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# Usefulness of [$^{18}$F]-DA and [$^{18}$F]-DOPA for PET imaging in a mouse model of pheochromocytoma

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## Abstract

**Purpose:** To evaluate the usefulness of [$^{18}$F]-6-fluorodopamine ([$^{18}$F]-DA) and [$^{18}$F]-L-6-fluoro-3,4-dihydroxyphenylalanine ([$^{18}$F]-DOPA) positron emission tomography (PET) in the detection of subcutaneous (s.c.) and metastatic pheochromocytoma in mice; to assess the expression of the norepinephrine transporter (NET) and vesicular monoamine transporters 1 and 2 (VMAT1 and VMAT2), all important for [$^{18}$F]-DA and [$^{18}$F]-DOPA uptake. Furthermore, to compare tumor detection by micro-computed tomography (microCT) to magnetic resonance imaging (MRI) in individual mouse.

**Methods:** $SUV_{max}$ values were calculated from [$^{18}$F]-DA and [$^{18}$F]-DOPA PET, tumor-to-liver ratios (TLR) were obtained and expression of NET, VMAT1 and VMAT2 was evaluated.

**Results:** [$^{18}$F]-DA detected less metastatic lesions compared to [$^{18}$F]-DOPA. TLR values for liver metastases were 2.26–2.71 for [$^{18}$F]-DOPA and 1.83–2.83 for [$^{18}$F]-DA. A limited uptake of [$^{18}$F]-DA was found in s.c. tumors (TLR=0.22-0.27) compared to [$^{18}$F]-DOPA (TLR=1.56-2.24). Overall, NET and VMAT2 were expressed in all organ and s.c. tumors. However, s.c. tumors lacked expression of VMAT1. We confirmed [$^{18}$F]-DA's high affinity for the NET for its uptake and VMAT1 and VMAT2 for its storage and retention in pheochromocytoma cell vesicles. In contrast, [$^{18}$F]-DOPA was found to utilize only VMAT2.

**Conclusion:** MRI was superior in the detection of all organ tumors compared to microCT and PET. [$^{18}$F]-DOPA had overall better sensitivity than [$^{18}$F]-DA for the detection of metastases. Subcutaneous tumors were localized only with [$^{18}$F]-DOPA, a finding that may reflect differences in expression of VMAT1 and VMAT2, perhaps similar to some patients with pheochromocytoma where [$^{18}$F]-DOPA provides better visualization of lesions than [$^{18}$F]-DA.



*Abbreviations:* PET, positron emission tomography; MPC cells, mouse pheochromocytoma cells; ROI, region of interest; FOV, field of view; TBR, tumor-to-background ratio; TLR, tumor-to-liver ratio; $SUV_{max}$, the maximum standardized uptake value; 2D-OSEM, 2-dimensional ordered-subsets expectation maximization; FWHM, full-width at half-maximum; [$^{18}$F]-DA, [$^{18}$F]-6-fluorodopamine; [$^{18}$F]-DOPA, [$^{18}$F]-L-6-fluoro-3,4-dihydroxyphenylalanine; VMAT1 and VMAT2, vesicular monoamine transporters 1 and 2; NET, membrane norepinephrine transporter; TH, tyrosine hydroxylase; MRI, magnetic resonance imaging; s.c., subcutaneous; i.v., intravenous.

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## 1. Introduction

In clinical practice, various imaging techniques are used to localize pheochromocytoma, a rare neuroendocrine tumor arising from chromaffin cells [1]. These tumors are characterized by the synthesis, storage, metabolism and release of catecholamines [2]. Even though metastatic pheochromocytoma is generally slow growing, the prognosis of patients with disseminated disease is often poor, with a 5-year survival rate of usually less than 50% [3,4]. This is due to the fact that, currently, there is no effective chemotherapeutic regimen [5]. Therefore, the development of animal models for testing new imaging methods and probes to visualize and monitor tumor growth, as well as other tumor characteristics, is crucial for new drug development.

In recent years, positron emission tomography (PET) has rapidly become a valuable diagnostic tool in the study of pheochromocytomas, particularly with the development of new targeted PET radiopharmaceuticals such as [$^{18}$F]-6-fluorodopamine ([$^{18}$F]-DA) [6,7] and [$^{18}$F]-L-6-fluoro-3,4-dihydroxyphenylalanine ([$^{18}$F]-DOPA) [8]. Functional imaging studies with [$^{18}$F]-DA and [$^{18}$F]-DOPA have the advantage over anatomical imaging of superior specificity for identification of pheochromocytomas [9,10]. Uptake of [$^{18}$F]-DA, a dopamine analogue, occurs via the cell membrane norepinephrine transporter (NET) [11]. Desipramine, a tricyclic anti-depressant, functions as an inhibitor of the uptake-1 mechanism involved in amine transport into cells [12] and has been used to evaluate the specificity of the NET transporter for [$^{18}$F]-DA uptake [13,14]. [$^{18}$F]-DOPA, an analogue of the dopamine precursor, DOPA, is incorporated into tumor cells via the aromatic amino acid transporter [15]. Upon entering the cells, [$^{18}$F]-DOPA is decarboxylated by the aromatic amino acid decarboxylase to [$^{18}$F]-DA and sequestered by vesicular monoamine transporters (VMAT1 and VMAT2) into catecholamine storage vesicles. The aromatic amino acid transporters, NET, VMAT1 and VMAT2 are specifically expressed in chromaffin cells and pheochromocytomas [16]. In this study, VMAT inhibitors, such as reserpine (VMAT1/VMAT2 inhibitor) and tetrabenazine (VMAT2 inhibitor) [17], have been used to evaluate the importance of [$^{18}$F]-DA and [$^{18}$F]-DOPA uptake in mouse pheochromocytoma (MPC) cells.

Recently, we have described an animal model of metastatic pheochromocytoma and introduced [$^{18}$F]-DA PET for the localization of liver lesions [18]. The aims of the present study were to compare the feasibility of a potentially more practical subcutaneous (s.c.) pheochromocytoma model against our previously established metastatic model, to evaluate [$^{18}$F]-DOPA PET as an alternative modality for localization of lesions compared to micro-computed tomography (microCT) and magnetic resonance imaging (MRI) and to assess the importance of NET and VMATs in entry and storage of [$^{18}$F]-DOPA and [$^{18}$F]-DA in cells and tumors. These findings were aimed to determine whether these pheochromocytoma models are comparable to clinical imaging scenarios and, thus, appropriate for potential experiment drug treatments. The metastatic model involved diffuse spread of tumors after tail vein injection of MPC cells and the s.c. model represented by s.c. tumors after s.c. implantation of MPC cells.

## 2. Materials and methods

### 2.1. Cell culture and in vitro assays of [$^{18}$F]-DA and [$^{18}$F]-DOPA uptake and storage

MPC cells were kindly provided by Dr. A.S. Tischler [19] and were maintained in Dulbecco's Modified Minimum Essential Medium (GIBCO, Grand Island, NY, USA) supplemented with 10% (v/v) fetal calf serum, 5% fetal bovine serum and 1% (v/v) penicillin/streptomycin and maintained at 37°C and 5% $CO_2$.

To confirm the ability of MPC cells to incorporate [$^{18}$F]-DA or [$^{18}$F]-DOPA, in vitro radionuclide assays were conducted. MPC cells were seeded onto collagen-coated 12-well plates at a density of $2\times10^5$ cells per well and incubated for 24 h. At the commencement of uptake assays, cells were washed three times with phosphate-buffered saline (PBS, pH 7.4). Cells were then incubated for 30 min with the non-selective monoamine transporter antagonists: desipramine (1 μM, Sigma Chemical, St. Louis, MO, USA); tetrabenazine (10 μM, Sigma Chemical), an inhibitor of VMAT 2 [20,21] and reserpine (10 μM, Serpasil, Siba, Summit, NJ) an inhibitor of both VMAT1 and VMAT2 [22]. Uptake studies involved incubation of cells with 0.024 MBq/ml of [$^{18}$F]-DA or 0.029 MBq/ml of [$^{18}$F]-DOPA. Cells were harvested after incubation periods of either 10 (to assess "uptake") or 120 min (to assess "storage and retention").

At the termination of the radionuclide assays, cells were counted, and the radioactivity was measured on a Packard gamma counter (United Technologies). The uptake of [$^{18}$F]-DA and [$^{18}$F]-DOPA was described as counts per minute per number of cells per sample and expressed as "% normalized ratio of controls". The uptake was calculated by subtracting uptake in treated cells from control (untreated) cells. All experiments were performed in triplicate and repeated twice.

### 2.2. Animal model of pheochromocytoma

All animal studies were conducted in accordance with the principles and procedures outlined in the National Institutes