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ORIGINAL ARTICLE

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Imaging the pharmacokinetics of [F-18]FAU in patients with tumors: PET studies

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Abstract *Purpose*: FAU (1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl) uracil) can be phosphorylated by thymidine kinase, methylated by thymidylate synthase, followed by DNA incorporation and thus functions as a DNA synthesis inhibitor. This first-in-human study of [F-18]FAU was conducted in cancer patients to determine its suitability for imaging and also to understand its pharmacokinetics as a potential antineoplastic agent. *Methods*: Six patients with colorectal ($n=3$) or breast cancer ($n=3$) were imaged with [F-18]FAU. Serial blood and urine samples were analyzed using HPLC to determine the clearance and metabolites. *Results*: Imaging showed that [F-18]FAU was concentrated in breast tumors and a lymph node metastasis (tumor-to-normal-breast-tissue-ratio 3.7–4.7). FAU retention in breast tumors was significantly higher than in normal breast tissues at 60 min and retained in tumor over 2.5 h post-injection. FAU was not retained above background in colorectal tumors. Increased activity was seen in the kidney and urinary bladder due to excretion. Decreased activity was seen in the bone marrow with a mean SUV 0.6. Over 95% of activity in the blood and urine was present as intact [F-18]FAU at the end of the study. *Conclusions*: Increased [F-18]FAU retention was shown in the breast tumors but not in colorectal tumors. The increased retention of FAU in the breast compared to bone marrow indicates that FAU may be useful as an unlabeled antineoplastic agent. The low retention in the marrow indicates that unlabeled FAU might lead to little marrow toxicity; however, the images were not of high contrast to consider FAU for diagnostic clinical imaging.

Keywords FAU · PET · Thymidine kinase · Proliferation

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Introduction

Positron emission tomography (PET) plays an important role in measuring drug pharmacokinetics and pharmacodynamics since it can directly reveal the distribution and the functional effects of a drug in a non-invasive manner [1–4]. Apart from elucidating such pharmacologic information, imaging radioisotope labeled chemotherapeutic agents can also assist in target selection and target validation and hence aid in determining which tumors readily take up and retain the drugs [5–9].

FAU (1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl) uracil) is being tested as a potential antineoplastic agent and an imaging agent for PET. Previous in vitro studies have demonstrated that FAU is phosphorylated by thymidine kinase (TK), methylated by thymidylate synthase (TS), and incorporated into DNA inducing cell growth inhibition. The inhibition of cell growth correlates with the fraction of FAU that is used to replace thymidine in DNA [10]. Thymidylate synthase is one of the common targets in the antineoplastic agent development and TS inhibitors are effective in cancer treatment [11]. However, many tumors are eventually resistant to the TS inhibitors, such as 5-fluorouracil (5FU), due to an over-expressed TS level [10]. While increased levels of TS may interfere with the efficacy of

thymidylate synthase inhibitors, such as 5FU, it can be utilized to enhance FAU methylation and DNA incorporation. Compared to non-proliferating tissues, proliferating tissue expresses increased levels of TS [12–17]. Therefore, labeling FAU with F-18 may be useful in imaging tumors and understanding the metabolism and pharmacokinetics of this potential chemotherapeutic agent. Our previous study in normal dogs demonstrated that the normal proliferating tissue, such as bone marrow, had much less retention of [F-18]FAU than the non-proliferating tissues [18]. Nevertheless, a pilot study in patients with tumors is still necessary to determine its suitability for imaging accumulation in tumors. In addition, this first-in-human study can also guide future studies that may evaluate the kinetics and mechanism of unlabeled FAU as an antineoplastic agent and ensure that FAU functions in a predictable fashion.

Materials and methods

Subjects

The human subject protocol for this study was reviewed and approved by the Human Investigation Committee of Wayne State University and Radioactive Drug Research Committee of Michigan (RDRC). All patients gave a written informed consent prior to each study. A total of six patients were studied, three of them had colorectal cancer (two with liver metastasis and one locally advanced rectal cancer) and the other three had breast cancer (two locally advanced primary breast cancers and one with lymph node metastasis).

PET image acquisition and image data analysis

We synthesized the [F-18]FAU as previously described [19] and intravenously injected 107.3–377.4 MBq of [F-18]FAU over 1 min into six patients. The dosages were determined by the radiation dosimetry estimates calculated from our prior animal biodistribution studies, which will be published separately. A transmission scan was acquired to correct photon attenuation for 15 min prior to tracer injection. Dynamic PET imaging was performed for 60 min with the field-of-view (FOV) over the area of the largest tumors located in the breast or upper abdomen followed by a whole body scan including six to seven bed positions using a Siemens Exact/HR tomograph. The whole body scans were started about 65 min post-injection and the total scan time was approximately 2.5 h. The [F-18]FAU retention in the region of interest (ROI) was quantified using the standardized uptake value (SUV) which is defined as the activity per gram in the tissue divided by the total injected activity per gram of the whole body weight. ROIs were defined from a summed image of 30–60 min over all dynamic frames at the location of the tumors and the normal tissues such as breast, liver, marrow, kidney, muscle, etc. Time–activity curves for all the defined ROIs were generated from the dynamic sequence (60 min) combining one additional time point beyond 60 min from the whole body scans for up to 2.5 h. Both the dynamic image sequence and the whole body scan were decay-corrected to the start-times of the scan.

Blood samples were obtained from an intravenous catheter, different from the one used for tracer injection, at serial time points between 0.25 min and 60 min along with one additional time point approximately 150 min post-injection and some blood samples (5, 10, 30, 60 and 150 min) were analyzed by HPLC for metabolites [18]. The urine samples obtained at 60 min and approximately 150 min post-injection were also analyzed for metabolites using HPLC. Meanwhile, all of the blood and urine samples were measured for the total radioactivity with a spectrometer (Packard) and quantified using SUV.

Results

FAU metabolism and clearance in patients with tumors

We studied six patients with tumors using [F-18]FAU and PET. The [F-18]FAU blood retention and clearance is demonstrated in Fig. 1. The activity of [F-18]FAU in the venous blood reached a mean SUV of 2.0 at 4 min post-injection and gradually decreased to 1.7 at 60 min and 1.4 at the end of the study (about 150 min post-injection). The activity in the urine ranged from an SUV of 12.5 to 100.2 at 60 min post-injection and 22.1–78.4 about 150 min post-injection. An average 10.4% (range, 3.0–22.9%) of the total injected radioactivity was excreted from urine at 60 min post-injection and an additional 7.4% (range, 2–13%) of total injected radioactivity was excreted at the end of each study (about 150 min post-injection). HPLC analysis demonstrates that over 95% of the activity in the blood and urine at 60 min and about 150 min was present as intact

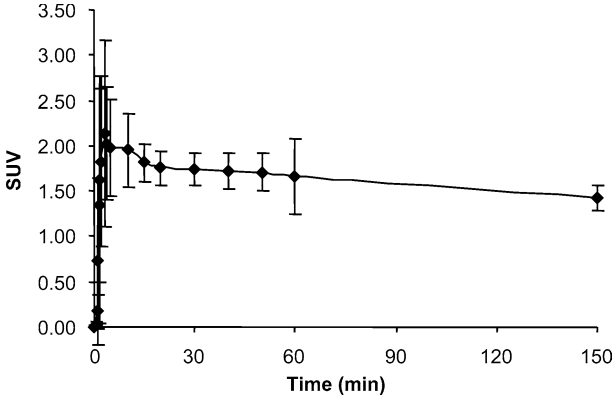

Fig. 1 [F-18]FAU blood time–activity curves in the patients with tumors (mean and standard deviations, $n = 6$)

FAU, which demonstrates that FAU is resistant to degradation in patients.

FAU PET characteristics and pharmacokinetics in tumors

We imaged three patients with breast cancer, two of which had locally advanced primary breast cancer and the third one had lymph node metastasis. We were able to visualize all the breast cancers with an increased FAU retention (SUV 1.2–1.5, background SUV 0.3). The breast tumor to background ratio 3.7–4.7 in the whole body image represented the [F-18]FAU accumulation from 70 min to 150 min post-injection (Fig. 2). We also imaged three patients with 5FU refractory colorectal cancer and liver metastasis who underwent colorectal cancer resection years prior to the PET scan. The colon cancers were not visible above background in the liver (mean SUV 2.0). The lung metastasis in one patient with colon cancer was not visualized with FAU but was seen with FDG 1 month later. In addition, low FAU retention in the bone marrow was noted in all six patients with a mean SUV 0.55 (range, 0.28–0.78) (Table 1). The marrow was seen as a negative image with higher retention in surrounding tissues. The mean marrow to muscle background ratio was 0.33 (range, 0.17–0.51) (Table 1). Furthermore, increased physiological uptake, due to the metabolism and the renal excretion of [F-18]FAU, could also be seen in the liver and kidneys with a mean SUV 2 and 3, respectively. Consistent with high radioactivity in the urine, increased activity was also observed in the urinary bladder with a SUV ranging 37.4–106.3. Finally, the radioactivity in the small intestine and stomach could not be distinguished from that in the background.

We were able to measure the time course of [F-18]FAU retention in two breast tumors and in the normal breast tissues. We also obtained time–activity curves for normal bone marrow from five patients. These dynamic data demonstrate that [F-18]FAU approaches the activity peak in the tumor with SUV 3.9 at 3 min and decreases to SUV 1.4 at 60 min, followed by a slightly increased FAU retention at 109 min post-injection with a SUV 1.5, which is 4.3-fold higher than in the normal breast tissue (Fig. 3, Table 1). In addition, FAU retention in the breast tumors was 2.4-fold higher than in the normal bone marrow (Table 1).

Discussion

FAU preferentially accumulates in the tumor but not normal proliferating tissues

While the physiological proliferating tissues such as bone marrow demonstrated a low FAU retention, all breast tumors, but none of the colorectal tumors, could be visualized with a significantly increased FAU retention (Figs. 2, 4). Recent in vitro studies demonstrated that FdUrd, a selective TS inhibitor, can decrease the incorporation of FAU into DNA, whereas increase the incorporation of FMAU into DNA through the salvage pathway. This evidence indicate that TS is a crucial factor for determining the accumulation of FAU into DNA. In addition, an in vivo study from the same group also showed that the tumor with a higher TS activity led to a higher incorporation of FAU into DNA [20]. Combined with the observation from previous studies that demonstrated increased levels of TS in breast cancer [21, 22], it seems reasonable to state that the visualization of [F-18]FAU in breast tumors in our human studies may reflect an increased TS expression to a certain degree. However, the increased uptake of [F-18]FAU relative to normal breast may be also due to increased extracellular volume, perfusion and vascular permeability in the tumor tissue. Therefore, further measurements need to be conducted to determine how much these non-specific factors can contribute to the level of FAU accumulation in the tumor. Nevertheless, we might conclude that labeling FAU with F-18 and PET may be useful in imaging tumors with a high TS expression and assessing the level of TS activity.

In addition, our previous study in the normal dogs also demonstrated a low FAU retention in the normal proliferating tissues such as bone marrow. These features indicate that FAU preferably accumulates in the tumors but not in the normal proliferating tissues, which might lead to tumor cytotoxicity with little harmful effect on the normal proliferating tissues. Therefore, unlabeled FAU deserves consideration as an

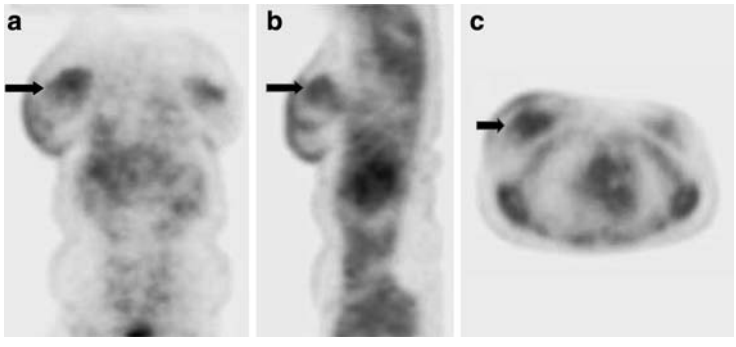

Fig. 2 Whole body image of a patient with locally advanced breast cancer. The image was obtained 70–150 min after the injection of 10.2 mCi of [F-18]FAU. **a** Coronal view; **b** Sagittal view; **c** transversal view. The *arrows* point to the tumor mass

Table 1 Tumor and bone marrow SUV summary

Tumor type	Tumor SUV	Normal breast SUV	Bone marrow SUV	Muscle SUV
Colorectal cancer metastasis	Not applicable	Not applicable	0.53	1.49
Rectal cancer	Not applicable	Not applicable	0.60	1.96
Colorectal cancer metastasis	Not applicable	Not applicable	0.28	1.67
Breast cancer	1.49 (breast)	0.30	0.46	1.19
Breast cancer	1.15 (breast)	0.30	0.65	2.08
Breast cancer, lymph metastasis	1.25 (lymph node)	Not applicable	0.78	1.52

antineoplastic agent for evaluation in breast cancer. Although the dynamic data demonstrates that FAU was gradually cleared from the tumors and the normal tissues after a quick uptake, FAU retention in the tumor was slightly increased from SUV 1.4 at 60 min to 1.5 at 109 min post- injection, which were fivefold higher than in the normal breast tissue (Fig. 3). Combined with the results obtained from previous studies by our group and others [10, 18, 20, 23], these data indicate that FAU can be incorporated into DNA and retained in the tumor cells although the phosphorylation and DNA incorporation are low and the rate is slow [18].

Apart from the importance of preferential retention in breast cancer, low retention in the normal proliferating tissues makes FAU particularly attractive. Cancer chemotherapy strives to cause a lethal cytotoxic lesion that can arrest tumor progression. Ideal anticancer drugs would specifically eradicate cancer cells without harming the normal tissues. Unfortunately, currently available anticancer drugs do not specifically target neoplastic cells, but rather affect all proliferating cells including the normal proliferating tissues such as bone marrow and the epithelium of the gastrointestinal tract. The actions on the normal proliferating tissues can lead to severe marrow toxicity and an adverse GI response and thus can be lethal to the patients. While work continues on developing effective targets and enhancing

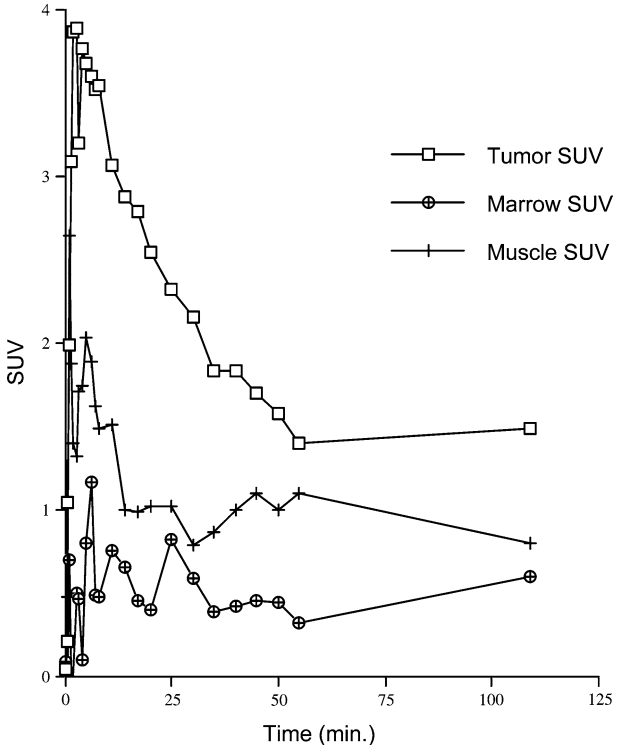

Fig. 3 [F-18]FAU retention time–activity curve from a patient with breast cancer, along with bone marrow and background curves

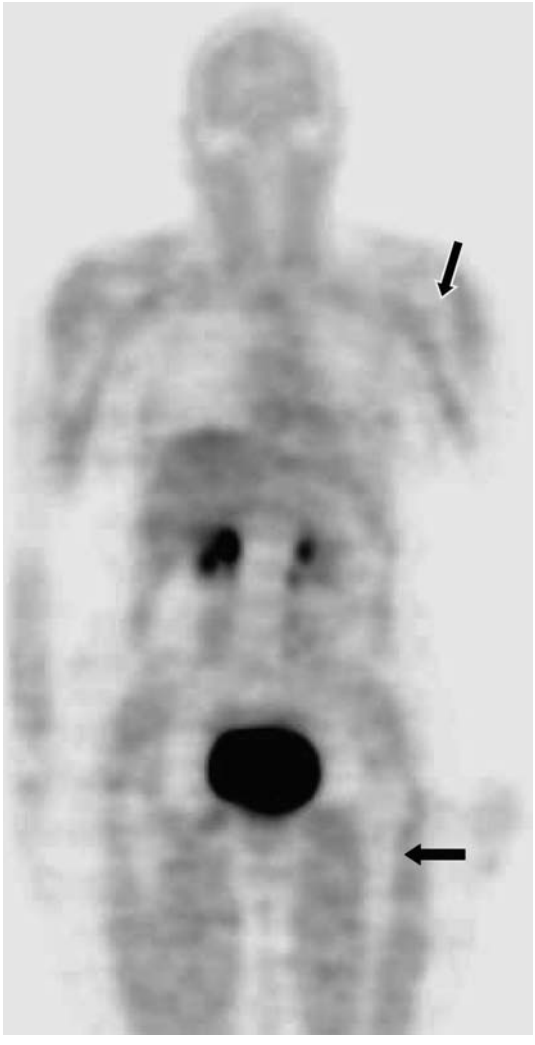

Fig. 4 Whole body image of a patient with colorectal cancer and liver metastasis. The image was obtained 70–150 min post injection after administering 2.9 mCi of [F-18]FAU. The *arrows* point to the low uptake of [F-18]FAU in bone marrow

the cytotoxicity to eradicate the tumors, an agent with little toxicity to the normal proliferating tissue would be particularly beneficial for cancer treatment. Our current results, along with the results obtained from previous dog PET imaging study, demonstrate low FAU retention in the normal marrow, which indicates that (unlabeled) FAU might lead to little marrow toxicity. In addition, our data also shows that the activity in the small intestine and stomach cannot be distinguished from background, which suggests that (unlabeled) FAU might cause little adverse GI tract response during the chemotherapy. The reason why FAU retention decreased and was lower in the bone marrow than that in the muscle cannot be readily explained by differences in TK1 since muscle is typically low in TK1. Nucleosides are hydrophilic molecules and require specialized transport proteins for permeation of cell membranes. Therefore, it is very much possible that variable FAU retention in tissues results from different nucleoside transporters expressed in the cell membrane and thus different rates of uptake and/or efflux. Therefore, alterations in transport need to be further explored to explain exclusion of FAU by the marrow.

Furthermore, we were unable to visualize any tumors in the colon or liver or rectum, which indicates that [F-18]FAU is not a good imaging agent even for the tumor refractory to 5FU. It has been reported that colorectal tumors responding to 5FU have low gene expression level of dihydropyrimidine dehydrogenase (DPD), TS, and thymidine phosphorylase (TP). 5-fluorouracil resistance can either result from drug-induced over-expression of TS overcoming the inhibitory effect of 5FU or due to increased levels of DPD or/and TP, which enhance 5FU degradation [24–29]. In our study, the levels of TS, DPD and TP were not determined prior to the PET imaging study. Although we intended to select the 5FU refractory colorectal tumors with a high expression level of TS, the three colorectal tumor patients that we imaged might have a high expression of either DPD or TP or the combination of both rather than a high expression of TS leading to 5FU resistance. Among three colorectal cancer patients, two patients with liver metastasis had the original colon tumor removed a couple of years prior to the imaging study and the third patient had locally advanced rectal tumor at the time of the study. The high uptake of [F-18]FAU in the liver and urinary bladder might interfere with the visualization of the tumors in the liver and rectum, respectively. Nevertheless, further imaging of tumors with a documented increased TS level is needed in order to reach a definitive conclusion.

FAU is resistant to degradation and excreted into the urine in the patients with tumors

Our data demonstrate that FAU distributes to the whole body in a uniform manner with the exception of the liver, kidneys and urinary bladder. Increased FAU uptake was seen in the liver due to metabolism. Increased uptake could also be seen in the kidneys and urinary bladder, which indicates that FAU is mainly eliminated through renal excretion (Fig. 4, Table 1). Despite a high renal excretion, the radioactivity in the blood circulation declined slightly from 60 min to 150 min post-injection with a mean SUV 1.5, which is beneficial for prolonging the systemic exposure component of drug delivery. In addition, a mean 95% of the radioactivity in the blood and urine at 60 min was present as intact FAU, which indicates that FAU is resistant to degradation.

In summary, we have imaged six patients with tumors with [F-18]FAU and PET. All tumors in the breast have significantly increased FAU retention and are visualized with [F-18]FAU. The normal proliferating tissues such as bone marrow have a low FAU retention and are visualized as a negative tissue to background contrast. In accord with previous in vitro and in vivo studies, we conclude that (unlabeled) FAU can be considered as a good antineoplastic agent for treatment of breast cancer. Furthermore, given its low marrow retention, it might have little marrow toxicity, but this would clearly need to be confirmed in clinical trials. However, despite the fact that the FAU retention is significantly increased in the breast cancer, image contrast is not sufficiently high for its use in routine clinical applications.

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