
Effect of Carbidopa on ^{18}F -FDOPA Uptake in Insulinoma: From Cell Culture to Small-Animal PET Imaging

Julien Detour^{1,2}, Alice Pierre^{1,2}, Frédéric Boisson³, Guillaume Kreutter^{4,5}, Thomas Lavaux⁶, Izzie Jacques Namer^{2,5,7}, Laurence Kessler^{4,5,8}, David Brasse³, Patrice Marchand³, and Alessio Imperiale^{2,3,5,7}

¹Department of Radiopharmacy, Strasbourg University Hospitals, Strasbourg, France; ²Department of Biophysics and Nuclear Medicine, Strasbourg University Hospitals, Strasbourg, France; ³Institut Pluridisciplinaire Hubert Curien (IPHC), CNRS/UMR7178, Strasbourg University, Strasbourg, France; ⁴EA7293, Vascular and Tissue Stress in Transplantation, Illkirch, France; ⁵Federation of Translational Medicine of Strasbourg (FMTS), Faculty of Medicine, Strasbourg University, Strasbourg, France; ⁶Department of Biochemistry and Molecular Biology, Strasbourg University Hospitals, Strasbourg, France; ⁷ICube, CNRS/UMR7357, Strasbourg University, Strasbourg, France; and ⁸Department of Diabetology, Strasbourg University Hospitals, Strasbourg, France

Patient premedication with carbidopa seems to improve the accuracy of 6- ^{18}F -fluoro-3,4-dihydroxy-L-phenylalanine (^{18}F -FDOPA) PET for insulinoma diagnosis. However, the risk of PET false-negative results in the presence of carbidopa is a concern. Consequently, we aimed to evaluate the effect of carbidopa on ^{18}F -FDOPA uptake in insulinoma β -cells and an insulinoma xenograft model in mice. **Methods:** ^{18}F -FDOPA in vitro accumulation was assessed in the murine β -cell line RIN-m5F. In vivo small-animal PET experiments were performed on tumor-bearing nude mice after subcutaneous injection of RIN-m5F cells. Experiments were conducted with and without carbidopa pretreatment. **Results:** Incubation of RIN-m5F cells with 80 μM carbidopa did not significantly affect the cellular accumulation of ^{18}F -FDOPA. Tumor xenografts were clearly detectable by small-animal PET in all cases. Insulinoma xenografts in carbidopa-treated mice showed significantly higher ^{18}F -FDOPA uptake than those in nontreated mice. Regardless of carbidopa premedication, the xenografts were characterized by an early increase in ^{18}F -FDOPA uptake and then a progressive reduction over time. **Conclusion:** Carbidopa did not influence in vitro ^{18}F -FDOPA accumulation in RIN-m5F cells but improved insulinoma imaging in vivo. Our findings increase current knowledge about the ^{18}F -FDOPA uptake profile of RIN-m5F cells and a related xenograft model. To our knowledge, the present work represents the first preclinical research specifically focused on insulinomas, with potential translational implications.

Key Words: ^{18}F -FDOPA; PET; insulinoma; carbidopa; small-animal PET; RIN-m5F

J Nucl Med 2017; 58:36–41

DOI: 10.2967/jnumed.116.180588

Insulinoma is a rare, usually solitary and benign neuroendocrine tumor (NET). It is characterized by inappropriate and uncontrolled insulin production and secretion, with consequent hypoglycemic symptoms and potentially lethal hypoglycemia. Early detection of the

tumor is crucial, allowing curative treatment by surgical enucleation. The localization of insulinoma remains challenging, and conventional imaging is the first method used for diagnostic investigation (1).

Functional imaging may be of particular interest when the results of conventional imaging are negative or inconclusive (2). Nevertheless, the gold standard examination remains to be defined. Radiolabeled glucagonlike peptide 1 analogs used for conventional scintigraphic studies or PET imaging have shown promising results because glucagonlike peptide 1 receptors are overexpressed in most insulinomas (3). However, these radiopharmaceuticals are not yet commercially available and are used in humans mostly in clinical trials.

6- ^{18}F -fluoro-3,4-dihydroxy-L-phenylalanine (^{18}F -FDOPA) is clinically approved and commercially available for PET imaging of insulinomas in many countries. Once internalized by specific cell membrane transporters, ^{18}F -FDOPA is decarboxylated to ^{18}F -dopamine by the aromatic amino acid decarboxylase (AADC) and accumulates in secretory vesicles. Therefore, the expression of AADC has a key role in tumor visualization by ^{18}F -FDOPA PET (4). However, the intense and prolonged radiotracer uptake in the mature exocrine pancreas represents the main drawback of ^{18}F -FDOPA PET and may explain the initial disappointing results for insulinoma detection (5). Furthermore, early PET images and patient premedication with carbidopa may improve the diagnostic accuracy of ^{18}F -FDOPA PET in adult patients with insulinoma-related hyperinsulinemic hypoglycemia (6,7). Carbidopa, an efficient inhibitor of AADC, is capable of drastically reducing physiologic pancreatic uptake, with a consequent increase in the tumor-to-background ratio (8). Moreover, carbidopa leads to better systemic radiotracer availability by reducing the peripheral conversion of ^{18}F -FDOPA to ^{18}F -dopamine and its renal excretion. However, no final consensus about the usefulness of carbidopa premedication before ^{18}F -FDOPA PET in patients with hyperinsulinemic hypoglycemia has been reached because of the potential reduction of tumoral uptake intensity (9). The risk of ^{18}F -FDOPA PET false-negative results could be high for tumors with low intracellular AADC activity (10), which are potentially more sensitive to the inhibitory effect of carbidopa.

The positive impact of carbidopa premedication has been demonstrated in both in vitro and in vivo studies with cell lines of human pancreatic NETs (BON cells) and a related tumor xenograft animal model (11,12). To the best of our knowledge, no preclinical data concerning the influence of carbidopa on ^{18}F -FDOPA uptake in insulinomas are available. Therefore, we aimed to evaluate the effect of carbidopa on ^{18}F -FDOPA uptake in the β -cell line RIN-m5F

Received Jul. 4, 2016; revision accepted Aug. 10, 2016.

For correspondence or reprints contact: Alessio Imperiale, Biophysics and Nuclear Medicine, Haute-pierre University Hospital, 1 Avenue Molière, 67098 Strasbourg Cedex, France.

E-mail: alessio.imperiale@chru-strasbourg.fr

Published online Sep. 8, 2016.

COPYRIGHT © 2017 by the Society of Nuclear Medicine and Molecular Imaging.

and a related insulinoma xenograft model developed in nude mice. To our knowledge, the present work represents the first preclinical research study focused on insulinomas, with a potential impact on the diagnostic and therapeutic management of patients in clinical practice.

MATERIALS AND METHODS

Radiotracer Production

Good manufacturing practices-compliant ^{18}F -FDOPA was obtained from Cisbio International (DOPACIS), and electrophilic substitution was performed in accordance with the method reported by de Vries et al. (13). A total activity of 200–240 MBq in 2.2 mL was received and further diluted with 0.9% sterile NaCl to reach an injection volume of 150 μL .

Cell Culture

In vitro experiments were performed with the β -cell line RIN-m5F (CRL-11605; American Type Culture Collection) (14). These cells produce and secrete insulin and produce AADC. Unlike the parental line, they do not produce somatostatin. Positive ^{18}F -FDOPA uptake in cell line RIN-m5F was previously reported (15). Cells were cultured in 75-cm² flasks containing 20 mL of RPMI 1640 medium without *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid but with glutamine (Invitrogen) (RPMI culture medium). The medium was supplemented with 10% fetal bovine serum (Gibco) and gentamicin (100 $\mu\text{g}/\text{mL}$; Panpharma). Cells were grown at 37°C in a humidified atmosphere containing 5% CO₂ and subcultured twice per week.

In Vitro Determination of ^{18}F -FDOPA Accumulation

Flasks were maintained at 37°C and incubated for 1 h either with or without 80 μM carbidopa (INRESA). In another condition, 2 h before the experiment, RPMI culture medium was replaced with an amino-acid-free medium composed of phosphate-buffered saline (0.9 mM CaCl₂, 0.5 mM MgCl₂, 2.6 mM KCl, 1.47 mM KH₂PO₄, 138 mM NaCl, 8 mM Na₂HPO₄; pH 7; Gibco) and glucose (0.75 mM D-glucose) (PBS-Glu medium). Then, 200 kBq of buffered ^{18}F -FDOPA dissolved in 100–150 μL of culture medium were added to each flask. After 5, 30, and 60 min, the culture medium or PBS-Glu medium was removed, and the cells were washed, harvested by trypsin treatment, and suspended in the same type and volume of culture medium (RPMI or PBS-Glu). A 2.5-mL volume of culture cells or of removed medium was transferred to 5-mL counting tubes, and radioactivity was measured in a calibrated γ -counter (Wallac, Perkin-Elmer). For nonspecific tracer adsorption, the protocol was reproduced identically without cells. The viability and number of cells were determined by the trypan blue exclusion technique with a Kova Glastic Slide 10 (Hycor Biomedical).

In a similar experiment with RPMI culture medium, cells were incubated separately with 10 mM 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH), described as an L-amino acid transporter inhibitor (16), and 10 μM tetrabenazine, described as an inhibitor of the vesicular monoamine transporter (17).

Animals

Twenty female athymic nude mice (24–28 g, 8–10 wk old; Charles River) were used for the entire experimental protocol. To optimize the management of mice with insulinoma xenografts, the carbidopa administration, and the PET imaging protocol, we used 10 of 20 animals (control group) to assess the morphologic evolution of the tumor xenograft (i.e., tumor size) and the related consequences of tumor insulin secretion on animal welfare (i.e., reduction in serum glycemia and the occurrence of lethal hypoglycemia). The remaining 10 mice (experimental group) were dedicated to in vivo small-animal PET investigations of insulinoma xenografts. Animals were kept at constant temperature (22°C) and humidity (40%) in 12-h light–dark cycles and were allowed free access to forage and water until the beginning of each imaging pro-

cedure. Animals were housed in individually ventilated cages with high-efficiency particulate air filters in the dedicated facility of the Institut Pluridisciplinaire Hubert Curien (IPHC), Strasbourg, France.

Mice with Tumor Xenografts

A cell suspension of 6×10^6 RIN-m5F cells in 100 μL of phosphate-buffered saline was injected subcutaneously into the right hind leg of mice under inhalation anesthesia (1.5%–2% isoflurane). Tumor width and length were measured (mm) with a caliper to calculate the tumor volume according to the following formula: volume (mm³) = (length \times width squared)/2. To evaluate insulin production, we assessed morning serum glycemia levels in blood samples collected from the tail vein of nonfasting mice with xenografts by using a commercial blood glucose monitoring system (Contour TS; Bayer).

^{18}F -FDOPA Small-Animal PET Imaging

Animals were scanned when the tumor reached a minimum dimension of 2.5 \times 2.5 mm or a volume of greater than 20 mm³. Mice were divided into 2 groups according to carbidopa pretreatment (i.e., carbidopa-treated or nontreated mice). Carbidopa was administered orally via a gavage needle at a dose of 20 μg in 100 μL of NaCl 1 h before the intravenous injection of ^{18}F -FDOPA. In all animals, ^{18}F -FDOPA was injected through the jugular vein, and PET images were acquired on a small-animal PET scanner (Iris; Inviscan) with a spatial resolution of less than 1.4 mm in the reconstructed PET images. As a preventive measure, 300 μL of 20% glucose solution were administered orally via a gavage needle 30 min before the imaging procedure. For small-animal PET imaging, ketamine–xylazine anesthesia was induced by an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (6 mg/kg) in a volume of 100 $\mu\text{L}/30$ g of body weight and was maintained with 1.5% isoflurane in medical air with a calibrated vaporizer for the entire procedure. Dynamic 3-dimensional PET acquisitions were performed for 20 min starting 2 min after the ^{18}F -FDOPA injection. During PET acquisition, animal temperature and respiration rate were monitored to assess the depth of anesthesia. Throughout the investigation, mice were kept at a constant temperature of 37°C.

Acquired list-mode PET data were binned into 4 frames of 5 min each and 20 frames of 1 min each. Data were reconstructed into a 201 \times 201 \times 120 three-dimensional volume by use of the iterative 3-dimensional ordered-subset expectation maximization algorithm with 6 iterations and 8 subsets. The voxel size was equal to 0.42 mm in the transverse plane and 0.85 mm in the axial direction. The calibration factor was included in the normalization file and applied during the reconstruction process. PET data were fully corrected for random coincidences, radioactive decay, and dead time. No attenuation or scatter corrections were applied.

^{18}F -FDOPA PET image analysis was performed with the AMIDE software package (<http://amide.sourceforge.net/>). The results of small-animal PET images were qualitatively interpreted as positive or negative. A focal nonphysiologic increase in radiotracer uptake in the area of tumor development was considered to be a positive PET result. For a semiquantitative assessment of tumoral ^{18}F -FDOPA uptake, an elliptical volume of interest (VOI) was drawn around the tumor on the image from the first 5 min of the PET acquisition. Tumoral VOI activity was first corrected with the mean voxel value measured in the contralateral thigh muscle. Afterward, only voxels with values higher than 40% of the maximum voxel value in the tumor were considered for further analysis. Elliptical VOIs were also drawn around the pancreas, liver, and mediastinum. Finally, time–activity curves were extracted from VOIs for the whole dynamic PET acquisition. SUVs were calculated from the mean voxel value within the VOIs. SUVs were recorded and used for generating time–activity curves.

All animal experiments were performed in accordance with European Institutes of Health Guidelines regarding the care and use

of animals for experimental procedures and were approved by the Alsace Regional Ethics Committee for Animal Experimentation (approval identification: APAFIS#2944).

Data Analysis

Differences between various groups were tested for statistical significance with nonparametric tests such as the Friedmann test for repeated measures, with adapted post hoc tests, and the Mann–Whitney *U* test for independent samples, as appropriate. The justification for such statistics was based on the small number of samples in some conditions. For convenience, the results are represented as the mean \pm SE of the mean. A *P* value of less than 0.05 was considered to be significant. Statistical analysis was performed with the SPSS Statistics (IBM) software package.

RESULTS

In Vitro Determination of ^{18}F -FDOPA Accumulation

Control Condition. The accumulation of ^{18}F -FDOPA in cells incubated in RPMI culture medium was almost constant over 60 min. Only a slight increase in ^{18}F -FDOPA uptake, ranging from 2.9%/10⁶ cells at 5 min to 4.5%/10⁶ cells at 60 min, was observed. In the control condition with amino acid–free PBS-Glu medium, cellular ^{18}F -FDOPA uptake decreased by about 3.7% over time, with values ranging from 6.8%/10⁶ cells at 5 min to 3.1%/10⁶ cells at 60 min (Fig. 1A). The highest amount of radiotracer uptake was observed at the earliest time point (5 min) and was more than twice as high as that in the RPMI culture medium condition. No statistically significant differences were observed within each culture condition over the 3 tested time points with standard culture medium or with amino acid–free PBS-Glu medium (*P* = 0.395 and *P* = 0.135, respectively). Nonetheless, statistical significance was observed between the 2 culture media in the control condition at the first (*P* < 0.01) and last (*P* < 0.05) time points.

Inhibition Experiments. Incubation of cells with 80 μM carbidopa did not significantly affect ^{18}F -FDOPA accumulation in RIN-m5F cells. As in the control condition, 2 different dynamic ^{18}F -FDOPA uptake patterns were observed according to the culture medium. In the standard condition (RPMI culture medium), cells showed quite

stable ^{18}F -FDOPA uptake over time. In PBS-Glu medium, ^{18}F -FDOPA uptake was highest at the earliest time point (5 min) and decreased over time, with values ranging from 4.6%/10⁶ cells at 5 min to 3.3%/10⁶ cells at 60 min (Fig. 1A). Overall, no statistically significant differences in ^{18}F -FDOPA uptake were observed between carbidopa-treated and nontreated cells at the 3 tested time points of incubation with RPMI culture medium or with PBS-Glu medium. BCH, a selective inhibitor of the L-amino acid transporter, drastically reduced the tracer uptake at all studied time points in a statistically significant manner (*P* < 0.05). Tetrabenazine led to a similar constant decrease in ^{18}F -FDOPA uptake over the studied period (*P* < 0.05) (Fig. 1B).

Mice with Tumor Xenografts and ^{18}F -FDOPA Small-Animal PET Imaging

Control Group. The subcutaneous tumor xenograft was visible about 2 wk after cellular inoculation of the RIN-m5F cell suspension, reaching a volume of at least 20 mm³ about 3 wk after cellular injection. Serial measurements of morning serum glycemia levels obtained before and after the subcutaneous cellular injection highlighted a progressive decrease in the blood glucose concentration in mice, confirming the secreting character of the insulinoma xenograft (Fig. 2A). Consequently, water was replaced with a 20% glucose solution 15 d after the subcutaneous injection of the RIN-5mF cell suspension in an attempt to limit the severity of hypoglycemic symptoms (decrease in spontaneous activity followed by no activity, tremor, tachypnea, or hypopnea) and to reduce the occurrence of lethal hypoglycemia in animals. No significant correlation was observed between the glycemia value and the insulinoma xenograft size. Pathologic examination of tumor xenografts was performed in 2 mice that died after a hypoglycemic crisis (Fig. 2B). Analysis revealed the presence of epithelioid and spindle cells staining for insulin (Figs. 2C and 2D).

Experimental Group. Despite the use of a 20% glucose solution instead of water, 4 animals died because of a hypoglycemic crisis within 12 d after inoculation of the RIN-m5F cell suspension and before the appearance of a measurable insulinoma xenograft. Hence, 6 mice underwent ^{18}F -FDOPA small-animal PET investigations.

Two experimental groups consisted of 3 mice each. Animals in the first group were pretreated with 20 μg of carbidopa about 1 h before the intravenous injection of ^{18}F -FDOPA (5.1 \pm 0.9 MBq in 150 μL). Mice in the second group received no premedication before intravenous ^{18}F -FDOPA administration (7.5 \pm 1.2 MBq in 150 μL). Small-animal PET studies were performed 3–4 wk after the inoculation of the RIN-m5F cell suspension, and the tumor xenograft volume ranged from 20 to 159 mm³. No significant difference in tumor size was observed between carbidopa-treated (77 \pm 42 mm³) and nontreated (53 \pm 22 mm³) mice, and the level of glycemia was less than 60 mg/dL in all cases. Glycemia levels and insulinoma sizes were not significantly correlated.

The tumor xenograft was clearly detectable in all mice regardless of carbidopa premedication (Fig. 3). The mean time-activity curves resulting from the tumor

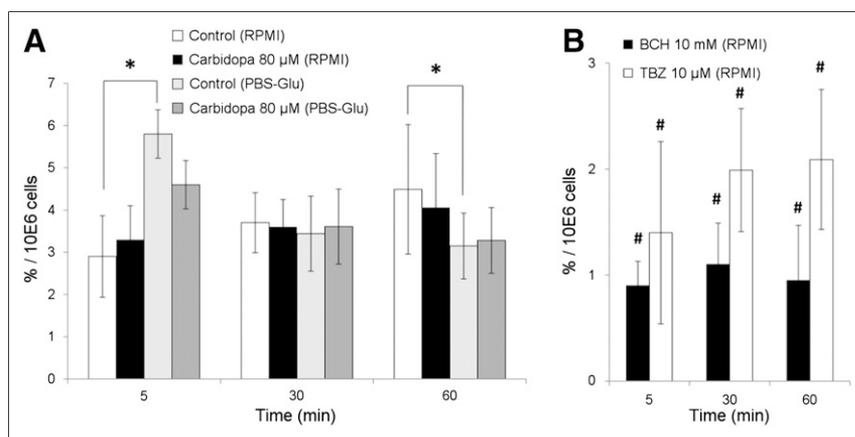


FIGURE 1. Accumulation of ^{18}F -FDOPA in RIN-5mF cells. Tracer accumulation was measured in classical RPMI culture medium and amino acid–free medium (PBS-Glu medium). Values represent mean percentage of uptake for 10⁶ cells \pm SE of mean. (A) Effect of carbidopa (80 μM) on ^{18}F -FDOPA uptake in RPMI culture medium (*n* = 12 for each condition) and in PBS-Glu medium (*n* = 4 for each condition). **P* < 0.05 (Mann–Whitney *U* test). (B) Effect of BCH (10 mM; *n* = 4) and tetrabenazine (TBZ; 10 μM ; *n* = 2) on ^{18}F -FDOPA uptake in RPMI culture medium. #*P* < 0.05 (Mann–Whitney *U* test).

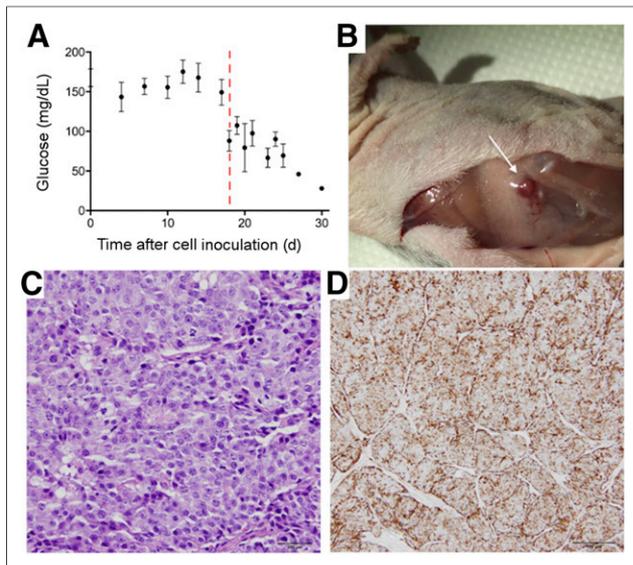


FIGURE 2. Functional and pathologic characterization of insulinoma xenografts. (A) Morning serum glycemia levels in blood samples collected from 10 nonfasting mice with xenografts. Dashed line represents introduction of 20% glucose solution instead of water. (B) Insulinoma xenograft of 1.4×1.3 mm (arrow) developed 15 d after subcutaneous injection of 6×10^6 RIN-5mF cells. Insulinoma-bearing mice died after hypoglycemic crisis (serum glycemia, 18 mg/dL). (C) Histologic analysis of RIN-5mF xenograft tumor showing epithelioid and spindle cells (hematoxylin–eosin stain, $\times 400$). (D) Immunohistochemical analysis of RIN-5mF xenograft tumor showing insulin-positive immunostaining.

xenografts in both carbidopa-treated mice and nontreated mice are shown in Figure 4. The dynamic profiles of tumoral ^{18}F -FDOPA uptake in carbidopa-treated and nontreated mice were similar. The insulinoma xenograft was characterized by an early increase in ^{18}F -FDOPA uptake after radiotracer injection, reaching a peak intensity about 5 min after radiotracer injection. Then, ^{18}F -FDOPA uptake progressively decreased over time until the end of the dynamic PET acquisition ($\sim 35\%$ reduction in the initial SUV). The tumor remained detectable during the whole PET examination in carbidopa-treated and nontreated animals (Fig. 3).

There was no negative influence of carbidopa on tumoral ^{18}F -FDOPA uptake in animals pretreated with carbidopa. On the contrary, insulinoma xenografts from carbidopa-treated mice showed significantly higher ^{18}F -FDOPA uptake ($P < 0.05$) over the whole PET acquisition than tumors from nontreated mice (Fig. 4).

Visually, carbidopa-treated mice showed increased ^{18}F -FDOPA uptake in abdominal organs, such as the pancreas and liver (Fig. 5); this finding could be explained by reduced peripheral radiotracer degradation by carbidopa and consequent increased availability for both the tumor and abdominal organs. Statistical significance was not reached, probably because of the small number of animals analyzed.

DISCUSSION

To the best of our knowledge, this is the first study evaluating the effect of carbidopa on ^{18}F -FDOPA uptake in β -cells and the related xenograft model in mice. The most important conclusions were that carbidopa did not induce a significant difference in ^{18}F -FDOPA uptake intensity in the β -cell line RIN-5mF and that no negative influence on PET detection of insulinoma xenografts in

mice premedicated with carbidopa was observed. On the contrary, carbidopa treatment significantly improved ^{18}F -FDOPA uptake in RIN-m5F tumor xenografts.

The accumulation of ^{18}F -FDOPA in RIN-5mF cells was sensitive to the presence of amino acids within the culture medium. In RPMI culture medium, ^{18}F -FDOPA cellular uptake was almost constant over 60 min. On the other hand, an important increase in ^{18}F -FDOPA uptake at the earliest time point (5 min) and then a progressive decrease were observed in amino acid–free medium. Early competition for cellular intake between ^{18}F -FDOPA and other amino acids present in RPMI culture medium could support this observation.

No statistically significant effect on the uptake of ^{18}F -FDOPA in RIN-5mF cells over time was observed, regardless of the culture medium. This finding indicates that the intracellular decarboxylation process in RIN-5mF cells was not considerably affected by the pharmacologic inhibition by carbidopa. The upregulation of AADC activity in NET cells supports this finding (10,17).

To our knowledge, there are no previously reported experimental data on the effect of carbidopa on the RIN-5mF cell line to allow further comparison. However, similar investigations were previously performed with BON cells. According to Neels et al. (11), the incubation of BON cells with carbidopa did not affect the accumulation of ^{18}F -FDOPA in full culture medium or PBS-Glu medium. Moreover, the accumulation of ^{18}F -FDOPA was more rapid and reached a much higher level in amino acid–free PBS-Glu medium than in standard culture medium. These results were partially confirmed by Kuik et al. (12), who recently reported a significant decrease in ^{18}F -FDOPA accumulation after carbidopa treatment only at late time points under conditions of amino acid depletion.

The experiment performed under BCH inhibition underlines the importance of the amino acid metabolic pathway in RIN-5mF cells. The deregulation of amino acid intake by specific pharmacologic agents targeting the L-amino acid transporter may emerge as an interesting therapeutic option, as previously observed during in vitro and in vivo studies of gliomas (18).

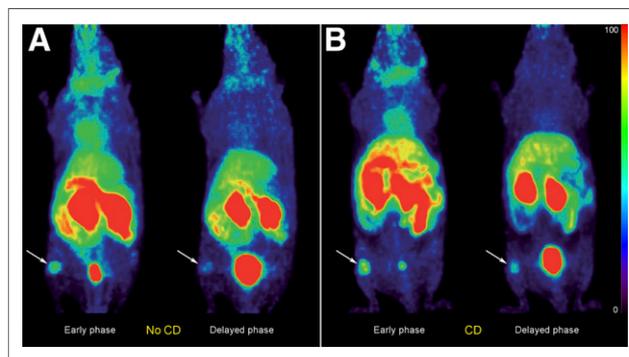


FIGURE 3. Representative anterior view of ^{18}F -FDOPA PET maximum-intensity-projection images obtained without (A) and with (B) carbidopa (CD) premedication of 2 nude mice with subcutaneous insulinoma xenografts of 38 and 52 mm^3 , respectively, in right hind legs (arrows). Images were reconstructed from summed time frames of 0–5 min (early phase) and 15–20 min (delayed phase) after intravenous ^{18}F -FDOPA injection. Insulinomas were detected regardless of carbidopa treatment. Moreover, tumor SUV in treated mice was higher than that in nontreated mice for both early and delayed PET acquisitions (SUVs: no CD, early phase, 0.94; no CD, delayed phase, 0.46; CD, early phase, 1.46; CD, delayed phase, 1.03).

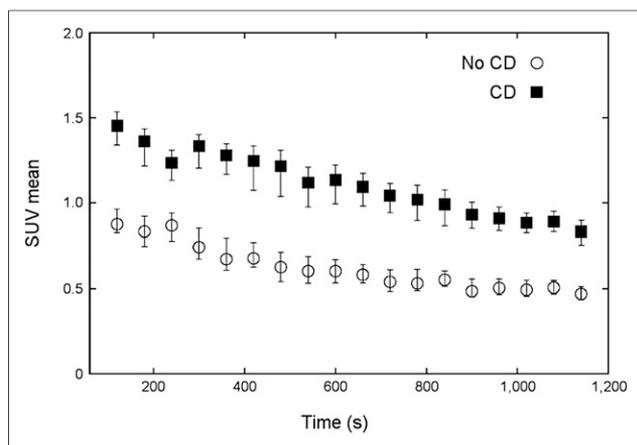


FIGURE 4. ^{18}F -FDOPA PET time-activity curves for insulinoma xenografts according to carbidopa (CD) premedication (no CD vs. CD). Tumors from CD-treated mice showed significantly higher ^{18}F -FDOPA uptake over whole PET acquisition than tumors from nontreated mice ($P < 0.05$). SUVs are reported as median and interquartile range.

Tetrabenazine led to a similar but less extensive decrease in ^{18}F -FDOPA uptake than BCH. In this experimental condition, ^{18}F -dopamine was not transported into cellular granules by vesicular monoamine transporters and thus was potentially cleared from cells.

The advantage of carbidopa administration has been suggested in mice with NET xenografts from BON cells and in patients with pancreatic NETs (11,19). Carbidopa improved image interpretation for ^{18}F -FDOPA PET by increasing tumoral uptake and decreasing physiologic pancreatic accumulation. We observed similar effects in nude mice bearing RIN-m5F insulinoma xenografts. Accordingly, under carbidopa inhibition, ^{18}F -FDOPA uptake was significantly higher than in nontreated mice.

In the present study, the carbidopa administration protocol was optimized before PET imaging. The combination of repeated anesthesia and the physical trauma from intraperitoneal injections of carbidopa was poorly tolerated in weakened mice with insulinoma-related chronic hypoglycemia. For this reason, in the experimental group, carbidopa was administered orally 1 h before small-animal PET imaging.

Overall, the absence of tumoral inhibition, the low physiologic pancreatic uptake, and the increase in ^{18}F -FDOPA availability could be considered the underlying effects of carbidopa premedication even in the RIN-m5F insulinoma xenograft model.

Regardless of carbidopa premedication, RIN-m5F insulinoma xenografts showed the highest uptake of ^{18}F -FDOPA within 5 min after radiotracer injection and then a continuous decrease until the end of the studied period. This kinetic pattern was close to that observed in vitro with a culture medium supplemented with D-glucose (PBS-Glu medium). The oral overload of glucose before small-animal PET imaging may help explain the results of the in vivo experiments.

We recently reported insulinomas showing pathologic ^{18}F -FDOPA uptake exclusively on early images in patients who underwent carbidopa-assisted PET/CT (6,7). In these cases, the influence of carbidopa on rapid tumoral washout could not be completely ruled out. In our preclinical model, the dynamic profiles of ^{18}F -FDOPA uptake in both carbidopa-treated mice and nontreated mice were similar. Hence, carbidopa premedication did not seem to be directly related to the dynamic pattern of ^{18}F -FDOPA uptake. Kauhanen et al. (9)

reported the disappearance of ^{18}F -FDOPA focal pancreatic uptake after carbidopa administration in 2 patients with histologically proven insulinoma and β -cell hyperplasia. In these cases, the negative PET result was more likely related to the nonoptimized PET acquisition protocol than to carbidopa-related tumoral inhibition. Parallel ^{18}F -FDOPA catabolic pathways through, for example, catechol O-methyltransferase warrant further investigation.

Significant differences between mice and humans in terms of methodologic issues regarding carbidopa dose, interspecies carbidopa metabolism, fasting conditions, and glucose charge before PET should be taken into consideration. Moreover, the growth pattern of our tumor xenografts was more rapid than that of human insulinomas, which are typically benign. Thus, caution in interpreting preclinical data is required. The malignant potential of our xenografts may have been amplified by the athymic status of the host (nude mice) and by the several cellular subcultures before subcutaneous implantation. However, 2 animals that underwent ^{18}F -FDG small-animal PET showed no significant radiotracer uptake in xenografts (data not shown). Such a finding is not typical for malignant tumors, including malignant insulinomas. Discordant results between in vivo and in vitro experiments have also been reported for evaluation of the effects of carbidopa on BON cells and related xenograft tumors (11).

Collantes et al. (15) reported the results of ^{18}F -FDOPA PET of 2 nude mice with RIN-m5F insulinoma xenografts. The PET results for both mice were positive, showing ^{18}F -FDOPA uptake about 60 min after intravenous ^{18}F -FDOPA injection. Neither dynamic PET acquisition nor carbidopa premedication was performed to allow further comparison with our results. Moreover, this group reported differential expression of type 2 vesicular monoamine transporters in RIN cells between in vitro and in vivo experiments; consequently, deductions about translation must be made with caution.

The small number of mice that underwent ^{18}F -FDOPA PET may represent a limitation of the present study. Moreover, no animal was examined twice, according to an intraindividual analysis, with and

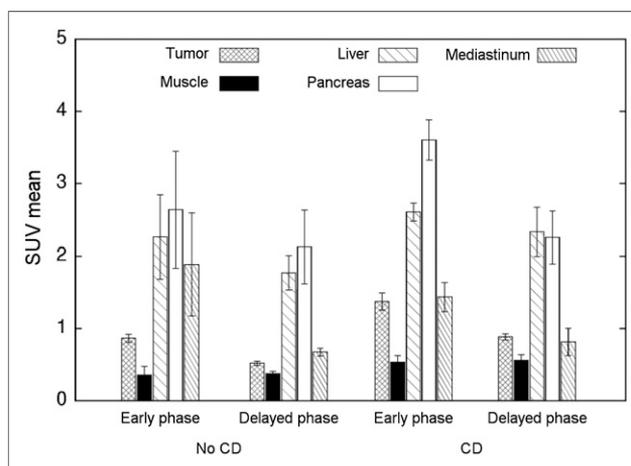


FIGURE 5. Biodistribution of ^{18}F -FDOPA in mice bearing insulinoma xenografts according to carbidopa (CD) premedication (no CD vs. CD). SUVs (mean \pm SE of mean) were obtained from summed time frames of 0–5 min (early phase) and 15–20 min (delayed phase) in dynamic PET study after ^{18}F -FDOPA injection. ^{18}F -FDOPA degradation in peripheral organs was inhibited by CD, with consequent increase in radiotracer availability and accumulation in abdominal organs. Statistical significance was not reached, probably because of small number of animals analyzed.

without carbidopa premedication. However, the high level of care required for the tumoral xenograft model, which was characterized by elevated mortality due to severe hypoglycemia before the appearance of measurable insulinoma xenografts, justified our study protocol.

CONCLUSION

Carbidopa did not influence in vitro ¹⁸F-FDOPA accumulation in RIN-m5F cells but improved tumor imaging in vivo. Our findings increase current knowledge about the ¹⁸F-FDOPA uptake profile of RIN-m5F cells and a related xenograft model. To our knowledge, the present work represents the first preclinical research specifically focused on insulinomas, with potential translational implications.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank Gerlinde Averous, MD, for pathological examinations of RIN-m5F insulinoma xenografts; Patrice Laquerriere, PhD, for assistance with statistics; and Lionel Thomas, PhD, and Bruno Jessel for technical support in animal care. Cisbio International-IBA Molecular is kindly acknowledged for providing ¹⁸F-FDOPA (DOPACIS).

REFERENCES

1. Okabayashi T, Shima Y, Sumiyoshi T, et al. Diagnosis and management of insulinoma. *World J Gastroenterol*. 2013;19:829–837.
2. Kauhane S, Seppänen M, Minn H, Nuutila P. Clinical PET imaging of insulinoma and beta-cell hyperplasia. *Curr Pharm Des*. 2010;16:1550–1560.
3. Christ E, Wild D, Reubi JC. Glucagon like peptide-1 receptor: an example of translational research in insulinomas—a review. *Endocrinol Metab Clin North Am*. 2010;39:791–800.
4. Koopmans KP, Neels ON, Kema IP, et al. Molecular imaging in neuroendocrine tumors: molecular uptake mechanisms and clinical results. *Crit Rev Oncol Hematol*. 2009;71:199–213.
5. Tessonnier L, Sebag F, Ghander C, et al. Limited value of ¹⁸F-F-DOPA PET to localize pancreatic insulin-secreting tumors in adults with hyperinsulinemic hypoglycemia. *J Clin Endocrinol Metab*. 2010;95:303–307.
6. Imperiale A, Sebag F, Vix M, et al. ¹⁸F-FDOPA PET/CT imaging of insulinoma revisited. *Eur J Nucl Med Mol Imaging*. 2015;42:409–418.
7. Imperiale A, Bahougne T, Goichot B, Bachellier P, Taïeb D, Namer IJ. Dynamic ¹⁸F-FDOPA PET findings after carbidopa premedication in 2 adult patients with insulinoma-related hyperinsulinemic hypoglycemia. *Clin Nucl Med*. 2015;40:682–684.
8. Koopmans KP, Neels OC, Kema IP, et al. Improved staging of patients with carcinoid and islet cell tumors with ¹⁸F-dihydroxy-phenyl-alanine and ¹¹C-5-hydroxy-tryptophan positron emission tomography. *J Clin Oncol*. 2008;26:1489–1495.
9. Kauhane S, Seppänen M, Nuutila P. Premedication with carbidopa masks positive finding of insulinoma and beta-cell hyperplasia in [¹⁸F]-dihydroxy-phenyl-alanine positron emission tomography. *J Clin Oncol*. 2008;26:5307–5308.
10. Uccella S, Cerutti R, Vigetti D, et al. Histidine decarboxylase, DOPA decarboxylase, and vesicular monoamine transporter 2 expression in neuroendocrine tumors: immunohistochemical study and gene expression analysis. *J Histochem Cytochem*. 2006;54:863–875.
11. Neels OC, Koopmans KP, Jager PL, et al. Manipulation of [¹¹C]-5-hydroxytryptophan and 6-[¹⁸F]fluoro-3,4-dihydroxy-L-phenylalanine accumulation in neuroendocrine tumor cells. *Cancer Res*. 2008;68:7183–7190.
12. Kuik WJ, Kema IP, Brouwers AH, et al. In vivo biodistribution of no-carrier-added 6-¹⁸F-fluoro-3,4-dihydroxy-L-phenylalanine (¹⁸F-DOPA), produced by a new nucleophilic substitution approach, compared with carrier-added ¹⁸F-DOPA, prepared by conventional electrophilic substitution. *J Nucl Med*. 2015;56:106–112.
13. de Vries EFJ, Luurtsema G, Brüsermann M, Elsinga PH, Vaalburg W. Fully automated synthesis module for the high yield one-pot preparation of 6-[¹⁸F]-fluoro-L-DOPA. *Appl Radiat Isot*. 1999;51:389–394.
14. Gazdar AF, Chick WL, Oie HK, et al. Continuous, clonal, insulin- and somatostatin-secreting cell lines established from a transplantable rat islet cell tumor. *Proc Natl Acad Sci USA*. 1980;77:3519–3523.
15. Collantes M, Barajas M, Quincoces G, et al. Lessons from ¹¹C-dihydrotetrabenazine imaging in a xenograft mouse model of rat insulinoma: is PET imaging of pancreatic beta cell mass feasible? *Q J Nucl Med Mol Imaging*. April 17, 2015 [Epub ahead of print].
16. Fuchs BC, Bode BP. Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? *Semin Cancer Biol*. 2005;15:254–266.
17. Zheng G, Dvoskin LP, Crooks PA. Vesicular monoamine transporter 2: role as a novel target for drug development. *AAPS J*. 2006;8:E682–E692.
18. Nawashiro H, Otani N, Shinomiya N, et al. L-type amino acid transporter 1 as a potential molecular target in human astrocytic tumors. *Int J Cancer*. 2006;119:484–492.
19. Orlefors H, Sundin A, Lu L, et al. Carbidopa pretreatment improves image interpretation and visualisation of carcinoid tumours with ¹¹C-5-hydroxytryptophan positron emission tomography. *Eur J Nucl Med Mol Imaging*. 2006;33:60–65.