Pre-clinical Evaluation of ^{225}Ac-DOTATOC Pharmacokinetics, Dosimetry, and Histopathology to Enable Phase-1 Clinical Trial in Patients with Neuroendocrine Tumors

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Abstract

Objectives: Evaluate pharmacokinetics of ^{225}Ac-DOTATOC with and without kidney protection (KP); to compare $^{225}AcNO_3$ or "free" ^{225}Ac derived from accelerator production versus stockpile extraction; to estimate predicted radiation absorbed dose (RAD) to humans receiving ^{225}Ac-DOTATOC; and to evaluate histopathology 90 days post-administration.

Methods: ^{225}AcNO3-accelerator, and ^{225}AcNO3-stockpile, or ^{225}Ac-DOTATOC prepared using ^{225}AcNO3-stockpile was administered IV to male Sprague Dawley rats, n= 5 per cohort per time point, with and without KP. At 1-hour to 90-days post-administration, rats were euthanized. Blood was collected for CBC and metabolic testing. Organs were collected, weighed, evaluated for radioactivity using a gamma counter and processed for histopathological examination. Cumulative organ radioactivity was used as the input function to estimate mean radiation absorbed tissue dose in humans (OLINDA 1.0). Mean Residence Times (Mbq-h/Mbq) were determined to allow estimation of RAD in mSv/MBq.

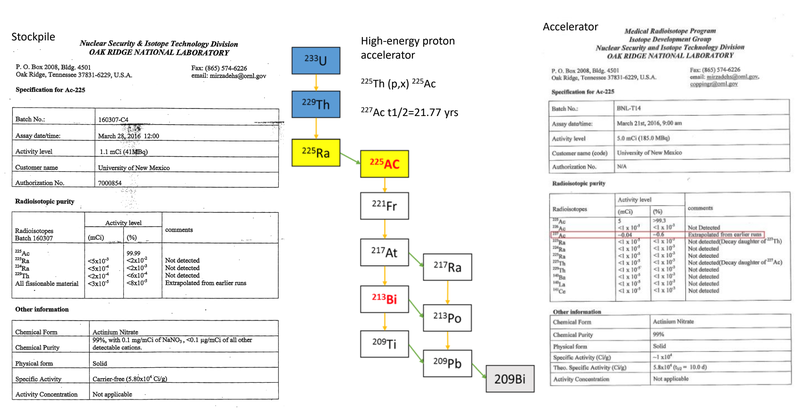
Results: ^{225}Ac-DOTATOC (10uCi +KP, 3uCi +KP, 10uCi, 3uCi), ^{225}AcNO3-accelerator, and ^{225}AcNO3-stockpile RAD to kidneys were (1.09E+02, 7.39E+01, 1.39E+02, 1.37E+02, 1.83E+02, 1.29+E02, respectively). KP decreased RAD 22% and 46% following 10uCi and 3uCi 225Ac-DOTATOC, respectively. ^{225}Ac-DOTATOC treated animals showed similar CBC to controls. Untargeted ^{225}AcNO3 from either accelerator or stockpile significantly decreased white and red blood cells, and overall survival. Three rats that received ^{225}AcNO3-stockpile and two rats that received ^{225}AcNO3-accelerator did not survive 90 days. The ^{225}AcNO3-stockpile and accelerator groups each had a single rat found dead which was not necropsied. Over the entire study, vehicle control rats continuously gained weight, while the groups receiving either ^{225}AcNO3-stockpile or ^{225}AcNO3-accelerator gained weight slower, with body weights remaining almost unchanged. There was no bone marrow hypoplasia in vehicle control, DOTATOC control, or 3 µCi-KP DOTATOC rats. Rats receiving 3 µCi+KP DOTATOC, 10 µCi+KP DOTATOC, and 10 µCi-KP DOTATOC developed mild to moderate bone marrow hypoplasia. All DOTATOC groups showed normal pattern of fat replacement in bone marrow consistent with normal aging. Bone marrow hypoplasia was marked to very marked in rats receiving ^{225}AcNO3-accelerator and was slightly less severe in ^{225}AcNO3-stockpile. All groups except control groups showed evidence of previous or ongoing renal tubular nephrosis. All groups except control groups and 3 µCi-KP DOTATOC group showed evidence of renal glomerulopathy; lesions were most severe in ^{225}AcNO3 stockpile and accelerator groups. Cardiac lesions of myofiber and epicardial mineralization were seen only in ^{225}AcNO3-accelerator group. The histological impact in control and ^{225}Ac-DOTATOC groups was negligible at all timepoints.

Conclusion: The estimated radiation absorbed dose from ^{225}Ac-DOTATOC was low in all critical organs. Accelerator produced ^{225}Ac contains ^{227}Ac (t½ ~ 21yrs) as a trace impurity, resulting in increased radiation dose when compared to stockpile-derived ^{225}AcNO3. The histopathological results show moderate impact from untargeted ^{225}AcNO3. The clinical impact is believed to be insignificant, since patients will receive targeted ^{225}Ac-DOTATOC which showed negligible toxicity at all timepoints.

Specific Aims

1. PK → MRT → radiation absorbed dose (RAD) following IV ^{225}Ac-DOTATOC in healthy rats
2. Estimate radiation absorbed dose in human subjects following IV ^{225}Ac-DOTATOC using OLINDA 1.0
3. Evaluate histopathology to determine the effects of dosage and kidney protection in normal rats up to 90 days PI.

^{225}Ac:Stockpile vs Accelerator Production



Methods

245 Male Sprague Dawley Rats, Crl: CD(SD) Charles River
n = 5 Rats per time point
^{225}Ac: IV bolus; Kidney Protection (KP): IP 30 minutes before ^{225}Ac
Analysis Method (Kirschner, 1975)

$$\frac{\text{frac}ID}{g}_{human} = \frac{\text{frac}ID}{g}_{rat} \cdot BW_{rat}/BW_{human}$$

- Animals sacrificed, tissues resected at times post iv injection
- Resected tissues were weighed
- Radioactivity in CPM by NaI(Tl) well-counter (4-pi 80hx75d mm)
- Tissues were recounted 100 days post sacrifice = 10x ^{225}Ac t½
- MRT values were entered in OLINDA 1.0 to generate dosimetry estimates for each group
- ^{227}Ac impurity contributed 2.0% +/- 0.1% of measured CPM observed in accelerator produced ^{225}Ac (at time of production)
- Enabled calculation of the relative CPM contributions from ^{225}Ac and ^{227}Ac) in both initial administered activity and subsequent organ measurements
- Single exponential fitting was used for organ AUC calculations

Study Design Cohorts

	^{225}Ac-DOTATOC				^{225}Ac-Nitrate			
Time Point	3uCi	+KP 3uCi	10uCi	+KP 10uCi	Accelerator	Stockpile	Accelerator-Recount 100 d	Stockpile-Recount 100 d
1 hr	X	X	X	X	X	X	X	X
3 hr					X	X		
4 hr	X	X	X	X				
24 hr	X	X	X	X	X	X		
48 hr	X	X	X	X				
72 hr					X	X		
96 hr					X	X		
7 d	X	X	X	X		X	X	X
30 d	X	X	X	X	X	X	X	X
90 d	X	X	X	X	X	X	X	X

Results

^{225}Ac Mean Residence Time (MBq-h/MBq)

	^{225}Ac-DOTATOC						^{225}Ac-Nitrate		
Organ	3uC.33i	+KP 3uCi	% Reduction	10uCi	+KP 10uCi	% Reduction	Accelerator	Stockpile	% Increase
Lower Large Intestine	1.85E-01	1.33E-01	28.11	1.47E-01	1.50E-01	-2.04	4.77E-01	3.19E-01	49.53
Small Intestine	4.56E-01	4.24E-01	7.02	4.39E-01	4.15E-01	5.47	1.78E+00	1.35E+00	31.85
Stomach	3.71E-01	2.86E-01	22.91	2.78E-01	2.88E-01	-3.6	1.59E+00	1.07E+00	48.6
Upper Large Intestine	2.70E-01	1.94E-01	28.15	2.14E-01	2.18E-01	-1.87	6.96E-01	4.65E-01	49.68
Heart Wall	9.65E-02	1.06E-01	-9.84	1.02E-01	1.34E-01	-31.37	6.24E-01	4.32E-01	44.44
Kidneys	2.46E+00	1.33E+00	45.93	2.49E+00	1.96E+00	21.29	3.29E+00	2.32E+00	41.81
Liver	5.40E+00	5.60E+00	-3.7	6.35E+00	7.72E+00	-21.57	6.80E+01	4.45E+01	52.81
Muscle	5.54E-01	5.59E-01	-0.9	9.21E-01	1.04E+00	-12.92	6.38E+00	5.53E+00	15.37
Pancreas	6.19E-01	5.99E-01	3.23	5.64E-01	3.45E-01	38.83	7.48E-02	7.91E-02	-5.44
Cortical Bone	1.22E+01	1.17E+01	4.1	1.45E+01	1.60E+01	-10.34	1.66E+02	1.45E+02	14.48
Trabecular Bone	3.04E+00	2.93E+00	3.62	3.62E+00	3.99E+00	-10.22	4.14E+01	3.63E+01	14.05
Spleen	8.95E-02	9.36E-02	-4.58	1.61E-01	1.51E-01	6.21	9.21E-01	8.66E-01	6.35
Testes	2.31E-03	1.99E-03	13.85	3.01E-03	3.43E-03	-13.95	1.74E-02	1.33E-02	30.83
Urinary Bladder Contents	3.66E-02	4.78E-02	-30.6	6.41E-02	5.29E-02	17.47	4.21E-01	3.93E-01	7.12

^{227}Ac Mean Residence Time (MBq-h/MBq)

	^{225}Ac-Nitrate + 100 days	
Organ	Accelerator-100	Stockpile-100
Lower Large Intestine	2.87E+01	0.00E+00
Small Intestine	1.51E+02	0.00E+00
Stomach	3.21E+02	0.00E+00
Upper Large Intestine	4.18E+01	0.00E+00
Heart Wall	6.40E+01	0.00E+00
Kidneys	5.52E+02	0.00E+00
Liver	6.21E+03	1.88E+04
Muscle	5.91E+01	0.00E+00
Pancreas	6.79E-02	0.00E+00
Cortical Bone	7.67E+04	0.00E+00
Trabecular Bone	1.92E+04	0.00E+00
Spleen	2.73E+02	0.00E+00
Testes	1.10E+01	0.00E+00
Urinary Bladder Contents	2.61E-01	0.00E+00

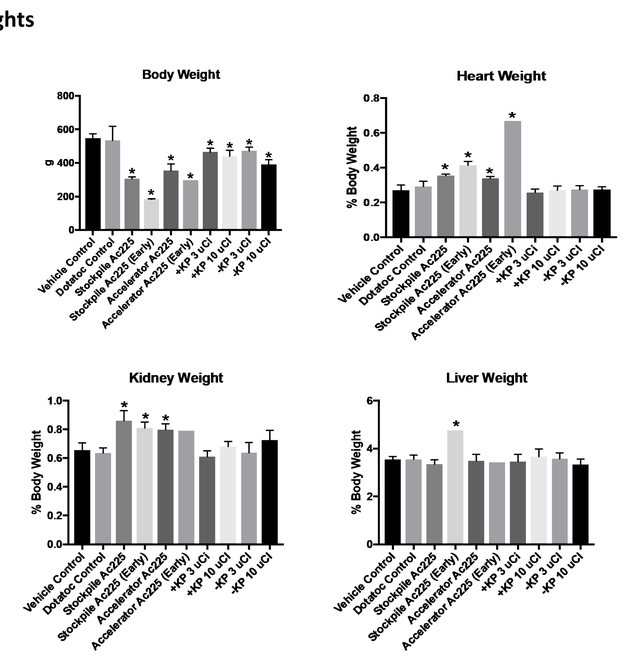
MRT ^{227}Ac > ^{225}Ac
Bone ^{227}Ac 15x ^{225}Ac
Due to late time points or "tail of pk", owing to ^{227}Ac t½ = 21.77 yrs

Organ	^{225}Ac (hr)	^{227}Ac (hr)
Lower Large Intestine	0.457	1.016
Small Intestine	1.69	4.645
Stomach	1.515	5.156
Upper Large Intestine	0.667	1.481
Heart Wall	0.596	1.314
Kidneys	3.12	9.988
Liver	59.715	157.391
Muscle	6.902	14.47
Pancreas	0.087	0.194
Cortical Bone	157.685	2606.94
Trabecular Bone	39.421	651.734
Spleen	0.888	3.129
Testes	0.017	0.291
Urinary Bladder Contents	0.573	0.84

^{225}Ac Absorbed Dose (mSv/MBq)

	^{225}Ac-DOTATOC						^{225}Ac-Nitrate		
Organ	3uCi	+KP 3uCi	% Reduction	10uCi	+KP 10uCi	% Reduction	Accelerator	Stockpile	% Increase
Adrenals	2.59E-03	2.26E-03	12.74	2.87E-03	2.94E-03	-2.44	2.14E-02	1.61E-02	32.92
Brain	7.27E-04	7.00E-04	3.71	8.68E-04	9.57E-04	-10.25	9.88E-03	8.65E-03	14.22
Breasts	3.82E-04	3.71E-04	2.88	4.46E-04	4.99E-04	-11.88	4.46E-03	3.50E-03	27.43
Gallbladder Wall	2.94E-03	2.78E-03	5.44	3.29E-03	3.65E-03	-10.94	2.86E-02	1.96E-02	45.92
LLI Wall	1.16E-01	8.35E-02	28.02	9.23E-02	9.40E-02	-1.84	3.04E-01	2.05E-01	48.29
Small Intestine	9.69E-02	9.01E-02	7.02	9.35E-02	8.85E-02	5.35	3.82E-01	2.90E-01	31.72
Stomach Wall	1.29E-01	9.94E-02	22.95	9.68E-02	1.00E-01	-3.31	5.54E-01	3.75E-01	47.73
ULI Wall	1.05E-01	7.58E-02	27.81	8.37E-02	8.53E-02	-1.91	2.78E-01	1.87E-01	48.66
Heart Wall	5.09E+00	5.59E+00	-9.82	5.37E+00	7.05E+00	-31.28	3.29E+01	2.28E+01	44.3
Kidneys	1.37E+02	7.39E+01	46.06	1.39E+02	1.09E+02	21.58	1.83E+02	1.29E+02	41.86
Liver	4.71E+01	4.89E+01	-3.82	5.54E+01	6.73E+01	-21.48	5.93E+02	3.88E+02	52.84
Lungs	9.95E-04	9.65E-04	3.02	1.16E-03	1.30E-03	-12.07	1.14E-02	8.73E-03	30.58
Muscle	3.30E-01	3.33E-01	-0.91	5.49E-01	6.22E-01	-13.3	3.80E+00	3.30E+00	15.15
Total Body	5.69E+00	5.32E+00	6.5	7.22E+00	6.63E+00	8.17	6.53E+01	5.36E+01	21.83
Effective Dose	1.18E+01	1.13E+01	4.24	1.51E+01	1.38E+01	8.61	1.41E+02	1.17E+02	20.51

Weights



- Significantly different from Vehicle Control by ANOVA.
- Examined over the entire study, vehicle control rats continuously gained weight, while ^{225}AcNO3-stockpile and ^{225}AcNO3-accelerator gained weight more slowly. Body weights remaining almost unchanged.
- For ^{225}AcNO3-stockpile & ^{225}Ac -accelerator groups, relative heart weights were increased suggesting that heart weight was maintained as body weight decreased.
- For ^{225}AcNO3-stockpile and ^{225}AcNO3-accelerator groups, relative kidney weights were increased, suggesting that kidney weight was maintained as body weight decreased.
- Except for the ^{225}AcNO3-stockpile group, relative liver weights did not differ from the vehicle control group. Since liver weight is usually proportional to the body weight, this suggests that no gross liver abnormalities were present.

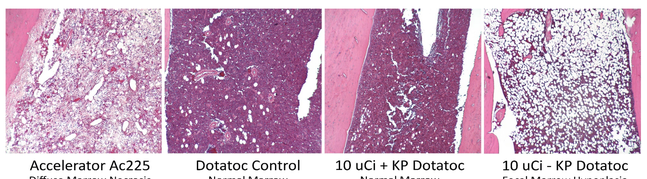
Histological Findings

- Livers in many rats, including control rats, had small foci of mononuclear cells, probably inflammatory cells. This did not appear treatment related.
- There was vey mild karyomegaly, with scattered enlarged hepatocyte nuclei in ^{225}AcNO3-accelerator and ^{225}AcNO3-stockpile groups. This is likely a regenerative change.
- Cardiac lesions were seen only in ^{225}AcNO3-accelerator treated rats. These changes included mineralization of individual myofibers (a degenerative change) and epicardial mineralization.

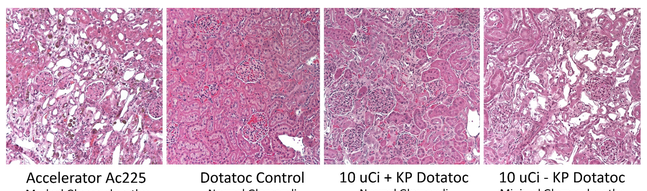
Results cont.

Histological Findings

- Bone marrow changes were more severe in the ^{225}AcNO3-accelerator and ^{225}Ac StockpileNO3 groups that died before the end of the study, suggesting that some bone marrow regeneration was taking place in rats that survived for the entire study. There was mild to moderate bone marrow hypoplasia in the 3 µCi+KP DOTATOC, 10 µCi+KP DOTATOC, and 10 µCi-KP DOTATOC groups.
- Glomerulopathy, characterized by hypocellularity, irregular mesangial thickening, and protein in kidney tubules, was observed in all groups except the vehicle control, DOTATOC control, and 3 µCi-KP DOTATOC groups.
- All groups except the two control groups showed evidence of tubular nephrosis, but the pattern of nephrosis in each group varied.

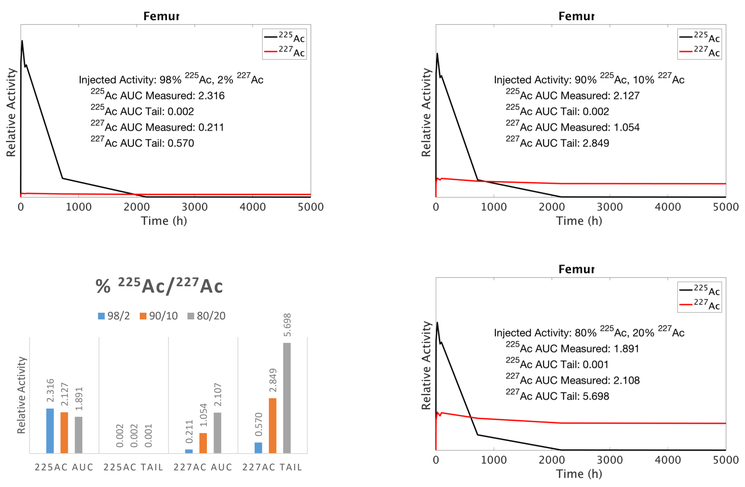


Accelerator Ac225 — Diffuse Marrow Necrosis
Dotatoc Control — Normal Marrow
10 uCi + KP Dotatoc — Normal Marrow
10 uCi - KP Dotatoc — Focal Marrow Hypoplasia



Accelerator Ac225 — Marked Glomerulopathy, Marked Tubular Necrosis
Dotatoc Control — Normal Glomeruli, Normal Tubules
10 uCi + KP Dotatoc — Normal Glomeruli, Mild Tubular Damage
10 uCi - KP Dotatoc — Minimal Glomerulopathy, Marked Tubular Necrosis

227Ac Impurity "Tailing" Effect on AUC



Conclusions

Toxicity
- ^{225}Ac-DOTATOC: 3 and 10 uCi +/- KP - No observed toxicity
- ^{225}AcNO3-stockpile and ^{225}AcNO3-accelerator - Marked lethargy; Decreased WBCs & RBCs; Decreased overall survival

Dosimetry
- MRT ^{227}Ac 3-15x ^{225}Ac; RAD ^{227}Ac <<<< ^{225}Ac in life
- ^{225}Ac emits several alpha particles. ^{227}Ac has 1.38% alpha decay, with multiple beta decay. This difference is critical in interpreting dosimetry results since MRT for ^{227}Ac are much longer, but absorbed dose estimates are much lower

Limitations
- Assumed that ^{227}Ac & ^{225}Ac in vivo biodistribution is identical
- Estimate of ^{227}Ac impurity based upon radioactive decay
- Lack intrinsic efficiency for ^{227}Ac

Histopathology
- Histological analysis of heart, kidney, spleen and bone tissues collected at the +90 day time point show moderate impact from untargeted ^{225}AcNO3 from both stockpile and accelerator production

Future

Dosimetry
- ^{227}Ac reference source to determine intrinsic efficiency
- Data reanalyzed using Olinda 2.0

Patient Study - 2019
- Phase I Trial of Intravenous ^{225}Ac-DOTATOC for Treatment of Somatostatin-Receptor Expressing Neuroendocrine Tumors - 2020

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