, and dog, respectively.

Comparisons of both *in vitro* and *in vivo* metabolite profiles between mice, rats, and dogs with those from humans will be made following the completion of the human radiolabel mass balance study.

References

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Table 3. Summary of Rambazole Metabolites Characterized or Identified by LCMS

Metabolite (R143191)	Structure	MW	Significance
Rambazole (R143191)		377	MP, MP (61.37%), RP, RF (44.63%), DF, DF (1.24.6%)
M3		393	MP, RP
M4 (R143191)		393	MP, MP (11.15%), RP, RF (5.78%), DF, DF (17.20%), DU (0.15%)
M8		555	DF (8.9-12%)
M9		569	MP, RP, DF (0.51.4%)
M10		731	DF (0.6-0.8%)
M11		489	RP
M12		487	RP
M13		391	MP, RP
M14		409	RF (4.8-5.1%)
M15		407	RF (2.4-6.4%)
M16		473	RP

*Denotes the 14C label position.
 †The hydroxyl group may locate in one of these four positions, which has not been determined.
 MP: mouse feces, MF: mouse plasma, RP: rat plasma, RF: rat feces, DP: dog plasma, DF: dog feces, DU: dog urine.
 ‡Values in brackets are percent of the administered dose.

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