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Comparison of *trans*-1-amino-3-[^{18}F]fluorocyclobutanecarboxylic acid (*anti*-[^{18}F]FACBC) accumulation in lymph node prostate cancer metastasis and lymphadenitis in rats

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ABSTRACT

Introduction: *Trans*-1-amino-3-[^{18}F]fluorocyclobutanecarboxylic acid (*anti*-[^{18}F]FACBC) is a positron emission tomography (PET) tracer used to visualize prostate cancer (PCa). In this study, we investigated the differences in *anti*-[^{18}F]FACBC accumulation between metastatic and inflamed lymph node (LN) lesions.
Methods: A PCa LN metastasis (PLM) model was developed by inoculating a rat PCa cell line, MAT-Ly-Lu-B2, into popliteal LNs of Copenhagen rats. Acute lymphadenitis (AL) was induced by injecting concanavalin A (Con A) into the hind footpad, and chronic lymphadenitis (CL) was induced by daily injection of Con A into the tissues surrounding the popliteal LNs for 2 weeks. Main lesions of all animal models were established in lumbar and/or inguinal LNs. Biodistribution and dynamic PET imaging data were acquired after tracer injection. T2-weighted magnetic resonance (MR) images were registered with PET images.
Results: In the biodistribution study, the uptake ratios of PLM-to-lymphadenitis in lesional lumbar and inguinal LNs were 0.97 − 1.57 and 1.47 − 2.08 at 15 and 60 min post-*anti*-[^{18}F]FACBC injection respectively. In PET imaging, the lesional lumbar LNs of CL and PLM, but not of AL, were visualized on *anti*-[^{18}F]FACBC-PET/MR fusion images without disturbance from radioactivity from urine, and the rank order of *anti*-[^{18}F]FACBC accumulation at 50 − 60 post-injection in lesional lumbar LNs was PLM > CL > AL.
Conclusions: *Anti*-[^{18}F]FACBC accumulation in LNs with PLM was higher than that in inflamed LNs.
Advances in knowledge: The study showed that although low but significant levels of *anti*-[^{18}F]FACBC uptake by chronic inflamed lesions might cause false-positives in *anti*-[^{18}F]FACBC-PET in some PCa patients, uptake of the tracer at acutely inflamed sites was minimal.
Implications for patient care: The findings of this study suggest the potential of *Anti*-[^{18}F]FACBC for distinguishing between tumors and acute inflammation in clinical practice.

1. Introduction

Prostate cancer (PCa) is the most common cancer in elderly men. In the United States, PCa is expected to account for 28% and 10%, respectively, of all cancer cases and deaths among men in 2013 [1]. These results indicate that PCa is highly prevalent among men but has relatively lower cancer-specific mortality risk than other cancers, e.g. lung cancer, which is the second most common cancer and has the highest mortality rate in the US [1].

While diagnosing PCa, it is important to determine whether the cancer has metastasized to other organs such as the lymph nodes (LNs) and bones, because this is the basis for the therapeutic strategy. According to Prostate Cancer Treatment (PDQ®) guidelines, radical treatments such as prostatectomy, external beam radiotherapy, and brachytherapy with or without hormonal therapy are administered to patients with localized PCa (stages I–III), while hormonal therapy is recommended for stage IV PCa patients with metastases [2]. Among PCa patients with clinically organ-confined cancer, 5–12% have metastases in regional LNs [3], and 10-year cancer specific survival rates after radical prostatectomy and pelvic LN dissection are worse in pelvic LN metastases patients [4].

Positron emission tomography (PET) with 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) can effectively detect primary tumors and their metastases. However, primary PCa imaging with [^{18}F]FDG is masked by high radioactivity in the urine due to rapid excretion of [^{18}F]FDG into the urinary bladder [5]. To overcome this, *trans*-1-amino-3-[^{18}F]fluorocyclobutanecarboxylic acid (*anti*-[^{18}F]FACBC), a synthetic amino acid PET tracer, has been used for PCa patients. *Anti*-[^{18}F]FACBC allows the visualization of cancer cells in the prostate bed and pelvic LNs [6–8] because of its relatively slow excretion into the urinary bladder. However, a recent clinical study showed no distinct separation between malignant and non-malignant sextants as

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ascertained by the standard uptake value (SUV) on *anti*-[^{18}F]FACBC-PET in some PCa patients [9]. To ascertain the reason, we previously conducted a series of in vitro experiments using rat inflammatory cells and a rat PCa cell line (MAT-Ly-Lu-B2) and demonstrated that *anti*-[^{18}F]FACBC is taken up by activated T and B lymphocytes as well as PCa cells, which could be the cause of the false-positives in PCa patients who undergo *anti*-[^{18}F]FACBC-PET [10]. However, the uptake of tracers in vivo could be different from that in vitro because of the complex interplay of many factors that exist in the body. Here, we compared the accumulation of *anti*-[^{18}F]FACBC in acute and chronic lymphadenitis with that in LN metastases of PCa using rat models.

2. Materials and methods

2.1. Chemicals

All reagents and tissue culture materials were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA) and Life Technologies Japan Ltd. (Tokyo, Japan) unless otherwise stated.

Anti-[^{18}F]FACBC was synthesized in our facility using an automated synthesis module based on the method by Nye et al. [11] with a minor modification: *syn*-1-*tert*-butylcarbamate-3-trifluoromethanesulfonoxy-cyclobutane-1-carboxylic ethyl ester was used instead of *syn*-1-*tert*-butylcarbamate-3-trifluoromethanesulfonoxy-cyclobutane-1-carboxylic methyl ester; after hydrolytic conversion of *anti*-1-tert-butylcarbamate-3-[^{18}F]fluoro-cyclobutane-1-carboxylic ethyl ester to *anti*-[^{18}F]FACBC, the alkaline solution, and then acidic solution was passed through an ion retardation resin. Finally, the radiochemical purity was >99.0%.

2.2. Animal models

All animal handling procedures and experimentation were conducted in accordance with the protocols approved by the committee on animal welfare at Nihon Medi-Physics Co. Ltd. Male Copenhagen rats (age, 7–13 weeks) were purchased from Japan SLC Inc. (Shizuoka, Japan) and used in the experiments. Animals were anesthetized with 1.5% isoflurane (Mylan Inc., Tokyo, Japan) or sodium thiopental (40 mg/kg; Mitsubishi Tanabe Pharma Corporation, Osaka, Japan).

MAT-Ly-Lu-B2, an androgen-independent rat PCa cell line, was obtained from the American Type Culture Collection and cultured as reported previously [12]. For the preparation of the PCa LN metastasis (PLM) model, MAT-Ly-Lu-B2 cells were suspended at 2.0×10^7 cells/mL in ice-cold phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} and mixed with the same volume of ice-cold Matrigel™ (Becton Dickinson, Franklin Lakes, NJ, USA); 5 μL cell suspension (5×10^4 cells) was injected into the popliteal LN of the right hind legs of rats. After 14–21 days of injection, the rats were used for biodistribution or PET/MR imaging experiments as described below.

We induced two types of lymphadenitis models: acute (AL) and chronic lymphadenitis (CL). For the preparation of the AL model, 0.1–0.2 mL of concanavalin A (Con A) dissolved at 15 mg/mL in ice-cold PBS was injected into the right hind leg footpad of rats (12 mg Con A/kg). At 12 h of Con A injection, the rats were used for experimentation. To prepare the CL model, Con A (20 mg/mL) in ice-cold PBS was mixed with the same volume of ice-cold Matrigel™, and 30–40 μL of the solution was injected into the surrounding tissues of the popliteal LN in the right hind legs of rats (2 mg Con A/kg). Rats received a Con A injection daily for 15 days and were used for experiments on the final Con A injection day.

After the experiments, the lumbar LNs and cysts were fixed in formalin, embedded in paraffin, cut into 3-μm-thick slices, and mounted on glass slides. The slices were then stained with hematoxylin–eosin by using standard methods, and pathological examination was performed with a BZ-3000 HS all-in-one fluorescence microscope (Keyence Corporation, Tokyo, Japan).

2.3. Biodistribution

Rats were fasted for 4–6 h prior to administering the tracer injection; 3.7–7.4 MBq *anti*-[^{18}F]FACBC was injected into the tail vein. Fifteen or sixty minutes after injection, the rats were sacrificed by drawing blood from the abdominal aorta. The right and left popliteal, lumbar, and inguinal LNs, and the gluteal muscle and bladder containing urine were dissected and weighed. In CL rats, a cyst formed at the Con A injected site was also separated from the surrounding tissues. Tissue radioactivity was measured using a single channel gamma counter (Ohyo Koken Kogyo Co. Ltd., Tokyo, Japan). Tracer excretion into urine and tracer accumulation in tissues were represented as the percentage of the injected dose (%ID) and %ID per gram of tissue (%ID/g), respectively.

2.4. Magnetic resonance imaging

To determine morphological and positional characteristics of the lumbar and inguinal LNs, rats were anesthetized with 1–2% isoflurane in 70% nitrous oxide and 30% oxygen, and magnetic resonance imaging (MRI) was performed as previously described [13] with a slight modification. T2-weighted images (T2WI) were obtained from the abdominal region including the lumbar/inguinal LNs and urinary bladder by using fast-spin echo sequences (repetition time [TR], ≥4567 ms; effective echo time [TE], 45 ms; echo train length, 8; matrix size, from 256 × 192 to 288 × 264; field of view, 64 mm × 48 mm; slice thickness, 1 mm) using a 2.0-T MRI System (BioSpec 2.0/31) (Bruker BioSpin MRI GmbH, Ettlingen, Baden-Württemberg, Germany) equipped with shielded gradients and a standard volume transmission/reception coil (Bruker-Biospin MRI GmbH). LNs were detected as moderately high intense T2 signal areas within round high-intensity fat signal. During MRI, the body temperature of rats was maintained at 37 ± 1 °C with an in-hand heater system and a respiration sensor was attached for breath-gating.

2.5. PET imaging

Following the MRI, PET imaging was performed with a small-animal PET scanner (eXplore Vista DR) (GE Healthcare, Waukesha, WI, US). The rats were fasted for 4–6 h prior to tracer injection, and anesthetized in the same way as for MRI. *Anti*-[^{18}F]FACBC (18.5 MBq) was injected into the tail vein, and dynamic imaging of the abdominal portion was performed 0–60 min after tracer injection. Frame duration was 600 s/frame. All PET images were reconstructed by FIRST 3D-ordered subset expectation maximization (OSEM) Graphical User Interface (GE Healthcare) with 3D OSEM algorithm. The slice thickness and transverse resolution were 0.775 mm and 0.3875 mm, respectively. Each reconstructed PET image was represented in SUV.

2.6. Coregistration of PET and MR images

Both transverse images from MR T2WI and PET were converted to Digital Imaging and Communications in Medicine (DICOM) format for co-registration by the FUSION software (DxMM workstation Opt. FUSION 7D-Standard; Medasys Japan Co. Ltd., Tokyo, Japan). PET and MR images were then coregistered using several internal soft tissue landmarks with characteristic anatomical and functional information. Image registration was performed with the same amount of translation and rotation for all continuous dynamic images.

Two volumes of interest (VOI) were positioned on dynamic PET/MR images: VOIs were positioned on decay-corrected PET images for right lumbar and left inguinal LNs as lesional and non-lesional LNs, respectively, with the referral of anatomical information on fusion