

ORIGINAL RESEARCH

Comparison of the Absorption, Brain and Prostate Distribution, and Elimination of CaNa_2 EDTA of Rectal Chelation Suppositories to Intravenous Administration

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ABSTRACT

Rectal suppositories were compared to IV administration of C14-labeled calcium disodium ethylenediaminetetraacetate (CaNa₂EDTA) to evaluate the absorption, brain and prostate tissue distribution, and excretion in rats. The absolute bioavailability of CaNa₂EDTA in blood following rectal dosing was 36.3% of the IV dose route, which confirmed that rectal dosing is an efficient method for delivering ethylenediaminetetraacetic acid (EDTA) to tissues. The ratio of radioactive residues of EDTA in tissues compared to blood, following IV or rectal dosing of C14 labeled CaNa₂EDTA, showed negligible brain localization. However, prostate tissues were found to have a mean ratio of 3.69 via the IV route and 13.6 rectally. The total recovery of C14 EDTA expressed as percent of administered dosed IV was a mean of 47.3% and 30.3% rectally at eight hours when the test was concluded. The suppository formulation of CaNa₂ appears to be well absorbed, delivering high levels of EDTA to prostate tissue.

INTRODUCTION

Heavy metal exposures in the twenty-first century are an established global health concern. The FDA has approved EDTA as a chelation agent for the removal of heavy metals. It has been placed on the FDA "Generally Recognized as Safe" (GRAS) list for the past sixty years. Extensive national and international clinical experiences demonstrate that acute and chronic human exposure to a wide range of heavy metals can be treated with considerable efficacy using EDTA. It is widely administered, with considerable cost to the patient, as an intravenous (IV) solution, which entails 15 to 30 sessions in a physician's office, taking two to five hours per visit. The transrectal delivery of several pharmacological agents is well established. Therefore, using a rat animal model, we set out to determine if the rectal administration of EDTA is absorbed, resulting in significant blood and tissue levels.

The pharmacodynamic effects of therapeutic agents differ widely in their route of administration, penetration, absorption, and distribution in body tissues. For medicinal agents to act, they must be absorbed and transported to the appropriate tissue or organ, penetrate to the responding cell surface or sub-cellular and interstitial space, and elicit a response or alter ongoing processes.¹ The parenteral and intramuscular forms of EDTA are well absorbed, but not very practical for routine usage.² Oral forms of EDTA have been shown to be poorly absorbed (2% to 5%), and topical and subcutaneous forms have been reported as not being

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absorbed at all.^{3,4,5,6,7,8,9,10} A relatively new alternative and more convenient route of administration is rectal suppository delivery of a proprietary suppository formula of EDTA (CaNa₂ EDTA, Detoxamin,[®] World Health Products, Draper, Utah), which is the basis of this pharmacokinetic (PK) study. Although IV EDTA dosing is well characterized and has been used for decades, little is known about the absorption of rectal suppositories.

In an effort to elucidate the absorption characteristics of CaNa₂ EDTA in a suppository form, a rat model was chosen. ¹⁴C-labeled EDTA Calcium Disodium salt was administered as a tracer in the suppository and in intravenous forms; blood, urine, and selected tissue levels were evaluated over eight hours.

MATERIALS AND METHODS

¹⁴C-labeled EDTA free acid (11.7 mCi/mmol, Lot No. 63151012, purity greater than 98%) was obtained from MP Biomedicals (Irvine, CA). For the IV dosing solution, ¹⁴C-labeled EDTA was added to normal saline to achieve concentrations needed to deliver a final dose of 7.53 μ Ci in approximately 1 gram. The rectal suppository (a proprietary suppository formula of EDTA, CaNa₂ EDTA, Detoxamin[®] Health Products, East Draper, Utah, Lot No. 228-190-0117) was prepared by adding ¹⁴C-labeled EDTA solution from Moravek to molten suppository. For the animal dose, approximately 100 μ L of the mixture (containing 23.7 μ Ci per dose) was taken up in a cylindrical glass pipette equipped with a plunger and allowed to cool to room temperature, where it re-solidified.

The radioactive concentration of the IV dosing solution was calculated by Liquid Scintillation Counting (LSC). The prepared dosing solutions were stored and refrigerated.

Ten male Sprague Dawley rats were obtained from Taconic, Oxnard, CA. Animals were 6 to 7 weeks old and weighed 157 to 187 grams on Day 1. The animal experiments were performed at the Biological Test Center (BTC), in Irvine, CA. Quarantine and care of animals were performed per BTC Standard Operating Procedures.

Prior to dosing, 10 animals were weighed. Cannulated animals (six animals to undergo IV dosing) were random-

ized for placement into Group A or B. Uncannulated animals (four animals to undergo rectal dosing) were not randomized and were placed into Group C. Treatment groups are presented below.

Animals were fasted (food withheld) for 16.5 to 19.5 hours before ¹⁴C-EDTA administration. Prior to dosing, rats were anesthetized with an intramuscular combination injection of ketamine hydrochloride (40-90 mg/kg) and xylazine (5-10 mg/kg). Water and feed were withheld from animals for four hours after ¹⁴C-EDTA administration, and then food and water were given *ad libitum*.

For Group C, the contents of the colon were removed before dosing by flushing with normal saline heated to 37°C. Rectal doses were administered via a 100 μ L glass cylindrical tube, gently heated to allow partial liquefaction of the suppository material. Blood samples of approximately 100 μ L were taken. Each sample was placed in combustion cones and stored frozen prior to combustion and LSC analysis. The time of blood collection was recorded.

A terminal blood sample was collected from all animals via heart puncture (1 hour \pm 5 minutes after dosing for Group A animals; 8 hours \pm 15 minutes after dosing for Group B and C animals). Each animal was anesthetized with an intramuscular combination injection of ketamine hydrochloride (40-90 mg/kg) and xylazine (5-10 mg/kg), and euthanized by exsanguination following heart puncture. As much blood as possible was collected from each rat into heparinized tubes. The time of blood collection was recorded. Four 100- μ L aliquots of whole blood were transferred to combustion cones. Two of the aliquots were combusted for determination of radioactivity by LSC, and two were kept frozen as reserve samples.

Absorbent paper was placed in the restrainers to collect urine 0 to 4 hours after dosing. Urine was collected from the individual metabolism cages 4 to 8 hours after dosing. For urine samples collected in absorbent paper, water was added to the paper and urine extracted. For urine samples collected from metabolism cages, the urine was freeze-trapped to avoid atmospheric oxidation, evaporation, and bacterial degradation, and the urine collection pan was rinsed with water.

Following euthanasia by exsanguination, the brain and prostate were collected from each animal. Prior to collec-

Group	No.	Treatment	¹⁴ C-EDTA Dose (μ Ci)	Route	Blood Collection Time points (Time After Dosing) ¹
A	2	¹⁴ C-EDTA	10	IV	1 hour
B	4	¹⁴ C-EDTA	10	IV	5, 15, 30 minutes; 1, 2, 4, 8 hours
C	3 ²	¹⁴ C-EDTA	20	Rectal	5, 15, 30 minutes; 1, 2, 4, 8 hours

1. Blood collection times were \pm 1 minute for the 5-minute time point; \pm 3 minutes for the 15- and 30-minute time points; \pm 5 minutes for the 1-hour time point; and \pm 15 minutes for the 2-, 4-, and 8-hour time points.

2. The fourth animal in group C, animal, No. 55905, was dead (attributed to anesthesia) 15 minutes after dosing.

tion, the brain was perfused with approximately 5 mL of saline via the carotid artery. Both organs were stored at -20° C. Following completion of blood kinetics analysis, brains and prostates were combusted for determination of radioactivity by LSC. Brains were homogenized prior to combustion, while prostates were directly combusted.

Duplicate aliquots of each urine sample (0.1 mL) and cage rinse sample (1 mL) were transferred to liquid scintillation counting vials and the amount of radioactivity determined by LSC; Insta-Gel was used as the scintillation fluid. Each of the rectal dosing solution samples, tail vein blood samples, and heart puncture blood samples in combustion cones were combusted. Brain and prostate samples were combusted. Combusted samples were trapped in Carbon-14 Cocktail (R.J. Harvey, Hillsdale, NJ) present in liquid scintillation counting vials, and the amount of radioactivity was determined by LSC.

Sample combustion was performed using a Harvey Sample Oxidizer, Model OX300 (Harvey Instrument, Hillsdale, NJ). All radioactivity measurements were performed using a Beckman Liquid Scintillation Spectrometer. Any radioactivity measurement of less than 100 dpm was considered close to background and was not repeated.

When applicable, summary statistics (mean and standard deviation) were prepared to characterize the data (i.e., radioactivity measurement and percent dose). PK parameters, including Area under the Curve (AUC), half-life, Maximum Concentration in blood (C_{max}), Time to Maximum Concentration (T_{max}), and bioavailability, were calculated using WinNonlin (Pharsight Corporation, Mountain View, CA).

RESULTS

Individual and mean (\pm SD) body weights and administered ^{14}C -EDTA doses are presented in Table 1. Radioactivity recovered from blood at different time intervals is presented in Figures 1 and 2. As shown in Figure 2, the absorption phase occurring within the first two hours after dosing for all three rectally-dosed animals was maximal, and the apparent biphasic absorption may have been related to additional material being released from the rectal suppository; the blood levels from the IV doses did not show a biphasic response.

Mean AUC, half-life, C_{max} , T_{max} , and bioavailability of derived radioactivity in blood are presented in Table 2. The T_{max} of EDTA following intravenous dosing occurred at 0.083 hours. The T_{max} of EDTA following rectal dosing occurred at 0.417 hours. The half-life of EDTA following intravenous dosing was 1.50 hours, and the half-life of EDTA following rectal dosing could not be calculated since a terminal elimination phase could not be determined. The absolute bioavailability of EDTA in blood following rectal

dosing was 36.3 compared to the IV bolus of 100%. Radioactivity recovered from urine at different time intervals is presented in Table 3. Following intravenous dosing, the amount of radioactivity excreted in urine decreased over the 8-hour study period (46.3% of dosed radioactivity excreted at the 0 to 4 hour interval, and 0.935% of dosed radioactivity excreted at the 4 to 8 hour interval). Following rectal dosing, the amount of radioactivity excreted in urine remained relatively constant over the 8-hour study period (15.8% of dosed radioactivity excreted at the 0 to 4 hour interval, and 14.4% of dosed radioactivity excreted at the 4 to 8 hour interval).

Radioactivity recovered from tissues (brain and prostate) expressed as a ratio of the radioactivity in blood is

Figure 1. EDTA levels in blood over time following intravenous administration of ^{14}C -EDTA.

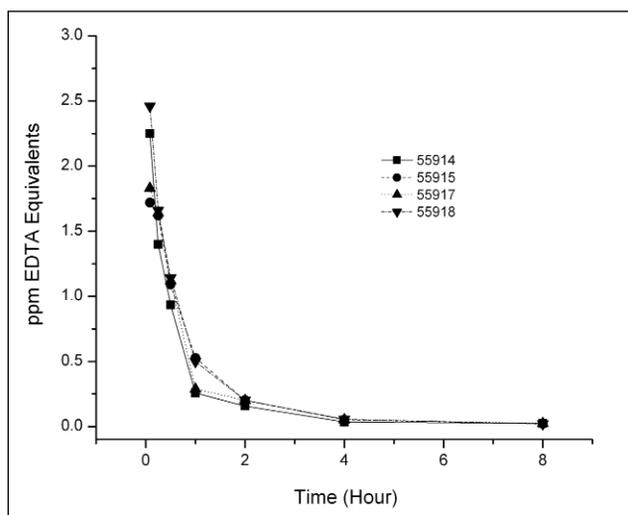


Figure 2. EDTA levels in blood over time following rectal administration of ^{14}C -EDTA.

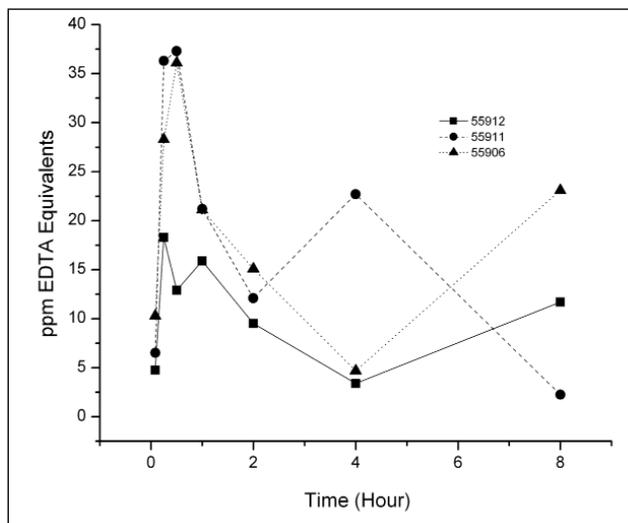


Table 1. Body Weights and Administered ¹⁴C-EDTA Doses

Group	Animal Number	Body Weight (kg)	Dosage Weight (g)	Dose (mg/kg)	Total Dose (μCi)	Total Dose (dpm)
A	55921	0.187	1.0019	1.31	7.64	16,954,958
A	55920	0.184	0.9906	1.31	7.55	16,763,730
	Mean:	0.186		1.31	7.59	16,859,344
	± SD:	0.002		0.00	0.06	135,218
B	55918	0.166	0.9899	1.45	7.55	16,751,884
B	55915	0.175	0.9776	1.36	7.45	16,543,734
B	55917	0.177	0.9718	1.34	7.41	16,445,582
B	55914	0.183	0.9804	1.31	7.47	16,591,118
	Mean:	0.175		1.37	7.47	16,583,079
	± SD:	0.007		0.06	0.06	127,819
C	55912	0.167	0.1100	214.4	24.6	54,534,057
C	55911	0.157	0.0970	201.1	21.7	48,089,123
C	55906	0.162	0.1120	225.0	25.0	55,525,585
	Mean:	0.162		213.5	23.7	52,716,255
	± SD:	0.005		12.0	1.8	4,037,765

Table 2. Mean AUC, half-life, C_{max}, T_{max}, and bioavailability of EDTA in blood following intravenous or rectal administration of ¹⁴C-EDTA

Group	Route	Stat.	Dose (mg/kg)	AUC (μg x Hr/mL)	AUC Inf (μg x Hr/mL)	Half-life (Hour)	C _{max} (μg/mL)	T _{max} (Hour)	Absolute Bioavailability (%)
B	Intravenous	MEAN	1.37	1.86	1.91	1.50	2.07	0.083	N/A
		SD	0.06	0.20	0.19	0.34	0.35	0.000	
		N	4	4	4	4	4	4	
C	Rectal	MEAN	213.5	105.8	307.3	N/A ¹	30.6	0.417	36.3
		SD	12.0	32.2	225.6	N/A ¹	10.6	0.144	
		N	3	3	3	3	3	3	

N/A = not applicable

$$\text{Absolute bioavailability (\%)} = \frac{(\text{AUC}_{\text{test}} \times \text{Dose}_{\text{ref}})}{(\text{AUC}_{\text{ref}} \times \text{Dose}_{\text{test}})} \times 100$$

Where “test” data is the rectal data, and “ref” (reference) data is the intravenous data.

1. The terminal elimination phase was not observed, therefore, the half-life could not be calculated.

References for the above formula are as follows:

- 1) Kwon Y. *Handbook of Essential Pharmacokinetics, Pharmacodynamics, and Drug Metabolism for Industrial Scientists*. New York: Kluwer Academic/Plenum Publishers, 2001.
- 2) Shargel L, Yu A. *Applied Biopharmaceutics and Pharmacokinetics, 4th ed.* Norwalk, Connecticut: Appleton & Lange, 1999

presented in Table 4 and Figure 3. The prostate retained higher levels of radioactivity than the brain following both intravenous and rectal dosing, with the highest level of radioactivity found in the prostate following rectal dosing.

The total recovery of radioactivity from urine and tissues expressed as percent of dose is presented in Tables 5 and 6. Total recovery represents the combined total percent of dose in urine and tissues. Following intravenous dosing, 41.4% and 47.3% of the radioactive dose was recovered 1 hour and 8 hours after dosing, respectively; of which virtually all was in urine. Following rectal dosing, 30.3% of the radioactive dose was recovered 8 hours after dosing, of which virtually all was also in urine.

DISCUSSION

This study has shown that the proprietary formula of Ca Na₂ EDTA has been effectively absorbed from the lower enteral route in rats, through the anal portal into the rectum or lower intestine to reach blood and tissue levels via rectal sup-

Figure 3. Ratio of tissue and blood radioactive residues (ppm) following intravenous or rectal administration of ¹⁴C-EDTA.

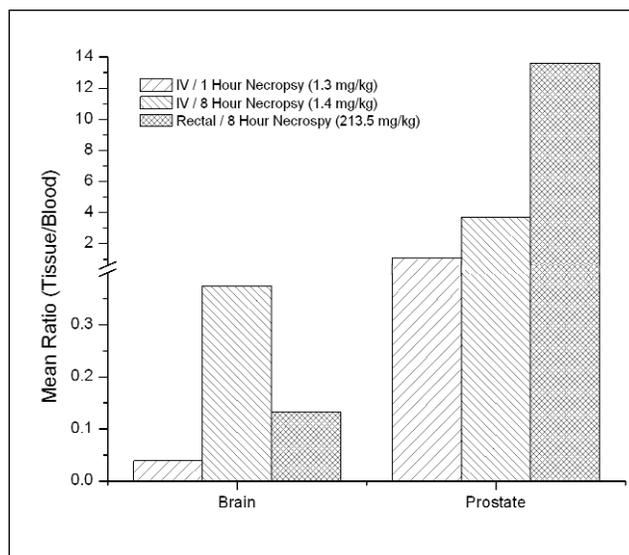


Table 3. ¹⁴C-EDTA-derived radioactivity excreted in urine expressed as percent of administered dose following intravenous or rectal administration of ¹⁴C-EDTA

Group A: IV (1 hour) Time Interval (Hour)	Animal No. 55921		Animal No. 55920		% Dose		Cum. % Dose	
	% Dose	Cum. % Dose	% Dose	Cum. % Dose	Mean Values	± S.D.	Mean Values	± S.D.
0-1	38.1	38.1	44.7	44.7	41.4	4.7	41.4	4.67
Total	38.1		44.7				41.4	4.67

Group B: IV (8 Hour) Time Interval (Hour)	Animal No. 55918		Animal No. 55915		Animal No. 55917		Animal No. 55914		% Dose		Cum. % Dose	
	% Dose	Cum. % Dose	Mean Values	± S.D.	Mean Values	± S.D.						
0 - 4	58.5	58.5	53.4	53.4	38.2	38.2	35.2	35.2	46.3	11.4	46.3	11.4
4 - 8	1.20	59.7	0.78	54.1	0.97	39.2	0.79	36.0	0.935	0.197	47.3	11.5
Total	59.7		54.1		39.2		36.0				47.3	11.5

Group C: Rectal (8 Hour) Time Interval (Hour)	Animal No. 55912		Animal No. 55911		Animal No. 55906		% Dose		Cum. % Dose	
	% Dose	Cum. % Dose	% Dose	Cum. % Dose	% Dose	Cum. % Dose	Mean Values	± S.D.	Mean Values	± S.D.
0 - 4	20.0	20.0	16.7	16.7	10.8	10.8	15.8	4.67	15.8	4.67
4 - 8	2.53	22.5	19.7	36.4	21.1	31.9	14.4	10.3	30.3	7.08
Total	22.5		36.4		31.9				30.3	7.08

positories. Bioavailability has now been established for this mode of administration in an animal model and is strong evidence that EDTA suppositories are an adequate and medically acceptable approach to providing the benefits of chelation.

Intravenous dosing resulted in greater elimination of radioactivity in urine at the 0 to 4 hour time point, but the percent of dose recovered drastically decreased by the 4 to 8 hour time point, while the level of recovery was relatively steady at both time points following rectal dosing. The slow and consistent movement of CaNa₂ EDTA via rectal administration may have lesser toxicity since there are significant blood and tissue levels to chelate metals without a high dose EDTA IV drip over many hours. These data point to the ability of rectal suppositories to deliver a continuous lower dose concentration of EDTA for longer periods of time compared with IV administration, allowing EDTA to bind metals efficiently and effectively.

In tissues, significant amounts of radioactivity were recovered from the prostate following intravenous or rectal dosing, with the highest level of dosed radioactivity (179.6 ppm) recovered 8 hours following rectal dosing. This observation of rectal administration, revealing higher amounts of EDTA in prostate tissue as compared to IV, can have far-reaching implications of a more complete distribution of EDTA into interstitial and intracellular spaces, further leading to more efficient chelation of compartmental-

ized heavy metal content with CaNa₂ suppositories.

EDTA is not bio-transformed in the body. It is excreted in hair, urine, feces, saliva, and perspiration. This study shows that animals excreted 47.3% and 30.3% of dosed radioactivity in urine during the 8 hours following intravenous and rectal dosing, respectively. The 30.3% excretion of EDTA in the urine corresponds closely to the rectal dose bioavailability calculated from the blood levels (36.3%).

Blood samples were taken over an 8-hour period, and during this time, the rectal administration showed high levels of absorbed EDTA with no apparent elimination phase observed. If further blood samples had been taken, the bioavailability calculated for rectally administered EDTA would have undoubtedly been much higher, since the bioavailability calculation presented here only used up to 8-hour blood level data. No extrapolation of the AUC could be done since the levels at 8 hours were actually increasing in two out of three animals. Further research is indicated over a longer time span to quantify the actual half life of the suppository form of administration.

CONCLUSIONS

This proprietary suppository formulation appears to be a viable dosing mechanism for delivery of CaNa₂ EDTA to the bloodstream in this rat model, showing substantial circulating levels of EDTA for least 8 hours after administra-

Table 4. Ratio of radioactive residues of EDTA in tissues (µg/g) to blood (µg/g) following intravenous or rectal administration of ¹⁴C-EDTA

Group A: IV (1 Hour)	Ratio of Radioactive Residues of EDTA			
Sample	Animal No: 55921	Animal No: 55920	Mean Values	± SD
Brain	0.039	0.039	0.039	0.000
Prostate	1.80	0.357	1.08	1.02

Group B: IV (8 Hour)	Ratio of Radioactive Residues of EDTA					
Sample	Animal No: 55918	Animal No: 55917	Animal No: 55915	Animal No: 55914	Mean Values	± SD
Brain	0.345	0.318	0.487	0.351	0.375	0.076
Prostate	6.70	2.01	3.07	2.98	3.69	2.06

Group C: Rectal (8 Hour)	Ratio of Radioactive Residues of EDTA				
Sample	Animal No: 55912	Animal No: 55911	Animal No: 55906	Mean Values	± SD
Brain	0.050	0.288	0.056	0.132	0.135
Prostate	8.90	14.4	17.4	13.6	4.31

Note: Brain was perfused with normal saline prior to collection.

Table 5. Total recovery of radioactivity expressed as percent of administered dose following intravenous or rectal administration of ¹⁴C-EDTA.

Group	Animal No.	% Administered Dose		
		Urine	Tissue	Total
A	55921	38.1	0.05	38.2
	55920	44.7	0.02	44.7
	Mean:	41.4	0.04	41.4
	± SD:	4.67	0.02	4.65
B	55918	59.7	0.02	59.8
	55915	54.1	0.01	54.2
	55917	39.2	0.01	39.2
	55914	36.0	0.02	36.0
	Mean:	47.3	0.02	47.3
	± SD:	11.5	0.01	11.5
C	55912	22.5	0.01	22.5
	55911	36.4	0.01	36.4
	55906	31.9	0.10	32.0
	Mean:	30.3	0.04	30.3
	± SD:	7.08	0.05	7.09

Table 6. Total recovery of radioactivity expressed as percent of administered dose following intravenous or rectal administration of ¹⁴C-EDTA.

Sample	% Administered Dose		
	Group A	Group B	Group C
Urine	41.4	47.3	30.3
Tissue	0.04	0.02	0.04
Total	41.4	47.3	30.3

tion. EDTA appears to be favorably distributed to the prostate, but not the brain, following both IV and rectal dosing. The excretion of rectal CaNa₂ EDTA administration in urine corresponds well with the rectal dose bioavailability of blood levels. The absolute bioavailability of EDTA in blood following rectal dosing was 36.3% within the 8-hour period. Additional testing is required to confirm and duplicate these results in humans.

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