IIA 5.1.1 Metabolism study - rats

IIA 5.1.1/XX

Previous evaluation:	No
Reference:	[redacted] (2019)
Report:	Pilot Pharmacokinetic Study of [^{14}C] Clomazone in Rats. [redacted] Study No. 036908-1. FMC Tracking No. 2018MET-CLZ4349
Guidelines:	OPPTS 870.7485
Deviations:	No
GLP:	No

Executive summary:

The pharmacokinetics of clomazone were studied in female Wistar rats administered [phenyl-U-^{14}C]clomazone. Experiments were performed to understand the pharmacokinetic behaviour of radioactive residues in plasma and red blood cells.

[Phenyl-U-^{14}C]-labelled clomazone suspended in 0.5% carboxmethylcellulose was administered to female rats as single oral doses (5, 50, 500, and 750 mg/kg bw). All rats received the appropriate levels of radioactivity.

The mean terminal elimination half-life for total radioactivity ranged from 7.91 to 10.6 h. The mean terminal elimination half-life for clomazone was 1.58 h for the 5 mg/kg dose and ranged from 10.4 to 14.5 h for the 50, 500, and 750 mg/kg doses. The total radioactivity AUC increased with increasing dose between 5 and 500 mg/kg and still increased at 750 mg/kg, but in a less than dose-proportional manner. This indicated absorption saturation at greater than 500 mg/kg. The increases in total radioactivity AUC_{Inf} were approximately dose proportional between 5 and 500 mg/kg.

Clomazone AUC_{Inf} increased with increasing doses between 5 and 500 mg/kg and appeared to plateau above 500 mg/kg, indicating saturation of clomazone exposure at greater than 500 mg/kg. The increases in AUC for clomazone were greater than dose proportional between 5 and 500 mg/kg. Comparison of total radioactivity and clomazone AUC showed that unchanged clomazone accounted for 2.01%, 6.89%, 12.92%, and 6.38% of the total radioactivity AUC for the 5, 50, 500, and 750 mg/kg doses, respectively, indicating the saturation of metabolism of clomazone at higher than 500 mg/kg dose levels.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Clomazone
   Lot/Batch #: [redacted]
   Purity: 99.0%
   Description: White crystalline solid
   CAS#: Not provided in report
   Stability of test compound: Test compound was shown to be stable under the conditions of the test.
2. Radiolabeled test material: ^{14}C-Clomazone technical
   Lot/Batch #: [Phenyl-U-^{14}C] CFQ42447
   Purity: [Phenyl-U-^{14}C]clomazone: 98.92%
   Specific activity: [Phenyl-U-^{14}C]clomazone: 61 mCi/mmol
   Description: White powder>
   Stability of test compound: Test compound was shown to be stable under the conditions of the test..
3. Vehicle and/or positive control: 0.5% carboxymethylcellulose (CMC) in water
4. Test animals
   Species: Rat
   Strain: Wistar
   Age at dosing: 11 weeks
   Weight at dosing: 168.5–196.2 g
   Source: [redacted]
   Acclimation period: Animals were acclimated to the facility for five days prior to test material administration.
   Diet: Certified rodent diet, *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: Prior to dose administration, animals were housed in stainless steel, wire-bottom cages; post dosing, animals were housed in Nalgene metabolism cages.
   Husbandry: Husbandry conditions were in accordance with the USPHS-NIH publication *Guide to the Care and Use of Laboratory Animals* (National Research Council, 2011).
5. Environmental conditions
   Temperature: 68–79°F
   Humidity: 30–70%
   Air changes: At least 10 changes/hour
   Photoperiod: Alternating 12-hour light and dark cycles

6. Preparation of the dosing solutions

   Rats were dosed with 5, 50, 500, or 750 mg/kg. Each animal received approximately 5 mL/kg body weight of [phenyl-U-^{14}C]clomazone for oral administration. The ^{14}C-clomazone radiolabelled material was diluted with unlabelled clomazone to the appropriate specific activity for each dose level. Then, 1518 µL of [^{14}C]clomazone solution was added to each vial. Additional acetonitrile was added to each vial as needed to ensure the isotopically diluted [^{14}C]clomazone was completely dissolved. The acetonitrile was removed under a slow stream of nitrogen and the [^{14}C]clomazone was suspended in the vehicle to the final dose volume of 7.2 mL.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

   21-May-2018 to 04-June-2018

2. Main study

   Animals were assigned to test groups according to Table 1:

Table 1
Study design: Pharmacokinetics of [^{14}C]clomazone in female rats

Experiment[a]	Dose level (mg/kg bw[b])	Label[c]	Number of animals	Time of sacrifice (hours)	Samples
			Female		
Pharmacokinetics	5	[phenyl-U-^{14}C]	2	96	Plasma, red blood cells
	50	[phenyl-U-^{14}C]	2		
	500	[phenyl-U-^{14}C]	2		
	750	[phenyl-U-^{14}C]	2		

a T_{max} and $T_{max}/2$ was determined experimentally based on pharmacokinetic data.
T_{max} = Time at maximum plasma concentration (C_{max})
$T_{max}/2$ = Time at half of plasma C_{max}
b bw = Body weight. Dose volume 5 mL/kg bw.
c [Phenyl-U-^{14}C]clomazone

Blood samples (target of 250 µL per sample) were collected from the jugular cannula from each animal at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours and again at termination by exsanguination under CO_2 anaesthesia at 96 hours, post-dose. The blood samples were places in tubes containing K_3EDTA as the anticoagulant.

Immediately following blood collection, the samples were inverted several times and held on wet ice pending centrifugation.

Samples were centrifuged with 60 minutes of collection under refrigeration (set to maintain 5ºC for 10 minutes at 2000g). Plasma samples were stored in labelled tubes at approximately -70ºC until analysed for total radioactivity by LSC and for clomazone concentration using LC/MS/MS method.

Urine and faecal samples were collected from each animal at 24-hour intervals following dosing. A cage wash was done for each cage following the collection of urine and faeces. The urine, faecal, and cage wash samples were pooled for disposal as radioactive waste and were not analysed for radiolabel content.

II. RESULTS AND DISCUSSION

The results for the pharmacokinetic experiment following a single oral gave administration of 5, 50, 500, and 750 mg/kg doses to female Wistar rats can be found in Table 2. The mean T_{max} for total radioactivity ranged from 0.25 to 0.38 h for the 5 and 50 mg/kg doses, indicating rapid absorption. The mean T_{max} for the 500 and 750 mg/kg doses was later (1.25-6 h), indicating delayed absorption at the higher dose levels. The mean T_{max} for clomazone showed similar trends (T_{max} of 0.25 h for the 5 and 50 mg/kg doses, T_{max} of 1.25-3 h for the 500 and 750 mg/kg doses).

The mean terminal elimination half-life for total radioactivity ranged from 7.91 to 10.6 h. The mean terminal elimination half-life for clomazone was 1.58 h for the 5 mg/kg dose and ranged from 10.4 to 14.5 h for the 50, 500, and 750 mg/kg doses.

The mean total radioactivity AUC_{Inf} values were 7540, 60008, 763789, and 899819 h*ng-eq/g for the 5, 50, 500, and 750 mg/kg dose levels, respectively. The total radioactivity AUC increased with increasing dose between 5 and 500 mg/kg and still increased at 750 mg/kg, but in a less than dose proportional manner, indicating saturated absorption at greater than 500 mg/kg. The increases in total radioactivity AUC_{Inf} were approximately dose proportional between 5 and 500 mg/kg.

Table 2
Pharmacokinetic parameters in plasma following single oral dose administration of [^{14}C]clomazone

Dose level (mg/kg)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng-eq/g)	AUC_{0-24} (h*nq-eq/g)	AUC_{last} (h*ng-eq/g)	AUC_{Inf} (h*ng-eq/g)	Clomazone/ Total Radioactivity AUC_{Inf} Ratio (%)
Total Radioactivity							
5	8.81	0.25	1355	6725	7266	7540	--
50	7.91	0.38	10216	54055	54055	60008	--
500	8.35	6.00	50843	622999	709333	763789	--
750	10.6	1.25	41566	527416	832294	899819	--
Clomazone							
5	1.58	0.25	169	146	144	145	2.01
50	14.2	0.25	2710	4092	4133	4146	6.89
500	14.5	3.00	16275	100552	101257	101347	12.92
750	10.4	1.25	13350	56291	57416	57503	6.38

The mean clomazone AUC_{Inf} values were 145, 4146, 101347, and 57503 h*ng/mL for the 5, 50, 500, and 750 mg/kg dose levels, respectively. Clomazone AUC_{Inf} increased with increasing dose between 5 and 500 mg/kg and appeared to plateau above 500 mg/kg, indicating saturation of clomazone exposure at greater than 500 mg/kg. The increases in AUC for clomazone were greater than dose proportional between 5 and 500 mg/kg.

Comparison of total radioactivity and clomazone AUC showed that unchanged clomazone accounted for 2.01%, 6.89%, 12.92%, and 6.38% of the total radioactivity AUC for the 5, 50, 500, and 750 mg/kg doses, respectively, indicating the saturation of metabolism of clomazone at higher than 500 mg/kg dose levels.

III. CONCLUSION

The mean T_{max} for total radioactivity ranged from 0.25 to 0.38 h for the 5 and 50 mg/kg doses, indicating rapid absorption. The mean T_{max} for the 500 and 750 mg/kg doses was later (1.25-6 h), indicating delayed absorption at the higher dose levels. The mean T_{max} for clomazone showed similar trends (T_{max} of 0.25 h for the 5 and 50 mg/kg doses, T_{max} of 1.25-3 h for the 500 and 750 mg/kg doses).

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(▇▇▇▇▇▇▇▇▇▇▇▇▇▇▇▇ 2018)