Journal of Veterinary Science & Medical Diagnosis

Research Article

A SCITECHNOL JOURNAL

Scintigraphic Imaging of B-cell Lymphoma in Three Dogs Using a Radio-labeled Somatostatin Analogue

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Abstract

Background: Somatostatin analogues have been used to image endocrine neoplasia in dogs and non-Hodgkin's lymphoma (NHL) in humans.

Hypothesis: The hypothesis of this study was that dogs with naturally-occurring NHL will demonstrate tumor-specific uptake of ^{111}In-DOTA-TATE on planar scintigraphy.

Methods: Three dogs with spontaneous B cell lymphoma received 3-5mCi (111-171MBq) of ^{111}In-DOTA-TATE injected intravenously and were imaged with a planar gamma camera at 1, 4 and 24 hours after injection. Regions of interest were drawn around disease sites and ratios constructed to muscle containing blood-pool within each image.

Results: All evaluated nodes concentrated the radiopharmaceutical at the 4 and 24 h time-points. Spleen uptake was also visible for all three dogs. Liver and gastrointestinal uptake was present as described in previous reports.

Conclusions and Clinical Importance: The somatostatin analogue ^{111}In-DOTA-TATE demonstrates tumor-specific uptake in canine B cell NHL. This agent could be used for imaging of dogs with NHL and as a platform for delivery of therapeutic radiopharmaceuticals.

Keywords

Somatostatin receptor; Somatostatin analogue; Molecular imaging; Planar scintigraphy

Abbreviations: DOTA: 1,4,7,10-tetraazacyclododecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid; NHL: Non-Hodgkin's Lymphoma; SPECT: Single Photon Emission Computed Tomography; SSTR2: Somatostatin Type 2 Receptors; SSTR5: Somatostatin Type 5 Receptors; TATE: Tyrosine-3-Octreotate

Introduction

The chelator-conjugated peptide 1,4,7,10-tetraazacyclododecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid (DOTA)-tyrosine-3-octreotate (TATE) is a peptide that binds type 2 and type 5 somatostatin receptors with high specificity [1]. It is similar to the product Octreoscan®, an

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FDA approved agent for imaging neuroendocrine tumors. Human non-Hodgkin lymphoma (NHL) expresses somatostatin receptors in approximately 80% of cases [2]. The targeting properties of radiolabeled somatostatin analogues could be exploited to deliver a payload of radioactivity for molecular imaging and/or therapy of NHL. Canine lymphoma has not been demonstrated to express these receptors.

Dogs express somatostatin type 2 receptors (SSTR2) in endocrine and gastrointestinal tissues suitable for imaging with ^{111}In-DOTA-TATE. Using ^{111}In-DTPA-D-Phe^1-octreotide, an SSTR2- and somatostatin type 5 receptor (SSTR5)-specific agent, investigators performed biodistribution studies in normal beagles as a preclinical evaluation for imaging endocrine disease [3]. Receptor-mediated uptake existed in the stomach, intestinal tract, gall bladder, pancreas and kidneys [3]. Germinal follicles of Peyer's patches in the intestines showed significant uptake, suggesting lymphoid accumulation of the agent, but specific cellular accumulation could not be demonstrated [3]. Similar agents have been used successfully in dogs to image gastrinoma and insulinoma [4-6]. Substitution of radiometals from a photon emitting nuclide to one which emits particle radiation could allow this targeted carrier to be applied with therapeutic intent.

The purpose of this study was to provide evidence that ^{111}In-DOTA-TATE-SSTR2 binding occurs *in vivo* in dogs with spontaneous B cell NHL. This evidence is necessary for further development of therapeutic radiopharmaceuticals based on this somatostatin analogue platform. There is no literature supporting for the presence of appropriate somatostatin receptors on the surface of canine NHL cells. These receptors could be assayed with immunohistochemistry or Western blot techniques, but the specificity of ^{111}In-DOTA-TATE-SSTR2 interaction is at least as high, and the biological data resulting from these scans is superior to an *in vitro* study. The hypothesis of this study was that dogs with naturally-occurring NHL will demonstrate tumor-specific uptake of ^{111}In-DOTA-TATE on planar scintigraphy with target to background ratios greater than 1.0 on region of interest (ROI) analysis.

Materials and Methods

Dose preparation

DOTA-TATE was labeled with ^{111}In as follows: 5mCi of ^{111}InCl$_3$ in 150 µL of 30 mM sodium acetate, 25 mM sodium ascorbate, pH 5.0, was incubated with 5 µg of DOTA-TATE at 99 °C for 30 min. Radiometal incorporation and radiochemical purity (typically >98%) were determined by radio-thin-layer chromatography (TLC). After sterile filtering, the radiolabeled peptide was diluted with normal saline for injection. Reverse-phase HPLC (RP-HPLC) was performed on a Waters 626 chromatograph equipped with a manual Rheodyne injector, 2487 dual wavelength UV detector, Bus SAT/IN analog-digital interface and Empower Pro software (Build No. 1154). A Phenomenex (Torrance, CA) Jupiter C18 column (5 µm, 4.6 × 250 mm) was used for purification and analysis of radiolabeled compounds. A gradient of 0-50% solvent B (solvent A, 0.1% TFA/H$_2$O; solvent B, 0.1% TFA/acetonitrile) over 30 min at a flow rate of 1.0 mL/min was used. UV detection was accomplished at 280 nm, and

Case	Hour	Mandibular	Superficial Cervical	Popliteal	Liver	Spleen	GI Tract
1	1	1.05	1.08	0.97	1.66	1.72	1.22
	4	1.14	1.18	1.29	1	2.06	1.94
	24	1.39	1.06	1.34	1.77	2.45	2.32
2	1	1	1.03	1.55	1.22	1.64	1.8
	4	1.67	1.61	1.6	2.28	2.4	2.32
	24	1.43	1.11	2.54	2.25	2.17	2.14
3	1	1.83	1.43	1.93	1.86	2.07	1.9
	4	1.08	1.07	1.43	2.45	2.64	2.5
	24	1.8	1.11	N/A	1.95	1.83	1.82
Mean	1	1.29	1.18	1.48*	1.58*	1.81*	1.64*
	4	1.30	1.29*	1.44	1.91	2.37*	2.25
	24	1.54*	1.09	1.94	1.99*	2.15	2.09*

Table 1: Lesion to muscle uptake ratios for target tissues of ^{111}In-DOTA-TATE in the mandibular, superficial cervical, and popliteal lymph nodes, and the liver, spleen, and gastrointestinal tract. Regions of interest for which raw counts were significantly different than background are marked with an asterisk (*).

radioactivity detection was accomplished using a NaI(Tl) radioactivity monitor (Canberra Meriden, CT).

Dose administration

A 22 Ga indwelling catheter was placed in each dog's cephalic vein. The complete dose of 3-5mCi (111-171MBq) of ^{111}In-DOTA-TATE was injected intravenously in a single bolus, followed by aspiration and flushing to ensure complete dose injection.

Image acquisition

Dogs were sedated according to the standard and IACUC approved protocol and images were acquired at 1h, 4h, and 24h following injection. Images included the right and left lateral head and neck, right and left lateral thorax, and right and left lateral abdomen using an Equistand II (Diagnostic Imaging, Middlesex, NJ) planar gamma camera with a medium energy all-purpose collimator (MEAP).

Image analysis

Images were converted to JPG file format and imported into ImageJ analysis software (National Institutes of Health, Bethesda, MD). Regions of interest were drawn around nodes, liver, spleen and gastrointestinal tract. The mean intensities within those regions were compared with a simultaneously acquired ROI overlying muscle in the same image. The result is the reported target to background ratio. Specific uptake would be expected to yield a target to background ratio greater than 1.0 on planar scintigraphy. Raw counts and intensity ratios were compared using a one-way ANOVA on ranks. Significance was set at $P \leq 0.05$. Images were also evaluated qualitatively by a board-certified veterinary oncologist trained in nuclear imaging (JCL). Dogs were released from radiation isolation 48hr after injection per protocol.

Results

Three dogs with naturally occurring B-cell lymphoma were enrolled in this pilot study. All dogs were spayed females, 8 years old, and of mixed breeding. Dog 1 was 44.3 kg, Dog 2 was 11.1 kg, and Dog 3 was 6.5 kg. Dog 1 was stage IV, based on physical examination, with palpable splenomegaly. No other routine imaging was performed on Dog 1. Dog 2 was stage V, based on palpable splenomegaly and 31,540 circulating large lymphocytes. Dog 2 had a mildly elevated serum alkaline phosphatase of 205 U/L (ref: 5-129 U/L). Dog 3 was stage IV, with palpable hepatosplenomegaly and ultrasound evidence of spleen and liver lymphoma infiltration. Each dog received a single intravenous injection between 3 and 5 mCi of ^{111}In-DOTA-TATE.
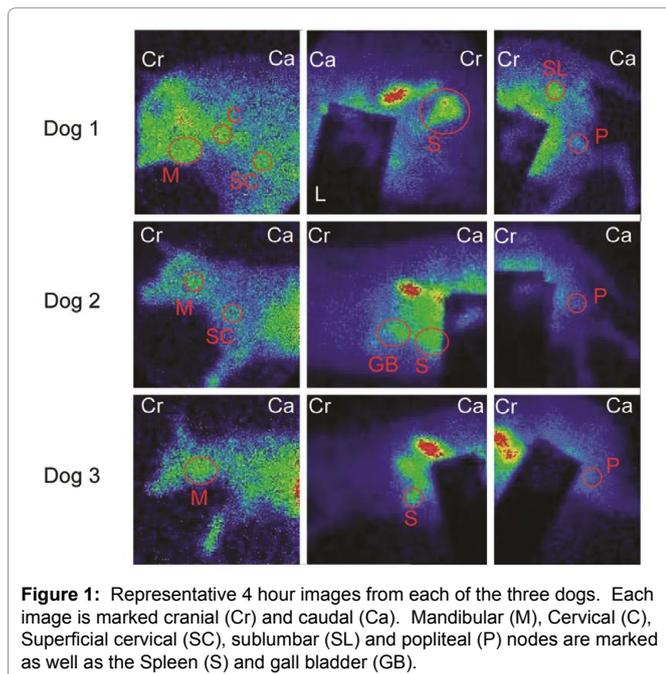
No dog had an adverse reaction of any kind to the injection. All dogs have subsequently been treated for their lymphoma and achieved complete remission. Regions of interest containing the lymph nodes, liver, spleen, and gastrointestinal tract were compared with regions of interest over muscular regions not containing lymph tissue in the field of view (Table 1). Uptake ratios ranged from 0.97 to 2.54 for lymph nodes, 1 to 2.45 for liver, 1.72 to 2.64 for spleen, and 1.22 to 2.32 for gastrointestinal tract. There was no single time-point at which the node values were consistently highest for any of the selected nodes. There was no significant difference in uptake ratio between time points for any tissue. Significant differences between raw counts of target tissue and background were present as shown in Table 1.

Discussion

The three dogs used in this study were female, eight years old, and having advanced, bulky and organ-based disease. As such, they do not represent a broad cross-section of canine B-cell lymphomas. Their body sizes also spanned a large weight range, which could affect distribution of the imaging agent, although the image quality was remarkably similar across all three dogs. However, as proof of principle, the results of these scans demonstrate the potential utility of radiolabeled-DOTA-TATE as an imaging or therapy agent. The scans demonstrate uptake in tissues affected by lymphoma, by both subjective and objective measures.

In each dog, the area of the mandibular lymph nodes was visually apparent. The objective uptake ratios, however, are mildly increased relative to muscle. The argument could be made that tumors have increased blood flow, explaining the increased ratio at the node regions. After the first hour, the ratio of heart activity to muscle activity was 1.0. If blood flow explained the increased activity visible in the lymph nodes, these, too, would be expected to return to background after one hour, when they were at least 39% above background at 24 hr in every case. In fact, in Dogs 1 and 2, lack of target to background difference likely explained the low node to muscle ratio at 1hr. It is more difficult to explain the low ratio at 4 hr for Dog 3. It should be noted that dog 3 had a large burden of disease in the liver and spleen. It is possible that these highly vascular lymphoma beds served to provide a first-pass effect to remove much of the radiopharmaceutical from the blood. Activity then accumulated slowly in the mandibular lymph nodes over time, after the 1hr blush subsided.

The superficial cervical node demonstrated consistent ratios above 1.0, but not as high as the mandibular node. The location of this node with a contiguous background of muscle mass and without air boundaries like the mandibular node would have consistently

Figure 1: Representative 4 hour images from each of the three dogs. Each image is marked cranial (Cr) and caudal (Ca). Mandibular (M), Cervical (C), Superficial cervical (SC), sublumbar (SL) and popliteal (P) nodes are marked as well as the Spleen (S) and gall bladder (GB).

lower uptake ratios than a more isolated node. The popliteal node demonstrated ratios greater than 1.29 in all cases except the 1h measurement of Dog 1. This was slightly surprising, as the node was not consistently evident on visual examination of the images. Ratios calculated on other portions of the leg, however, were approximately 1.0, confirming the presence of uptake in the popliteal region.

Organ ratios tended to be higher than node ratios. Splenic involvement was suspected by palpation or documented by ultrasound imaging in all three dogs. Liver involvement was only suspected in Dogs 2 and 3. It is to be noted that liver uptake ratios were increased relative to background in all dogs. This would suggest that there was some hepatic clearance in these dogs not related to the localization of the lymphoma. This is consistent with the finding of liver uptake in five Beagle dogs imaged using ^{111}In-DTPA-D-Phe[1]-octreotide [3]. In this study, ratios were higher in the dogs with suspected liver involvement, confirming the subjective impression of a liver outline on the images. Spleen uptake was high in all dogs. It is likely that this is a direct result of the lymphoma present in the spleen. Robben and others found no splenic uptake present in normal dogs [3]. Future studies need to repeat scans in remission from lymphoma to document resolution of splenic uptake. The data presented here cannot rule out spleen uptake by mechanisms other than lymphoma present. Robben and others also found gastrointestinal uptake in the normal dogs that appeared receptor-mediated [3]. Similar to that finding, the dogs in this study had gastrointestinal uptake ratios well above 1.0 at all time points. This should be expected in dogs with no gastrointestinal lymphoma disease burden in future studies. On average, the uptake ratios for nodes reported here were lower than those reported in a study of ^{111}In-DOTA-DPhe[1]-Tyr[3]-octreotide and ^{111}In DOTA-lanreotide in humans [7]. These agents yielded ratios, when positive, ranging from 2.34+/-0.94 to 2.49+/-0.58. Included in the target to background ratios of these measurements were both nodes and organ lesions, with no specifics listed. It is possible that the organ ratios were higher than the node ratios in humans as presently observed in dogs [7].

The subjective evaluation of the images of Dog 1 revealed multiple visible nodes from the 1h time-point. In fact, the zygomatic and sublumbar node involvement had not been noted until they were detected on the scan images. It is also unlikely that the cervical node involvement would have been detected without the scan. Depreotide scanning in children with either Hodgkin's lymphoma or NHL revealed lesions that changed the stage and therapy plan in 3 of 11 patients [8]. Lesions were also missed by depreotide scanning in these patients, but none would have altered staging [8]. Staging with ^{111}In-pentetreotide resulted in detection of lesions in 10 patients that altered the clinical course in 5 of them [9]. Similar to the depreotide study, 19 patients had lesions detected by conventional means that were missed on nuclear scintigraphy [9].

Dog 2 did not have nodes as clearly visible as dog 1. This result may be due to this dog's significantly smaller size. The liver and spleen activity was clearly visible in this dog, with a clear gallbladder image on lateral abdominal views at 4hr. Gallbladder activity was detected in normal beagles, so it was likely a normal finding in this case [3]. Popliteal node activity was present in this dog, as measured by uptake ratios, but the nodes were not clearly visible subjectively. This finding was likely a result of the small size of the patient as well. Subjectively, the cardiac silhouette was particularly prominent in this patient. The presence of an active leukemia component of this dog's disease would suggest the possibility that this increased blood-pool activity was receptor mediated. When compared to the other dogs, however, there was not a significant increase in heart activity at any time point. The images of dog 3 were subjectively very similar to those of dog 2. The presence of hepatosplenomegaly in both dogs, and their similar small size, likely contributes to the similarity of the scans.

The results of this study confirm the hypothesis that the uptake of a radiolabeled somatostatin analogue in canine B cell NHL occurred at levels allowing non-invasive detection with planar scintigraphy. This result forms the basis for future development of imaging and therapy agents using SSTR2-specific ligands. In addition, the internalizing nature of somatostatin analogues make them suitable for intracellular delivery of imaging and therapeutic nuclides as well as molecules directed at intracellular targets. This study also mimics the presence of SSTR2 receptors on the surface of human B cell NHL cells, supporting the use of dogs as a pre-clinical model for human research. Future proof-of-principle studies using novel constructs with somatostatin analogue targeting could be evaluated in dogs with naturally-occurring disease. Imaging biodistribution and dosimetry could be modeled in dogs before translation to human patients. Replacing the radionuclide with a positron-emitting species could allow highly accurate dosimetry using three-dimensional modeling (Figure 1).

Acknowledgement

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