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ScienceDirect
Nuclear Medicine and Biology 36 (2009) 801–809
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

Imaging of peripheral-type benzodiazepine receptor in tumor: in vitro binding and in vivo biodistribution of *N*-benzyl-*N*-[^{11}C]methyl-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9*H*-purin-9-yl)acetamide

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Received 20 February 2009; received in revised form 30 April 2009; accepted 12 May 2009

Abstract

Introduction: The aim of this study was to evaluate *N*-benzyl-*N*-[^{11}C]methyl-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9*H*-purin-9-yl) acetamide ([^{11}C]DAC) as a novel peripheral-type benzodiazepine receptor (PBR) ligand for tumor imaging.
Methods: [^{11}C]DAC was synthesized by the reaction of a desmethyl precursor with [^{11}C]CH_3I. In vitro uptake of [^{11}C]DAC was examined in PBR-expressing C6 glioma and intact murine fibrosarcoma (NFSa) cells. In vivo distribution of [^{11}C]DAC was determined using NFSa-bearing mice and small-animal positron emission tomography (PET).
Results: [^{11}C]DAC showed specific binding to PBR in C6 glioma cells, a standard cell line with high PBR expression. Specific binding of [^{11}C]DAC was also confirmed in NFSa cells, a target tumor cell line in this study. Results of PET experiments using NFSa-bearing mice, showed that [^{11}C]DAC was taken up specifically into the tumor, and pretreatment with PK11195 abolished the uptake.
Conclusions: [^{11}C]DAC was taken up into PBR-expressing NFSa. [^{11}C]DAC is a promising PET ligand that can be used for imaging PBR in tumor-bearing mice.

Keywords: Tumor imaging; [^{11}C]DAC; PBR; PET; NFSa

1. Introduction

Positron emission tomography (PET) is an important diagnostic tool in oncology, and the most widely used radiopharmaceutical for PET is 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG). Although [^{18}F]FDG PET has been identified as a suitable clinical tool for tumor detection, [^{18}F]FDG uptake in some cases shows false-positive or false-negative. When [^{18}F]FDG is taken up into the inflamed area, it is false positive. Decreased uptake of [^{18}F]FDG has been identified in hyperglycemic patients. Because of the urinary excretion of [^{18}F]FDG, tumors in the abdomen (e.g., prostate tumors) are relatively poorly imaged [1] and become false-negatives. Therefore, tumor heterogeneity makes it difficult to reach agreement on the information obtained from [^{18}F]FDG uptake. Thus, there is a need to develop novel tumor imaging agents that may overcome some of the shortcomings of [^{18}F]FDG.

The peripheral-type benzodiazepine receptor (PBR) was identified in 1977 when scientists were searching for benzodiazepine binding sites in peripheral tissues [2]. It is mainly located in the adrenals, kidneys, lungs, heart and central nervous system, and is known to play important roles in cell growth, differentiation and apoptosis [3]. The fundamental functions of PBR are as follows: (1) cholesterol binding followed by cholesterol transport, which is crucial in steroid

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doi:10.1016/j.nucmedbio.2009.05.002

and bile salt biosynthesis; (2) protein import, which is important for membrane biogenesis and (3) porphyrin binding and transport, which is involved in heme biosynthesis [3]. Overexpression of PBR has been identified in various tumors, such as gliomas [4,5], astrocytomas [6], breast cancer [7,8], colorectal cancer [9,10], hepatomas [11], leukemia [12] and fibrosarcomas [13]. PBRs in normal tissue cells are located on the mitochondrial membrane; however, in some PBR-over-expressed tumor cells, this receptor is located on the nucleus and not only on the mitochondria. Nuclear localization of PBR and PBR-mediated cholesterol transport into the nucleus has been reported to be involved in cell proliferation [7,12]. Previous studies have also identified an anti-cancer effect of PBR ligands, such as PK11195, Ro5-4864 and FGIN-1-27. These ligands induce apoptosis through caspase activation from the release of cytochrome C [14,15]. Furthermore, PK11195 could make chemotherapeutic drugs more effective by inhibiting ATP-binding Cassette (ABC) transporters [16]. These findings suggest that PBR binding in tumors may become a promising biomarker for PET imaging of tumors.

[^{11}C]PK11195 was first used for clinical imaging in a patient with glioblastoma. Tumor retention of [^{11}C]PK11195 was demonstrated to be partly due to in vivo specific binding to PBR. However, nonspecific binding has also been detected [17]. AC-5216 was recently synthesized as a new in vitro potent ligand for PBR in glioma cells [18], and radiolabeled [^{11}C]AC-5216 was evaluated for imaging PBR in tumors [19]. An ex vivo autoradiography study of tumor-bearing mice did not identify sufficient specific binding of [^{11}C]AC-5216 in tumors [19], which might have been due to high nonspecific binding, high lipophilicity (cLogD=3.46) [20] and rapid clearance of this ligand.

The aim of this study was to evaluate a novel PET ligand, *N*-benzyl-*N*-[^{11}C]methyl-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9*H*-purin-9-yl)acetamide ([^{11}C]DAC; Fig. 1) and to determine the uptake and binding of [^{11}C]DAC to PBR in tumor cells and tumor-bearing mice. In this study, we first determined the inhibitory affinity of DAC for C6 glioma cells, a standard cell line with high PBR density [18,21,22]. We then characterized [^{11}C]DAC in vitro and in vivo using intact murine fibrosarcoma (NFSa) cells, a target tumor cell line in this study, and NFSa-bearing mice.

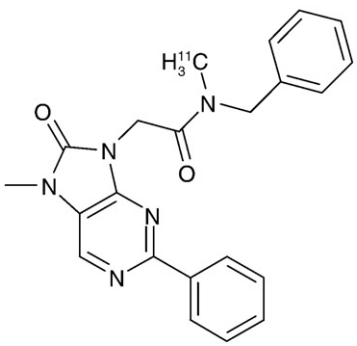

Fig. 1. Chemical structure of [^{11}C]DAC.

2. Material and methods

2.1. General

All chemicals and solvents were of analytical or high-performance liquid chromatography (HPLC) grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Wako Pure Chemical (Osaka, Japan). DAC and the desmethyl precursor [23] were synthesized in our laboratory. ^{11}C was produced by a ^{14}N (p, α) ^{11}C nuclear reaction in a CYPRIS HM18 cyclotron (Sumitomo Heavy Industries, Tokyo, Japan). A dose calibrator (IGC-3R Curiemeter; Aloka, Tokyo, Japan) was used for all radioactivity measurements, unless otherwise stated. Reverse-phase HPLC was performed using a Jasco system (Jasco, Tokyo, Japan), and effluent radioactivity was measured with a NaI (Tl) scintillation detector system. Animal experiments were performed according to the recommendations of the Committee for the Care and Use of Laboratory Animals, National Institute of Radiological Sciences.

2.2. Radiosynthesis of [^{11}C]DAC

[^{11}C]CH_3I for radiosynthesis was produced from cyclotron-produced [^{11}C]CO_2, as described previously [20]. Briefly, [^{11}C]CO_2 was bubbled into 40 mM $LiAlH_4$ in anhydrous tetrahydrofuran (THF) (500 μl). After removing THF, the remaining complexes were treated with 57% hydroiodic acid (300 μl). [^{11}C]CH_3I was transferred under helium gas flow with heating into a reactor containing desmethyl precursor (1 mg) and NaH (10 μl, 0.5 g/20 ml *N*. *N*-dimethylformamide (DMF)) in anhydrous DMF (300 μl) and then cooled to −15°C to −20°C. After radioactivity reached a plateau, the reactor was warmed to 70°C and maintained for 5 min. The reaction mixture was applied to a semipreparative HPLC system. HPLC purification was performed on a CAPCELL PAK C_{18} column (10 mm ∅×250 mm; Siseido, Tokyo, Japan) using a mobile phase of CH_3CN/H_2O (6/4) at a flow rate of 4.0 ml/min. The radioactive fraction of [^{11}C]DAC (retention time 7.5 min) was collected, evaporated, redissolved in 5 ml normal sterile saline and passed through a 0.22-μm Millipore filter (Waters, Milford, MA, USA) for analysis and animal experiments.

Radiochemical purity of [^{11}C]DAC was assayed by analytical HPLC, using a CAPCELL PAK C_{18} column (4.6 mm ID×250 mm) with CH_3CN/H_2O (6/4) as the mobile phase, a retention time of 4.5 min and a flow rate of 1.0 mL/min. The identity of [^{11}C]DAC was confirmed by coinjection with an authentic sample. Specific activity of [^{11}C]DAC was calculated by comparing the assayed radioactivity to the carrier at an ultraviolet (UV) peak of 254 nm.

2.3. Mice

Male C3H/HeMsNrsf mice (9–10 weeks old) were used for this study. The mice were produced and maintained in a specific pathogen-free facility in our institute and were housed in groups. The tumor was a syngeneic NFSa fibrosarcoma; its 16th through 18th generations were