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Monitoring tumor response with [^{18}F]FMAU in a sarcoma-bearing mouse model after liposomal vinorelbine treatment☆

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ABSTRACT

Objective: Previous studies have shown that the accumulation level of FMAU in tumor is proportional to its proliferation rate. This study demonstrated that 2′-deoxy-2′-[^{18}F]fluoro-β-D-arabinofuranosyluracil ([^{18}F]FMAU) is a promising PET probe for noninvasively monitoring the therapeutic efficacy of 6% PEGylated liposomal vinorelbine (lipo-VNB) in a subcutaneous murine NG4TL4 sarcoma mouse model.
Methods: Female syngenic FVB/N mice were inoculated with NG4TL4 cells in the right flank. After tumor size reached 150 ± 50 mm^3 (day 0), lipo-VNB (5 mg/kg) was intravenously administered on days 0, 3 and 6. To monitor the therapeutic efficacy of lipo-VNB, [^{18}F]FMAU PET was employed to evaluate the proliferation rate of tumor, and it was compared with that observed from [^{18}F]FDG/[^{18}F]fluoroacetate PET. The expression of proliferating cell nuclear antigen (PCNA) in tumor during treatment was determined by semiquantitative analysis of immunohistochemical staining.
Results: A significant inhibition ($p < 0.001$) in tumor growth was observed on day 3 after a single dose treatment. The tumor-to-muscle ratio (*T/M*) derived from [^{18}F]FMAU-PET images of lipo-VNB-treated group declined from 2.33 ± 0.16 to 1.26 ± 0.03 after three doses of treatment, while that of the control remained steady. The retarded proliferation rate of lipo-VNB-treated sarcoma was confirmed by PCNA immunohistochemistry staining. However, both [^{18}F]FDG and [^{18}F]fluoroacetate microPET imaging did not show significant difference in *T/M* between the therapeutic and the control groups throughout the entire experimental period.
Conclusion: Lipo-VNB can effectively impede the growth of NG4TL4 sarcoma. [^{18}F]FMAU PET is an appropriate modality for early monitoring of the tumor response during the treatment course of lipo-VNB.

1. Introduction

Accurate and early information of proliferation rate would be valuable for reflecting the response to chemotherapy or guiding optimal clinical management. Recent studies have demonstrated that positron emission tomography (PET) with a proliferation rate-specific radiotracer, such as 3′-deoxy-3-[^{18}F]fluorothymidine ([^{18}F]FLT), is capable of assessing the therapeutic efficacy of various tumor treatments [1–3]. FLT is trapped in cells that express high activity of thymidine kinase 1 (TK1) which is responsible for the phosphorylation of thymidine analogues to fulfill the high demand for DNA replication during growth. However, only a very small proportion of FLT is incorporated into DNA sequence [4].

The fluorine substitution at deoxyribose prevents cleavage of the sugar from thymine by phosphorylase, making both [^{18}F]FLT and 2′-deoxy-2′-^{18}F-fluoro-β-D-arabinofuranosyluracil ([^{18}F]FMAU) highly resistant to breakdown. Previous studies have shown that [^{18}F]FMAU resists degradation in living subjects when compared with [^{11}C]thymidine. More than 95% of intact [^{18}F]FMAU was observed in blood and urine at 1 h post injection [5]. Alauddin et al. have developed a multi-step synthetic method for the preparation of [^{18}F]FMAU [6]. FMAU is preferentially phosphorylated by thymidine kinase 2 (TK2), a mitochondrial enzyme, and displays elevated radioactivity accumulation in high TK2-expressing normal organs, such as heart, kidney and liver [7]. For tumor detection, Tehrani et al. and Sun et al.

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demonstrated that [^{18}F]FMAU, as a proliferation probe, can clearly delineate the tumors in brain, prostate, thorax and bone in patients [8,9]. However, employing [^{18}F]FMAU-PET for monitoring the therapeutic efficacy of tumor treatment has never been reported.

Vinorelbine (VNB), a semi-synthetic Vinca alkaloid, can suppress the dynamic behavior of spindle microtubule in the metaphase during mitosis and leads to the following apoptosis by inducing the activity of p53 and bcl-2-associated protein kinase [10,11]. VNB has been shown to be effective in various tumor types [12,13]. Liposomal encapsulation may dramatically improve the pharmacokinetic profile and tumor accumulation of chemodrug through the enhanced permeability and retention (EPR) effect. In order to reduce the capture of liposomes in reticuloendothelial system (RES)-rich organs, modification of liposomes with polyethylene glycol (PEG) was usually employed to ensure long-term systemic circulation of the PEGylated liposomes and rapid clearance from RES organs [14]. Drummond et al. have explored a stable and long-circulating PEGylated liposomal vinorelbine with high efficacy but low acute toxicity in a C-26 murine colon carcinoma tumor-bearing immunocompetent mouse model [15]. This study assessed the therapeutic efficacy of 6% PEGylated liposomal vinorelbine (lipo-VNB) in an NG4TL4 sarcoma-bearing mouse model and demonstrated that, compared with [^{18}F]FDG and [^{18}F]fluoroacetate ([^{18}F]FAc, for evaluation of lipid synthesis of tumor) [16,17], [^{18}F]FMAU is a more suitable PET probe for the assessment of responses in tumor in the early stage of lipo-VNB treatment.

2. Materials and methods

2.1. Materials

Cell-culture materials were obtained from GIBCO™ (Grand Island, NY, USA). Column (40 × 8 mm) and Sephadex™ G-50 Fine were purchased form GE Healthcare (Waukesha, WI, USA). 8-Hydroxyquinoline (oxine) was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). [^{18}F]HF and [^{111}In]$InCl_3$ were obtained from the National PET/Cyclotron Center of Veterans General Hospital (Taipei, Taiwan) and the Institute of Nuclear Energy Research (Taoyuan, Taiwan), respectively. The 6% pegylated liposomal vinorelbine (lipo-VNB) was kindly provided by the Taiwan Liposome Company (Taipei, Taiwan). All other chemicals were purchased from Merck & Co., Inc. (Whitehouse Station, NJ, USA).

2.2. Radiotracer preparations

The preparation of [^{18}F]FAc and [^{111}In]lipo-VNB, a radioactive surrogate of lipo-VNB, was detailed in our previous reports [18,19]. [^{18}F]FMAU was synthesized following the method described by Alauddin et al. [6]. The radiochemical yield (decay corrected) was 55% ~ 60% for [^{18}F]FAc, 85% ~ 90% for [^{111}In]-lipo-VNB and 15% ~ 20% for [^{18}F]FMAU. The radiochemical purity of these radiotracers was all greater than 95%. [^{18}F]FDG was prepared using an automated [^{18}F]FDG synthetic system (TracerLab MX, GE Healthcare, WI, USA) at National PET/Cyclotron Center of Veterans General Hospital, Taipei, Taiwan.

2.3. Cell culture and Tumor xenografts in mice

NG4TL4 sarcoma and GM2147 normal human fibroblast cell lines were cultured in Minimal Essential Medium, supplemented with 10% fetal bovine serum (Thermo, MA, USA), and 1% Penicillin–Streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University (Taipei, Taiwan). About 2×10^5 NG4TL4 sarcoma cells were inoculated in the right flank to produce subcutaneous tumor in a six-week-old female syngenic FVB/N mouse under anesthetization (pentobarbital 70 mg/kg, intraperitoneally).

2.4. In vitro cell viability assay

NG4TL4 sarcoma cells were plated in a 96-well microplate (3.5×10^3 cells/well) overnight and then sequential concentrations of VNB or lipo-VNB were added to the cells. Cell viability after a 24-h incubation was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma, USA) assay. Each sample was repeated at least five times. Half inhibition concentration (IC_{50}) was used to express the anti-proliferative effect of VNB and lipo-VNB.

2.5. Flow cytometry

For cell cycle analysis, 3×10^6 NG4TL4 cells were seeded in 10-cm-diameter Petri dishes (n = 5) and exposed to 0.75 and 2.5 μg/mL of VNB for 24 h. Cells were trypsinized and fixed with 70% ethanol for 2 h at −20 °C and then stained with a solution containing 20 μg/mL propidium iodide, 0.2 mg/mL RNase A, and 0.1% Triton X-100 for 30 min in the dark at ambient temperature. Cell cycle distribution was performed by FACSCalibur flow cytometry (BD Medical, NJ, USA) and calculated by using FlowJo software (Tree Star, Ashland, OR, USA). Experiments were repeated thrice independently.

2.6. In vitro cellular uptake assays

The in vitro accumulation studies were conducted following previous published methods [20,21]. Briefly, either NG4TL4 or GM2147 fibroblast cells (3×10^6) were seeded into 15-cm dishes containing 15 mL of culture medium until 70% confluence. The culture medium was replaced with 12 mL of the medium that contained [^{18}F]FMAU (0.5 μCi/mL). At designated time points (10, 30, 60 and 120 min post-incubation), the cells were harvested by gentle scraping and pelleted by centrifugation. The radioactivity was measured using a gamma counter (1470 WIZARD Gamma Counter, Wallac, Finland). The cellular uptake was expressed in cell-to-medium ratio (C/M) according to the following equation.

$$Cell-to-medium\ ratio = \frac{Radioactivity\ of\ cell\ pellet\,(cpm)/\ Net\ weight\ of\ cell\ pellet\,(g)}{Radioactivity\ of\ medium\,(cpm)/\ Net\ weight\ of\ medium\,(g)}$$

2.7. Treatment protocol

NG4TL4 sarcoma-bearing FVB/N mice with tumor size of 150 ± 50 mm^3 (about 12 days post NG4TL4 cells inoculation) were selected for the trial on the day of treatment initiation (day 0). Tumor-bearing mice were randomly assigned to the therapeutic and the control groups and were treated following the treatment protocol shown in Scheme 1. Lipo-VNB (5 mg/kg of body weight) and normal saline were individually administered to mice of the therapeutic and control groups via the tail vein on days 0, 3 and 6. Tumor growth was followed by caliper measurement made perpendicular to the tumor. The tumor volume was estimated from the formula: $V = 0.523 \times L \times W \times T$, where V, L, W, and T are the volume, length, width, and thickness of tumor, respectively. The average increment of tumor volume was expressed as $100\% \times (V_n - V_0)/V_0$, where V_0 and V_n are the tumor volume on day 0 and on each measuring day, respectively. For ethical considerations, mice with a tumor volume ≥2000 mm^3 were euthanized. To monitor the changes in tumor cell proliferation, glucose metabolism and lipid synthesis during treatment, [^{18}F]FMAU, [^{18}F]FDG and [^{18}F]FAc PET scanning was conducted to the lipo-VNB-