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A Pilot Trial Evaluating Zoledronic Acid Induced Changes in [¹⁸F]FMAU-Positron Emission Tomography Imaging of Bone Metastases in Prostate Cancer

Ulka N. Vaishampayan¹, Omid S. Tehrani², Jawana M. Lawhorn-Crews¹, Lance K. Heilbrun¹, Kimberlee Dobson¹, Daryn Smith¹, Brenda Dickow¹, Anthony F. Shields¹

¹Karmanos Cancer Institute, Wayne State University, 4233 Hudson Weber Cancer Center, 4100 John R, Detroit, MI, 48201, USA

²University of California San Francisco, Fresno, CA, USA

Abstract

Purpose: We conducted a pilot trial utilizing [¹⁸F]FMAU [1-(2'-deoxy-2'-[¹⁸F]fluoro-β-D-arabinofuranosyl) thymine] as a tumor tracer in positron emission tomography (PET) and evaluated its reproducibility, and changes in maximum and peak standardized uptake value (SUV_{max} and SUV_{peak}) with zoledronic acid treatment in castrate resistant prostate cancer (CRPC) patients with bone metastases (BM).

Procedures: Eligible patients had CRPC with radiographic evidence of BM and creatinine clearance >30 ml/min. Two baseline [¹⁸F]FMAU-PET scans (about 1 week apart, range 2–12 days) were obtained for testing reproducibility. Zoledronic acid 4 mg was infused over 15 min within 1 week after second scan and a third PET scan was obtained 7 days later. The bony lesion with the highest uptake on the first scan was compared with later scans. Bone turnover markers and prostate-specific antigen (PSA) were obtained pre- and post-therapy. PET response was defined as decline in SUV_{mean} of 15 % after zoledronic acid.

Results: Eleven patients were evaluated, median age was 65 years, five were African-American and six were Caucasian, and median PSA level was 36.3 ng/ml (range 1.0–1209.3). Notably, the range of absolute percent SUV_{max} changes varied between 0.77 and 54.7, and only nine measurements were greater than one (1.09–2.19). Zoledronic acid did not appreciably change FMAU uptake. No clinical response was noted. Urine N-telopeptide (NTx) was markedly decreased in all patients after zoledronic acid and serum bone-specific alkaline phosphatase (BSAP) registered a modest change. Urine NTx correlated more closely with SUV_{max} than serum BSAP.

Conclusions: FMAU tracer was able to detect bone metastases in CRPC patients but uptake was highly variable in bony lesions. Zoledronic acid did not produce an appreciable change in scans. Future investigations of FMAU tracer as a marker of early response in CRPC is recommended.

Correspondence to: Ulka Vaishampayan; vaishamu@karmanos.org.

Conflict of Interest

The authors declare that they have no conflict of interest.

Keywords

Castrate resistant prostate cancer; Zoledronic acid; Prostate-specific antigen; Positron emission tomography; Imaging

Introduction

Eighty to ninety percent of patients with metastatic prostate cancer have bone involvement as the only site of metastases. Zoledronic acid is an approved treatment for castrate resistant prostate cancer (CRPC) with bone metastases (BM). It decreases skeletal-related events such as bone pain, and the risk of pathologic fractures or neuropathic compression [1, 2]. Zoledronate has shown in vitro antitumor activity, but clinical antitumor efficacy in prostate cancer has not been observed [3, 4]. ^{99m}Tc -labeled diphosphonate bone scan is the most widely used imaging technique for detection of BM. Newer techniques such as sodium [^{18}F]fluoride positron emission tomography (PET) imaging are highly sensitive in detecting BM in CRPC [5–7]. Both techniques measure the bone turn over and remodeling instead of the tumor metabolic activity. 2-Deoxy-2-[^{18}F]fluoro-D-glucose (FDG) has low uptake in CRPC and has limited value in detecting BM [8]. [^{18}F]FMAU [(1-(2'-deoxy-2'-[^{18}F]fluoro- β -D-arabinofuranosyl) thymine], a thymidine analog, is a novel tracer for PET imaging and is retained by mitochondrial thymidine kinase. We have previously demonstrated that [^{18}F]FMAU-PET can successfully detect BM in CRPC [9, 10]. During the development of this tracer for evaluating response, it would be critical to assess the impact of the other supportive care medications on the imaging.

Bisphosphonates such as zoledronic acid comprise a routinely used agent in CRPC with bone metastases. Zoledronic acid is frequently used in conjunction with other anticancer therapies in CRPC and has demonstrated lack of response or survival benefit. Pilot data from this proposed trial was to help collect data regarding reproducibility of PET scans in metastatic prostate cancer as well as evaluate the ability of PET scans to detect therapeutic response. If the trial demonstrated potential utility of PET scans in detecting therapeutic effect, then further investigation would be proposed to validate the findings for clinical application. FMAU scanning is an attractive option for early detection of response or clinical benefit for CRPC with bone metastases.

We proposed the evaluation of zoledronic acid affecting FMAU to assess the impact of this therapy. We conducted an open-label pilot study to evaluate reproducibility and changes induced by zoledronic acid in maximum and peak standardized uptake values (SUV_{max}, SUV_{peak}) obtained on [^{18}F]FMAU-PET imaging in patients with BM from CRPC.

Methods

Patients and Treatment

Eligible patients had evidence of CRPC with BM and disease progression as determined by rising prostate-specific antigen (PSA) (at least two values at least 1 week apart) or by new lesions identified clinically or on scans, or by progression of existing lesions on computed tomography (CT scan) or magnetic resonance imaging (MRI). Patients had to have a

minimum creatinine clearance of 60 ml/min calculated by the Cockcroft-Gault method and measured within 2 weeks of registration. Institutional Review Board approval was obtained annually and all patients reviewed and signed an informed consent for the study. Two pre-therapy [^{18}F]FMAU-PET scans were obtained and therapy was started within 7 days of the second scan. Zoledronic acid at a dose of 4 mg was administered intravenously over 15 min and post-treatment PET scans were obtained 1 week post-treatment.

Radiochemistry and Imaging

The methods were described in our prior publication [10]. Briefly, [^{18}F]FMAU was synthesized with purity greater than 98 % and specific activity greater than 111 GBq/mol as previously described. After positioning the patient on the PET/CT machine (GE, DSTE Discovery PET/CT, 2006), an intravenous dose of [^{18}F]FMAU (mean 9.35, range 4.1–10.17 mCi) was given. An attenuation scan, followed by tomographic imaging and venous blood sampling, was obtained after injection. After the [^{18}F]FMAU infusion, static images were obtained in two consecutive periods, over the first 5 min and then from 5 to 11 min post-injection over the thorax extending into the pelvic region. This was followed by another attenuation and static PET imaging of the neck to pelvic region (whole body scan), followed by CT imaging. Images were analyzed by OsiriX (v.2.7.5.32; University of California Los Angeles; 2004). SUV were used to measure the uptake in static images collected between 5 and 11 min post-injection. Whole body bone scans were done on all patients and were reviewed, before selecting bone lesions for SUV analysis on [^{18}F]FMAU scans. In patients who did not get all static images for comparison, whole body PET scans were used to measure tumor uptake. SUVmax was defined as the pixel with the highest SUV, and SUVpeak was defined as the average SUV over a circle of 1-cm radius around the pixel with the highest SUV. Background activity was measured as SUV of the subcutaneous fat with average SUV on a circle with radius 2cm. Due to the diffuse involvement of the bone by CRPC, active lesions were found by screening the lesions in the first scan. The lesions with the highest SUVmax were identified by one operator by screening through different planes and confirmed by another operator. SUVmax and SUVpeak were measured on the target lesion in three consecutive planes, and the average measurement of the three planes for each parameter were used for further analysis. The same lesions identified as the target lesions were followed for comparison in the second and third PET images.

Laboratory Studies

Serum markers, including serum bone specific alkaline phosphatase (BSAP) and N-terminal telopeptide of collagen type I (NTx), were assessed pre- and post-treatment. NTx was also assessed from 24-h urine collection sample using the Vitros ECI Immunodiagnostic System competitive assay (Johnson & Johnson Ortho-Clinical Diagnostics, Raritan, NJ). Urinary marker levels were normalized relative to urinary creatinine levels. Serum BSAP levels were assessed using a chemical inhibition and differential inactivation assay. Urine and serum markers were measured pre-therapy, and 1 and 5 weeks post-zoledronic acid.

Statistical Design and Analysis

PET response was pre-defined as a decrease in SUV max of 15 % from scan 1 to scan 3, and from scan 2 to scan 3. The primary statistical objective was to estimate the PET response rate with 80 % confidence. The sample size of 11 patients was the minimum required to have an exact, lower one-sided 80 % confidence limit >0.10 , assuming there were as few as two PET responders. That would provide evidence that the true, unknown PET response rate was $>10\%$. Point estimates and Wilson type two-sided 80 % confidence interval (CI) estimates of PET response rates were calculated.

The secondary statistical objectives were to evaluate the changes in serum BSAP and urine NTx which are reported descriptively. Pearson correlation coefficients (r) and R^2 values were calculated between the change in SUV from scan 1 to scan 3, and the change in SUV from scan 2 to scan 3. Pearson correlation coefficients (r) were calculated between each biomarker and each SUV variable. Two-sided 80 % CI estimates for r were calculated from the bias-adjusted version of the Fisher's Z transformation of r . As this was an exploratory investigation in a small pilot study, only point and CI estimates were calculated, and no statistical tests or p values were calculated. There were 72 pairs of variables so analyzed: (3 SUV time points [described earlier] + 3 SUV percent changes [among each pair of the 3 time points]) * (2 SUV measurements [mean, max]) * (2 biomarkers [urine NTx, serum BSAP]) * (3 biomarker measurements [pre-Zometa therapy, post-Zometa therapy, pre-/post-percent change]) = $6 \times 2 \times 2 \times 3 = 72$ pairs. Point and CI estimates of some of the stronger correlations are reported in a table. The source data for one such relationship pair were illustrated graphically in a scatterplot with an overlay of a linear regression model, along with its parabolic 80 % CI's for predicting the mean SUV and for predicting a single SUV value (Fig. 4).

Results

Patient Characteristics

Characteristics of the patients in this study are summarized in Table 1. Eleven of the 12 eligible patients were evaluable; one patient withdrew consent. Median age was 65 years (55–71 years), five patients were African-American and six were Caucasians. All patients had bone metastases and two had visceral, and two had soft tissue or lymph node metastases. At the time of enrollment, six patients had progression by rising PSA levels; four had new bone lesions on bone scans showing progression of the disease, and one had progression in the form of tumor invading spinal canal.

Median PSA level was 36.3 ng/ml (range 1–1209.3 ng/ml) and median baseline creatinine clearance was 105 ml/min (range 73.29–179.31 ml/min). No clinical response was noted in the study duration and three patients showed minimal decline in PSA; $<1\%$ in two patients and 15 % in one patient. All other patients demonstrated a PSA progression. For completeness (given the small size of our pilot study), the individual data values of PSA, biomarkers, PET scan location, and SUV measurements are presented in Table 1.

Image Analysis

Specifics of tumor imaging are provided in Table 2. Six tumors had persistent SUVmax above two, and only one had consistent values higher than three. Changes in tumor uptake were measured both by absolute and relative (i.e., percentage) changes between all three pairs of scans (33 comparisons). The range of SUVmax values were between 1.09 and 7.57, with only nine measurements from four patients above three (range 3.07 to 7.57). Figs. 1 and 2 show the examples of [^{18}F]FMAU uptake visualized on PET imaging scans of bone lesions.

The median absolute difference in SUVpeak between scans 1 and 2 was 9.3 % (range 1.2 to 57.0 %). The median absolute difference in SUVpeak between scans 2 and 3 was 15.0 % (range 0.9 to 30.0 %). Only one patient had a decrease in SUVpeak more than 15 % which was predefined as a positive response in the protocol. The patient with the >15 % decline was treated with dexamethasone (patient 2).

As shown in Table 1, tumor uptake before zoledronic acid treatment showed variable pre-therapy uptake (SUVmax median absolute change 8.2 %; range 1.6 to 50.2 %). Changes in tumor uptake were calculated between the third scan and each of the pre-therapy scans. The median absolute change in tumor SUVmax between scans 1 and 3 was 11.2 % (range 0.8 to 54.7 %). The median absolute change in tumor SUVmax difference between scans 2 and 3 was 14.5 % (range 1.2 to 33.5 %). There was a correlation between percent change of scan 1 and scan 2 versus scan 1 and scan 3 with ($R^2 = 0.79$) and (P value = 0.0003) (Fig. 3).

Bone Turnover Markers

Ten patients had samples collected for pre- and post-treatment serum BSAP and all showed an increase in levels. Urine NTx was decreased in all seven patients who had pre- and post-samples collected (median decrease of 57 % compared to pre-treatment levels (range 20–82 % decline)). Of the 72 Pearson correlations (r) calculated between a bone resorption marker and an SUV variable, 8 such r values had an 80 % CI that excluded zero (Table 2). Overall, urine NTx seemed somewhat more correlated with SUV (either SUVmean or SUVmax, or a percent change in SUVmean) than did serum BSAP. BSAP was moderately correlated only with percent change in SUV. Pre-/post-Zometa therapy, percent change in BSAP was the only *negative* moderate correlation with (percent change in) SUV.

The strongest r value (0.77) involving urine NTx was in relationship with SUVmax at scan 1 (Fig. 4). The one patient with a very high urine NTx level (and highest SUVmax at scan 1) might have been a data point exerting undue influence on the correlation analysis. As a sensitivity analysis, that point was excluded but there was only a small change in the resulting estimate of r 0.77 that became 0.70 (Fig. 4).

Discussion

The need for better imaging techniques to detect response in bone metastases is a current unmet need in metastatic prostate cancer. Technetium bone scans are inadequate to detect changes in bone tumors and in a disease with 90 % of the cancers presenting with bone only

metastases, this represents a serious handicap in the evaluation of novel therapies, or in therapeutic clinical decision-making of when to switch therapies. The PSA level is frequently used as a marker in metastatic CRPC, but for targeted therapies, or in poorly differentiated tumors that do not produce PSA, it is seriously flawed. [^{18}F]FMAU is a novel tracer that has the advantage of a non-renal route of excretion and hence can visualize the prostate and pelvic area clearly. In addition, it has the ability to specifically delineate bone tumors without demonstrating uptake in areas of degenerative joint disease or old injuries as noted in technetium bone scans and fluoride PET scans.

The clinical applications of this tracer in metastatic prostate cancer are promising. However, reproducibility has to be established and readings have to be standardized. Further validation testing is needed in the setting of effective therapies with established clinical response rates. Medications such as steroids may reduce inflammation and bone turnover and have an impact on the [^{18}F]FMAU PET scan findings. It is plausible that multiple medications, especially bisphosphonates or RANK ligand inhibitors that alter the bone turnover, can affect the vascular uptake and have unknown effects on the results of multiple imaging modalities which are used for therapeutic decisions. The importance of the above pilot study is that it revealed that [^{18}F]FMAU uptake is not altered by bisphosphonate therapy. This will enable detection of changes in [^{18}F]FMAU uptake that can be attributed to the anticancer therapy being evaluated. In addition, an interesting finding of correlation between the bone turnover markers such as baseline urine Ntx levels and the SUV max was noted; however, this was not seen with serum BSAP levels.

The main limitation of the study was the variability noted in pre-therapy tumor imaging. Reasons for this may be the heterogeneity of the tumors, different rates of disease progression, or use of medications such as dexamethasone. However, it is noteworthy that only six tumors had consistent SUVmax above two, and there was only one tumor with SUVmax more than three. Notably, the range of absolute percent SUVmax changes varied between 0.77 and 54.7, and only nine measurements were greater than one (1.09–2.19). These values show that tracer uptakes and inter-scan variations were close to the background noise level.

In conclusion, this study suggests that treatment with zoledronic acid in prostate cancer patients with bone metastases does not lead to an appreciable change in the [^{18}F]FMAU uptake on PET imaging.

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Fig. 1.

a Scan 1 and scan 2 [^{18}F] FMAU (PET/ CT) images were both obtained pre-therapy for reproducibility and **b** scan 3 image was obtained 1 week post-treatment of zoledronic acid. The right pelvic bone lesion (*black arrow*) with the highest SUVmax on scan 1 was obtained and compared to the same anatomic location on scans 2 and 3. The SUV max percent change is as follows: scan 1 vs scan 2 -23.3% , scan 2 vs scan 3 $+17.1\%$, and scan 1 vs scan 3 -10.2% .

Fig. 2.

a Scan 1 and scan 2 [^{18}F] FMAU (PET/ CT) images were obtained pre-therapy for reproducibility and **b** scan 3 image was obtained 1 week post-treatment of zoledronic acid. The right rib bone lesion (*black arrow*) with the highest SUVmax on scan 1 was obtained and compared to the same anatomic location on scans 2 and 3. The SUV max percent change is as follows: scan 1 vs scan 2 -50.1% , scan 2 vs scan 3 $+12.1\%$, and scan 1 vs scan 3 -44.0% .

Fig. 3. Correlation between percent changes in SUVpeak in scan 1 vs scan 2 and scan 1 vs scan 3 of index bone lesions in metastatic CRPC; $R^2 = 0.79$ and P value = 0.0003.

Fig. 4. Scatterplot of SUVmax from scan 1 vs pre-therapy urine NTx. The *solid line* is the linear regression model which is $\text{scan 1 SUVmax} = 2.0719 + 0.0039 \times \text{pre-therapy urine NTx}$. The *lines* at the outer edges of the shaded band define the 80 % confidence limits for the predicted mean of scan 1 SUVmax for a given value of pre-therapy urine NTx. The *dashed lines* define the 80 % prediction limits for a predicted individual value of scan 1 SUVmax for an individual patient with a given value of pre-therapy urine NTx.

Table 1.PSA, biomarker, PET scan location, and SUV data by individual patient. ($N = 11$)

Patient	PSA_preRx	PSA_postRx	BSAP_preRx	BSAP_postRx	NTx_preRx	NTx_postRx	Location of PET scan	SUV1 max	SUV2 max	SUV3 max	SUV1 peak	SUV2 peak	SUV3 peak
a_1	207.4	N/A	107	N/A	433	N/A	Pelvis bone	3.57	2.74	3.21	3.14	2.06	2.46
a_2	1209.3	797.1	24.5	35.3	157	46	Pelvis bone	3.23	3.07	2.08	2.96	2.73	1.93
a_3	1.0	2.9	15.5	17.5	21	9	Vertebra	1.59	1.46	1.24	1.48	1.36	1.16
4	25.2	35.6	11.7	11.4	35	N/A	Right ischium prostate	2.60	2.79	2.58	2.40	2.55	2.29
a_5	49.5	749.8	13.3	13.2	N/A	23	Marrow	6.03	6.47	7.57	5.38	6.02	6.99
6	12.8	17.5	7.2	9	22	7	Marrow	1.82	1.79	1.92	1.73	1.58	1.80
7	36.3	30.8	24.5	17.2	34	6	Rib	2.19	1.09	1.23	1.84	0.97	1.02
a_8	282.2	280	88.2	82.1	139	31	Femur	2.53	1.70	2.27	2.24	1.57	2.04
9	407.5	1154.4	32	33	N/A	99	Pelvis bone	2.30	3.11	3.56	1.78	2.79	3.25
10	21.6	64.1	18.1	15.4	18	8	Iliac crest	1.84	1.80	1.86	1.66	1.64	1.69
11	6.0	5.9	11.4	8.7	38	19	Rib	2.78	2.44	2.47	2.47	2.24	2.22

PreRx = before Zometa treatment. SUV1, SUV2, SUV3 measurement times are described in the "Methods" section

N/A not available

 a_i Patients received dexamethasone

Table 2.

Selected correlations between bone resorption markers and SUV levels

Marker	SUV measure	N	r	80 % LCL	80 % UCL
Urine NTx pre-therapy	SUVmax from scan 1	9	0.77	0.47	0.91
Urine NTx pre-therapy	SUVmean from scan 1	9	0.75	0.43	0.91
Urine NTx post-therapy	% change in SUVmean from scan 1 to scan 2	9	0.74	0.40	0.90
Urine NTx post-therapy	% change in SUVmean from scan 1 to scan 3	9	0.68	0.30	0.88
% change in urine NTx pre-/post-therapy	% change in SUVmean from scan 1 to scan 2	7	0.77	0.37	0.93
Serum BSAP pre-therapy	% change in SUVmax from scan 2 to scan 3	11	0.50	0.10	0.77
Serum BSAP pre-therapy	% change in SUVmean from scan 2 to scan 3	11	0.52	0.12	0.77
% change in serum BSAP pre-/post-therapy	% change in SUVmax from scan 2 to scan 3	10	-0.55	-0.80	-0.13

From a total of 72 correlations. N varies due to occasional missing data

r Pearson's correlation coefficient after Fisher's Z transformation and bias adjustment. LCL lower confidence limit of the two-sided 80 % confidence interval for r; UCL upper confidence limit of the two-sided 80 % confidence interval for r