Report

Study Title	Pilot Hyperammonemia Induction Study in Male Beagle Dogs
Testing Facility	Frontage Laboratories, Inc. 10845 Wellness Way Concord OH 44077
Sponsor	Callitas Therapeutics, Inc. 187 Pavilion Pkwy, #200 Newport, KY 41071
Study Number	037543
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Signatures

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Study Director

A. L. O'Leary **Date:** 05 Dec 2019

Ann L. O'Leary, Ph.D.
Frontage Laboratories, Inc.

Testing Facility Management

Date: 05 DEC 2019

Frontage Laboratories, Inc.

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Introduction

Objective

The objectives of this study were to:

1) dose the two male beagle dogs with ammonium acetate to induce and maintain hyperammonemia (Dose Event 1), and
2) evaluate the efficacy of AST-120 to reduce blood ammonia levels in hyperammonemic dogs (Dose Event 2 and 3).

Responsible Personnel

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Study Details

Test Article Name	AST-120
Lot	171M17
Pretreatment	ammonium acetate (Sigma, Lot MKCJ6670)
Strain / Species / Sex	Beagle dog / *Canis familiaris* / male
Routes of Administration	Intravenous (IV) – ammonium acetate Oral gavage (PO) – AST-120

Treatment Groups

Dose Event	Dose Group	No. of Animals	Treatment	Route	Dose (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Dose Schedule
1	1	2 M	ammonium acetate	IV	30	30	1	0 and 3 hr

Dose Event	Dose Group	No. of Animals	Treatment	Route	Dose (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Dose Schedule
2	1	2 M	ammonium acetate	IV	90	90	1	0 and 3 hr
3	1	2M	ammonium acetate	IV	90	90	1	0 and 3 hr
	1	2M	AST-120	PO	500	100	5	1 hr after each ammonium acetate dose

Total Number of Animals	2 males
Age Range of Animals	32.8 to 33.1 months
Weight Range of Animals (Day 1)	9.80 – 10.80 kg
Day of Dosing	
Dose Event 1 (Day 1)	22-Aug-2019
Dose Event 2 (Day 19)	09-Sep-2019
Dose Event 3 (Day 22)	12-Sep-2019
Experimental Completion (Day 30)	20-Sep-2019

Pretreatment and Test Article Dose Formulation

Frequency of Preparation	On the day of dose administration
Pretreatment Formulation	Dose Event 1: Ammonium acetate was prepared at a concentration of 30 mg/mL in sterile saline. The dose was filter-sterilized through a 0.22-µm filter after preparation. Dose Event 2 and 3: Ammonium acetate was prepared at a concentration of 90 mg/mL as described above.

Test Article Formulation	AST-120 was prepared at a concentration of 100 mg/mL in deionized water. The preparation was stirred during dosing.

Dose Administration Details

Dose Administration	Dose Events 1 and 2: The intravenous (IV) dose was administered as a bolus injection into a cephalic vein using an indwelling catheter. After dose administration at 0 and 3 hour, but before the catheter was removed from the animal, the catheter was flushed with approximately 1 mL of sterile saline. Dose Event 3: Ammonium acetate at 90 mg/kg was administered by IV injection to animal 3299113 and immediately after AST-120 was dosed by oral gavage. Within seconds of the test article dose, the animal vomited the dose. The same dosing regimen was conducted on animal 3308350 and this animal also vomited the dose within seconds. No other adverse signs were observed in the animals. To determine if the test article caused the emesis, a facility stock dog was dosed with AST-120 at 500 mg/kg without administration of ammonium acetate; this animal did not vomit. This suggested that the ammonium acetate was likely the cause of the emesis. The decision was made to increase the interval between ammonium acetate and AST-120 administrations. At 1 hr after the first administration of ammonium acetate the animals were dosed with 500 mg/kg of AST-120 and no emesis occurred. At 2 hr after the test article was dosed, the second dose of ammonium acetate was administered. Both animals had small amounts of emesis with no visible test

article. Finally, at 1 hr after the second ammonium acetate dose, the test article was dosed again and no emesis occurred.

Sample Collection (Plasma Ammonia and Clinical Chemistry)

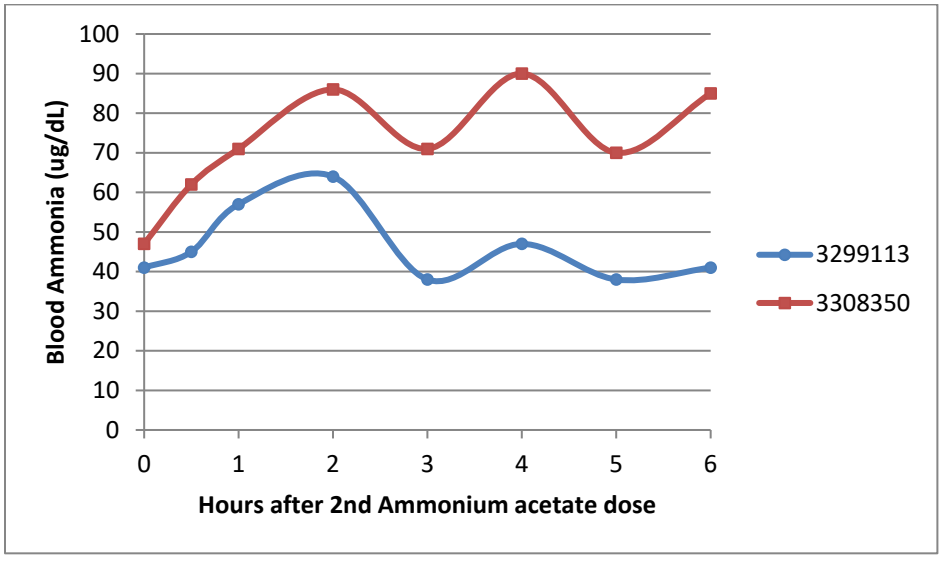
Sample Location	Jugular vessel
Volume of Blood Drawn	Target volume of 3 mL/sample
Whole Blood Conditions	Samples were inverted several times following collection and were held on wet ice until centrifuged.
Anticoagulant	Pre-cooled EDTA
Separation Method	Within 15 minutes of collection, samples were separated in a centrifuge set at 5 °C for 10 minutes at 2000 x g.
Collection Time Points for Ammonia Analysis	Predose (prior to the first dose of ammonium acetate), and 0.5, 1, 2, 3, 4, 5, and 6 hr after the second dose of ammonium acetate
Sample Analysis for Ammonia Concentration	After the final collection for each Dose Event, plasma samples were shipped for analysis, on dry ice, via FedEx overnight delivery to: Cornell University College of Veterinary Medicine Animal Health Diagnostic Center 240 Farrier Rd. Ithaca, NY 14853 Phone: 607-253-3900

Serum Chemistry – Liver Function Screen	At 6 hr after the second AST-120 dose, a target volume of 2 mL of blood was collected from each animal in tubes without anticoagulant. Serum was collected and analyzed for liver function. Parameters: Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Alkaline phosphatase (ALKP) Gamma-glutamyltransferase (GGT) Total bilirubin (TBILI)

Results and Discussion

Dose Event 1. Plasma ammonia levels increased in both animals dosed with 30 mg/kg ammonium acetate by IV injection. The increase was more pronounced in animal 3308350 than in animal 3299113 (Figure 1). Peak increases occurred at 2 and 4 hr after the second dose. At 2 hr after the second dose, plasma ammonia levels increased 56 and 85% in animals 3299113 and 3308350, respectively. Increases of 15 and 92% were observed at 4 hr after the second dose in animal numbers 3299113 and 3308350, respectively (Table 1).

Figure 1: Plasma ammonia levels in animals dosed with 30 mg/kg of ammonium acetate in Dose Event 1.



The 30 mg/kg dose of ammonium acetate was well tolerated in the two animals. No effects were observed and liver panel parameters in serum collected at 6 hr after the second ammonium dose were within historical values (Table 3).

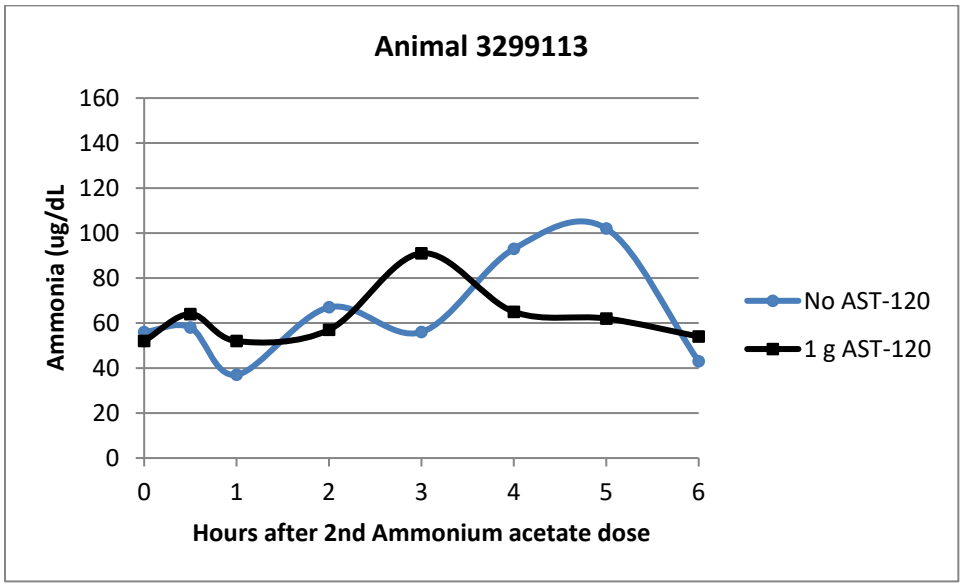
Dose Event 2. The 90 mg/kg dose by IV injection resulted in higher ammonia plasma levels compared to the 30 mg/kg dose (Figure 2). As in dose Event 1, the increase was more pronounced in animal 3308350. The maximum plasma ammonia levels, 102 µg/dL for animal 3299113 and 136 µg/dL for animal 3308350, occurred at 5 hr after the second dose of ammonium acetate (Table 2). The higher dose was tolerated by the animals, with no abnormal observations and the liver panel parameters were within historical values (Table 3).

Dose Event 3. When dosed at 1 hr after administration of the ammonium acetate, the two doses of AST-120 at 500 mg/kg reduced plasma ammonia levels in both animals (Figure 2). Plasma ammonia levels in the AST-120-treated animals at 5-hr time after the second ammonium acetate administration were reduced by 39 and 49% compared to the levels at the same time point in Dose Event 2 (Table 2).

Conclusions

Plasma ammonia was reduced up to 39 and 49% in two dogs administered two doses of AST-120 at 500 mg/kg by oral gavage after ammonium acetate pretreatment.

Figure 2: Plasma ammonia levels in animals dosed with 90 mg/kg ammonium acetate (Dose Event 2) and then with ammonium acetate and AST-120 (Dose Event 3)



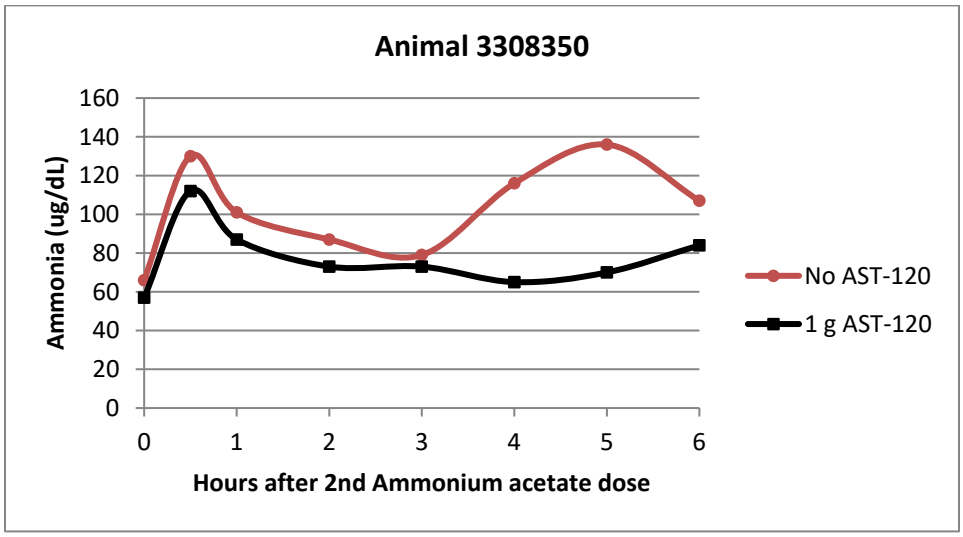


Table 1: Plasma ammonia levels in animal numbers 3299113 and 3308350 dosed with 30 or 90 mg/kg of ammonium acetate.

Time[1] (hr)	Dose Event 1 – 30 mg/kg				Dose Event 2 – 90 mg/kg			
	3299113		3308350		3299113		3308350	
	µg/dL	% change[2]	µg/dL	% change	µg/dL	% change	µg/dL	% change
pretreatment	41	-	47	-	56	-	66	-
0.5	45	9.8	62	31.9	58	3.6	130	97.0
1	57	39.0	71	51.1	37	-33.9	101	53.0
2	64	56.1	86	83.0	67	19.6	87	31.8
3	38	-7.3	71	51.1	56	0.0	79	19.7
4	47	14.6	90	91.5	93	66.1	116	75.8
5	38	-7.3	70	48.9	102	82.1	136	106.1
6	41	0.0	85	80.9	43	-23.2	107	62.1

[1]Pretreatment blood was collected prior to dosing; times are hours after the 2nd ammonium acetate treatment

[2]% change from pretreatment blood ammonia levels

Table 2: Plasma ammonia levels in animal numbers 3299113 and 3308350 dosed with 90 mg/kg ammonium acetate either alone or in combination with AST-120 treatments.

Time[1] (hr)	3299113			3308350		
	No AST-120 µg/dL	1 g AST-120	% change[2]	No AST-120 µg/dL	1 g AST-120 µg/dL	% change
pretreatment	56	52	-7	66	57	-14
0.5	58	64	10	130	112	-14
1	37	52	41	101	87	-14
2	67	57	-15	87	73	-16
3	56	91	63	79	73	-8
4	93	65	-30	116	65	-44
5	102	62	-39	136	70	-49
6	43	54	26	107	84	-21

[1]Hours after the second ammonium acetate dose.

[2]% change from no AST-120 dose.

Table 3: Liver panel parameters.

Dose Event	Animal No.	Ammonia Dose (mg/kg)	AST-120 Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALKP (U/L)	TBILI (mg/dL)	GGT (U/L)
1	3299113	30	0	28	40	52	0.0	0
	3308350	30	0	39	44	52	0.0	1
2	3299113	90	0	31	36	55	0.0	0
	3308350	90	0	43	48	57	0.0	0
3	3299113	90	500	36	36	60	0.0	0
	3308350	90	500	60	59	67	0.0	0

ALT = alanine aminotransferase
AST = aspartate aminotransferase
ALKP = alkaline phosphatase
TBILI = total bilirubin
GGT = gamma-glutamyl transferase