Feline oral squamous cell carcinoma is one of the most refractory feline malignancies. Most patients succumb due to failure in local tumor control. 2-^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG PET) is increasingly being used for veterinary oncology staging as it highlights areas with higher glucose metabolism. The goal of the current prospective study was to compare gross tumor volume measurements using ^{18}F-FDG PET vs. those using computed tomography (CT) for stereotactic radiation therapy planning in cats with oral squamous cell carcinoma. Twelve cats with confirmed oral squamous cell carcinoma underwent pretreatment ^{18}F-FDG PET/CT. Gross tumor volumes based on contrast-enhanced CT and ^{18}F-FDG PET were measured and compared among cats. Mean PET gross tumor volume was significantly smaller than mean CT gross tumor volume in the mandibular/maxillary squamous cell carcinoma group (n = 8, P = 0.002) and for the total number of patients (n = 12, P = 0.006), but not in the lingual/laryngeal group (n = 4, P = 0.57). Mismatch fraction analysis revealed that most of the lingual/laryngeal patients had a large region of high-^{18}F-FDG activity outside of the CT gross tumor volume. This mismatch fraction was significantly greater in the lingual/laryngeal group than the mandibular/maxillary group (P = 0.028). The effect of poor spatial resolution of PET imaging was greater when the absolute tumor volume was small. Findings from this study indicated that ^{18}F-FDG PET warrants further investigation as a supplemental imaging modality in cats with oral squamous cell carcinoma because it detected regions of possible primary tumor that were not detected on CT images. © 2013 Veterinary Radiology & Ultrasound.

Key words: ^{18}F-FDG PET/CT, feline oral squamous cell carcinoma, gross tumor volume, radiation therapy planning.

Introduction

Accurate measurement of gross tumor volume is important for successful radiation therapy in cats with oral squamous cell carcinoma because geographically missed tumor cells could lead to a failure in local tumor control. Computer-based three-dimensional (3D) planning tools typically use electron density maps to calculate radiation attenuation associated with tissue heterogeneity and to date, computed tomography (CT) has been the only imaging modality that could provide this information. However, CT has some limitations when it is used for delineation of gross tumor volume in that it does not always allow discrimination of infiltrative tumors from surrounding nontumor reactive regions or normal adjacent tissues. To address the limitations of CT for oncologic staging and radiation therapy planning, imaging modalities that are metabolically or functionally based are the current standard in human medicine and are increasingly being used for veterinary patients. Positron emission tomography (PET) is one of the most commonly used functional imaging studies and 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) is one of the most commonly used radioisotopes. This glucose-analog radiotracer accumulates in areas with high glucose uptake and high cellular metabolism. Increased glucose metabolism can be seen in tumor tissues as well as areas of inflammation or infection. Some normal organs such as the brain and the cardiac muscle also have high glucose metabolism.

Recent technological advances allow coregistration of PET images with CT images (PET/CT) so that functional and anatomical images can be viewed simultaneously (Fig. 1). Several human studies have concluded that metabolic gross tumor volume based solely on ^{18}F-FDG PET is generally smaller than gross tumor volume
Feline oral squamous cell carcinoma has been proposed to be the feline counterpart of human head and neck squamous cell carcinoma. This tumor is the most common malignancy seen in the feline oral cavity, accounting for approximately 65% of all feline oral tumors. Risk factors such as environmental tobacco smoke, high intake of canned food, and the use of flea collars have been previously identified. Treatment outcome for cats with oral squamous cell carcinoma is often disappointing and most patients die or are euthanized due to failure in local tumor control even after multimodal treatment.

Some veterinary reports have described the use of 18F-FDG PET for dogs and cats in the fields of oncology, physiology, and behavioral medicine. No veterinary studies were found that described the use of this new imaging modality for radiation therapy planning. The objective of this prospective study was to compare PET and CT gross tumor volume measurements for stereotactic radiation therapy planning in a cohort of feline patients with oral squamous cell carcinoma. Based on previous studies in humans, we hypothesized that 18F-FDG PET would allow detection of additional possible tumor regions and that gross tumor volumes based solely on PET imaging would not completely overlap with gross tumor volumes based on CT imaging.

Materials and Methods

Cats

Client-owned feline patients with histopathologically confirmed oral squamous cell carcinoma were prospectively recruited during the period of January 2010 and July 2011. All cats were presented for treatment at the Animal Cancer Center, Colorado State University. The study protocol was approved by and conducted in accordance with requirements of the institutional animal care and use committee. Each cat was assigned to a mandibular/maxillary or a lingual/laryngeal group based on the location of the grossly visible tumor.

PET-CT Scanning Procedures

All patients were fasted at least 12 h. The anesthetic protocol was tailored to individual patients’ medical and physical conditions so as to maintain the best standard of care. An identical standardized drug scheme was not used for all patients in the study. Preanesthetic drugs included atropine and either methadone or hydromorphone, and anesthetic induction agents included ketamine or propofol, with or without midazolam or diazepam. Upon anesthetic induction, patients were instrumented with two intravenous catheters, an intra-arterial catheter for blood pressure monitoring, and a urinary catheter with a bag. One intravenous catheter was dedicated exclusively for injection of the radioactive isotope. Anesthesia was maintained with isoflurane or sevoflurane inhalation, typically with 1–2% of admixture rate. Normal body temperature was maintained by appropriate warming devices (Bair Hugger, Arizant Healthcare, Eden Prairie, MN) as needed. Patient positioning for PET/CT scanning was done to provide concurrent immobilization and CT simulation for radiation therapy treatment using a thermoplastische bite block (Patterson Medical, Cedarburg, WI) and mask...
system (Civco Systems, Orange City, IA) as previously reported. Dorsal or ventral positioning was chosen for the best exposure and access to the tumor and regional lymph nodes for modulated teletherapy beams and modifiers. Extended urinary catheter tubing was placed through the two bores of the PET/CT gantries and the urinary bag was placed in a lead container behind the PET camera. Whole body PET/CT imaging was performed using the same scanner for all cats (Philips Gemini TF Big Bore 16-slice scanner, Philips Medical Systems, Andover, MA). Orthogonal pilot views were obtained to prescribe the CT and PET series. Immediately after positioning and planning, the cats were injected intravenously with 0.17 mCi per kg ± 10% 18F-FDG.

After injection of the radioisotope, a volumetric (helicical) low-dose CT scan was obtained precontrast through the body and reconstructed at 5.0 mm contiguous intervals at 600 mm field of view for the PET acquisition and 2.0 mm contiguous intervals at 350 mm field of view for improved resolution and radiation therapy planning. The following CT acquisition parameters were used: 120 KV, 100 mAs/slice, 0.75 s rotation time, 0.813 pitch, and 0.75 mm 16 detector width. A second whole-body helical low-dose CT pass after injection with 0.7 g/kg of iohexol (Omnipaque TM 350, GE Healthcare, Princeton, NJ) contrast media was acquired using the scan parameters and reconstruction protocol above.

The animal was maintained under anesthesia and in position until 1 h post-18F-FDG injection, at which time the PET data collection was initiated. The PET acquisition consisted of eight to nine frames (beds), each 18.0 cm in cranial-caudal length. Acquisition time was 1.5 min per bed. The range of times for the collection of the PET series was 12–13.5 min. Nonattenuation-corrected PET images were reconstructed using the 3D-row action maximum likelihood algorithm (RAMLA) method. CT attenuation-corrected images of the PET data were reconstructed using the line-of-response TruFlight (LOR-TF) RAMLA method (identified as BLOB-OS-TF for this scanner) and the precontrast CT dataset. After image acquisition, all patients were recovered from anesthesia and isolated until their radiation level reached the institutional safety requirement.

Measurement of Gross Tumor Volumes

For each cat, postcontrast CT and PET image datasets were exported to the PET/CT scanner’s image viewing workstation (Extended brilliance workspace, EBW, version 4.5.3, Philips Healthcare Nederland B.V., Veenpluis, the Netherlands) and loaded into the radiation therapy planning software (Eclipse treatment planning system, TPS, version 8.6.0, Varian Medical Systems, Palo Alto, CA). Using a freehand contouring tool, gross tumor volume based exclusively on postcontrast CT images was contoured by one of the authors who had expertise in radiation therapy planning (H.Y.). A board-certified veterinary radiologist (E.K.R.) interpreted the CT images independently and modified the contours as needed for final determination of CT gross tumor volume for each cat. The PET gross tumor volume was then calculated based solely on the 18F-FDG PET images, using a modified thresholding technique and the workstation’s image analysis software. Thresholding was done by two board certified veterinary radiologists (S.L.K. and E.K.R.) and was based on selection of the standardized uptake value that completely surrounded the entire tumor and best delineated the FDG avidity. This was done while reviewing the 18F-FDG PET uptake pattern on images using a standard window level setting. Once the standardized uptake value threshold was determined, the workstation’s software was used to automatically draw a volume of interest (VOI) around the tumor and calculate PET gross tumor volume. Modifications in the VOI were made as needed to eliminate regions outside of the patient body (such as the oral cavity and endotracheal tube) that exhibited artifactual hypermetabolic activity. After the PET gross tumor volume was finalized for each cat, the first author (H.Y.) copied the VOI onto the postcontrast CT image in the treatment planning system and used this for subsequent comparisons.

Comparisons between CT and PET Gross Tumor Volumes

All statistical tests were selected and performed by a statistical consultant, using commercially available software (SigmaPlot version 12, Systat Software, San Jose, CA and GraphPad Prism version 5.03, GraphPad Software, La Jolla, CA). A P-value < 0.05 was considered statistically significant for all tests. Once the CT and PET VOI’s for each cat were imported into the treatment planning system, gross tumor volumes based on each VOI were calculated using the “measure volume” tool in the treatment planning system. A two-tailed paired t-test was used to compare mean values for CT vs. PET gross tumor volumes. Comparisons were performed for all cats and for cats assigned to mandibular/maxillary and lingual/laryngeal groups.

The degree of CT and PET gross tumor volume overlapping was calculated using a mismatch fraction as described in a previous study. By using the “Boolean operators” tool in the treatment planning system, a structure defined as “only in PET gross tumor volume but not in CT gross tumor volume” was created. Then a mismatch fraction defined as “mismatch PET gross tumor volume to CT gross tumor volume” was calculated using the following formula:

\[
\frac{(\text{volume of PET gross tumor volume that is not overlapped by CT gross tumor volume})}{(\text{entire CT gross tumor volume}) \times 100.}
\]
The Mann–Whitney rank sum test was then used to compare mismatch fractions for cats assigned to mandibular/maxillary and lingual/laryngeal groups.

Results

Cats

Twelve feline patients with oral squamous cell carcinoma were included in this study. All patients had no history of previous treatment except for one (patient no. 11 had tumor recurrence after surgical debulking). Breeds represented were domestic short hair (8), domestic longhair (3), and Siamese (1). Eight cats were neutered males and four were neutered females. Average patient age and body weight at the time of diagnosis were 12 years old and 4.8 kg, respectively. Clinical stages for tumors were stage II (n = 6), stage III (n = 3), and stage IV (n = 3), based on a previously published feline oral squamous cell carcinoma staging scheme. Eight cats were assigned to the mandibular/maxillary group and four were assigned to the lingual/laryngeal group.

Comparisons of Gross Tumor Volumes

Gross tumor volumes measured for each patient using PET are summarized in Fig. 2A. The average of the mean standardized uptake values for PET gross tumor volumes was 5.5 (standard deviation (SD) = 1.7) and the median of the mean standardized uptake values was 5.2. The average of the maximum standardized uptake values was 10.1 (SD = 5.03) and the median of the maximum standardized uptake values was 8.95. The selected standardized uptake value threshold that best encompassed the PET gross tumor volumes ranged from 2.4 to 3.8 (median value 3.2). Mean CT gross tumor volume (14.7, SD = 9.6) was significantly larger than mean PET gross tumor volume (11.0, SD = 7.5) (P = 0.006) when all 12 cats were analyzed. For the eight cats in the mandibular/maxillary group, mean CT gross tumor volume (18.0, SD = 7.9) was significantly larger than PET gross tumor volume (13.2, SD = 7.7) (P = 0.002). For the four cats in the lingual/laryngeal group, no statistically significant difference was found between mean CT gross tumor volume (8.1, SD = 10.3) and mean PET gross tumor volume (6.5, SD = 5.5) (P = 0.57). Mismatch fractions defined as “mismatch PET gross tumor volume to CT gross tumor volume” for each patient are summarized in Fig. 2A. Cats in the lingual/laryngeal group (median = 41.6, mean = 53.9, SD = 52.0) had a significantly higher mismatch fraction than cats in the mandibular/maxillary group (median = 4.2, mean = 8.3, SD = 11.5) (P = 0.028, Fig. 2B).

Discussion

In this study, we incorporated 18F-FDG PET into the radiation treatment plans for 12 feline patients with oral squamous cell carcinoma with the ultimate goal of improving targeted delivery. Our protocols were based on those previously described for humans with head and neck squamous cell carcinoma. The use of FDG-PET/CT yielded gross tumor volumes that, in some feline patients, were asymmetrically or only partially overlapping with the CT gross tumor volumes. This finding supported our hypothesis that the information provided by 18F-FDG PET would lead to the detection of additional possible tumor areas outside of the volumes indicated by CT alone. The use of metabolic 18F-FDG PET imaging to better detect all potential tumor regions may be particularly critical for stereotactic radiation therapy planning in order to avoid geographical miss because failure in local tumor control is the most common reason for death/euthanasia in feline patients with oral squamous cell carcinoma. Affected cats in this study were to be treated with stereotactic radiation therapy, which employs a high dose per fraction to tumor and has a very sharp drop-off at tumor margins to spare surrounding tissues. Target tumor volumes in stereotactic radiation therapy do not employ an additional microscopic tumor margin, which is called clinical target volume and is used in conventional radiation therapy.

Our finding that CT gross tumor volume was significantly larger than PET gross tumor volume was consistent with some reports describing radiation treatment planning for humans with head and neck squamous cell carcinoma. In one of those studies, it was concluded that 18F-FDG-PET/CT was the more accurate imaging tool compared to CT/MRI, even though gross tumor volumes derived from PET were significantly larger than surgically excised tumor volumes. In our feline study, a reference standard such as a surgically or microscopically determined tumor volume was not available as all cats were treated with radiation alone. We also did not measure gross tumor volumes more than one time using more than one observer. Therefore possible effects of outside factors such as thresholding methods, resolution of the imaging device, size of the patient, and degree of contrast enhancement on calculated gross tumor volumes remain unknown.

Both CT gross tumor volumes and PET gross tumor volumes in the lingual/laryngeal group of cats tended to be smaller than gross tumor volumes in the mandibular/maxillary group. While not statistically significant, this difference between two tumor groups may warrant further investigation in terms of the usefulness of this imaging modality for radiation therapy planning of small anatomic regions. It is possible that 18F-FDG PET could overestimate the metabolic volume more when a tumor is small due to PET imaging’s relatively poor spatial resolution compared to CT and MRI. The in-plane and longitudinal resolution at best was 5 mm with our system. The effect of resolution could be especially exaggerated when determining tumor volumes for anatomic structures of the
feline head that differ greatly in size (i.e., maxilla/mandible compared to the smaller tongue). Also, a hypermetabolic region reconstructed by the PET system could extend into the air cavity adjacent to an oral mass due to the increased range of positrons in air and could require modification of contours drawn for oral tumors (Fig. 3). The current resolution limits of PET as well as the presence of hypermetabolic peritumor reactions could therefore preclude its use for determining accurate margins for small tumors and small patients. In spite of this limitation, we propose that PET’s greatest advantage may be in providing increased conspicuity of hypermetabolic regions outside of the primary tumor. In our study, the mismatch fraction between PET and CT gross tumor volumes was significantly larger for the lingual/laryngeal group of cats. Patients with lingual/laryngeal squamous cell carcinoma had 18F-FDG avid regions that were not overtly abnormal on postcontrast CT images (Fig. 4). In cats, as in people, there is high vascularity in the lingual/sublingual area. This high vascularity can make accurate differentiation of contrast enhanced abnormal regions challenging. A recent study describing the usefulness of CT for characterizing feline oral squamous cell carcinoma reported that this tumor showed varying types of contrast enhancement patterns, mass effect and a high frequency of adjacent osteolysis and that CT examination more accurately identified mass extension than radiographs. Our findings were discordant with this previous study in that we encountered feline oral squamous cell carcinoma patients without direct bone involvement, with minimal contrast enhancement and with minimal mass effect. It is therefore possible that, depending on the location, CT examination alone may have accurately
detected tumoral changes in one area but missed changes in other parts of the same tumor. It is also possible that, because $^{18}$F-FDG tracers are not cancer-specific, some of the hypermetabolic regions detected in PET scans of our cats could have contained a mixture of tumor cells and reactive tissues. Full volume histopathology was not performed in any of our cats. However, even in a terminal study, it would be difficult to perfectly correlate imaging and histopathological findings on a millimeter or submillimeter level and to exactly confirm the cell composition of a given area seen on images.

The lower specificity of $^{18}$F-FDG can also make interpretation of standardized uptake values challenging. In human oncology studies, standardized uptake value has been reported to be a valuable tool for certain tumor types to distinguish tumor grades, benign from metabolic tumors, and to quantitatively assess treatment response.$^{1,3,13,35}$ Many reports have attempted to find an optimum protocol for standardized uptake value thresholding and delineating radiation therapy target volumes for human cancer patients, but currently there are no widely accepted, standardized methods.$^{4,36}$ For that reason, we chose to define the volumes subjectively using a consensus of two observers and to determine standardized uptake value threshold levels individually for each patient instead of using an arbitrary, fixed, universal standardized uptake value threshold. This was more time consuming, but the goal was to use a technique that could still be simply implemented in a clinical setting.

Clinically, PET imaging has other drawbacks that may limit its widespread use in feline oral squamous cell carcinoma patients. First, the protocol for PET/CT imaging requires a prolonged period of anesthesia. This is an important consideration given that many feline squamous cell carcinoma patients are debilitated, geriatric, and may have concurrent medical issues such as renal failure. Second, radioisotopes and imaging equipment are not widely available. Third, the cost of examination is high and may be prohibitive for some feline owners.

In conclusion, although there are some important limitations of $^{18}$F-FDG PET for stereotactic radiation therapy planning in cats with oral squamous cell carcinoma, this imaging modality allowed us to detect potential tumoral regions that were not detected using contrast-enhanced CT, especially in the patients with lingual/laryngeal squamous cell carcinoma. Until a cancer-specific tracer is developed and spatial resolution is improved, we recommend combining results of postcontrast CT and $^{18}$F-FDG PET imaging with results of clinical examination for stereotactic radiation therapy planning in order to include all potential tumor regions within the treatment volume. Future, controlled prospective studies are needed to determine whether radiation therapy treatments based on $^{18}$F-FDG PET imaging yield increased survival times and success rates of local tumor control in feline oral squamous cell carcinoma patients.

ACKNOWLEDGMENT

The authors would like to thank Morris Animal Foundation, the Winn Feline Foundation, the Department of Environmental and Radiological Sciences, and CSU Animal Cancer Center for financial support. The authors would also like to thank Dr. Ann Hess for statistical analysis; Ms. Billie Arceneaux and Mr. Jeff Stewart for technical support in PET/CT imaging; and Drs. Jenna Burton, Kelly Carlsten, and Kristen Weishaar for clinical trials coordination.

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