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O-(2-\(^{18}\)F-fluoroethyl)-L-tyrosine (\(^{18}\)F-FET) uptake in insulinoma: first results from a xenograft mouse model and from human☆☆☆☆

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**A B S T R A C T**

**Introduction:** Herein we have evaluated the uptake of O-(2-\(^{18}\)F-fluoroethyl)-L-tyrosine (\(^{18}\)F-FET) in insulinoma in comparison with those of 6-\(^{18}\)F-fluoro-3,4-dihydroxy-L-phenylalanine (\(^{18}\)F-FDOPA) providing first data from both murine xenograft model and one patient with proved endogenous hyperinsulinemic hypoglycemia.

**Methods:** Dynamic \(^{18}\)F-FET and carbidopa-assisted \(^{18}\)F-FDOPA PET were performed on tumor-bearing nude mice after subcutaneous injection of RIN-m5F murine beta cells and on a 30-year-old man with type-1 multiple endocrine neoplasia and hyperinsulinemic hypoglycemia defined by a positive fasting test.

**Results:** Seven and three nude mice bearing a RIN-m5F insulinoma xenograft were respectively studied by \(^{18}\)F-FET and \(^{18}\)F-FDOPA PET. Insulinoma xenograft was detected in all the imaged animals. Xenograft was characterized by an early but moderate increase of \(^{18}\)F-FET uptake followed by a slight decline of uptake intensity during the 20 min dynamic acquisition. Tumoral radiotracer peak intensity and the highest tumoral-to-background contrast were reached about 5 minutes after \(^{18}\)F-FET iv. injection (mean SUV: 1.21 ± 0.10). The biodistribution of \(^{18}\)F-FET and \(^{18}\)F-FDOPA and their dynamic tumoral uptake profile and intensity were similar. In the examined patient, \(^{18}\)F-FDOPA and \(^{18}\)F-FET PET/CT showed one concordant focal area of well-defined increased uptake in the pancreatic tail corresponding to 11 mm histologically proved insulinoma. The SUVmax tumor to liver ratio was 1.5, 1.1 for \(^{18}\)F-FDOPA, 1.1, 1.1 for \(^{18}\)F-FET at early (0-5 min post injection) and delayed (5-20 min post injection) PET/CT acquisition, respectively. Despite the relatively low tumoral uptake intensity, insulinoma was clearly identified due to the low background in the pancreas. At the contrary, no \(^{18}\)F-FDOPA or \(^{18}\)F-FET tumoral uptake was revealed on whole-body PET/CT images performed about 30 min after radiotracer administration. Note of worth, the dynamic uptake pattern of \(^{18}\)F-FET and \(^{18}\)F-FDOPA were similar between human insulinoma and mice xenograft tumor.

**Conclusion:** \(^{18}\)F-FET PET compared equally to \(^{18}\)F-FDOPA PET in a preclinical RIN-m5F murine model of insulinoma and in one patient with insulinoma-related hypoglycemia. However, in both cases, the tumoral uptake intensity was moderate and the tumor was only visible until 20 min after radiotracer injection. Hence, caution should be taken before asserting the translational relevance of our results in the clinical practices. However, the structural analogies between \(^{18}\)F-FET and \(^{18}\)F-FDOPA as well as the limited pancreatic uptake of \(^{18}\)F-FET in human, encourage evaluating \(^{18}\)F-FET as diagnostic radiotracer for insulinoma detection in further prospective studies involving large cohorts of patients.

**1. Introduction**

Insulinoma is a pancreatic insulin-secreting neuroendocrine tumor (NET), usually solitary and benign. The uncontrolled insulin secretion may be responsible of severe hypoglycemia and patient death. Early
FDOPA as internalized in cells [18F-FET is not a choice. 68Ga-DOTATATE or carbidopa-assisted 18F-FDOPA PET/CT are hypoglycemia the molecular imaging is usually advocated to localize insulinoma in cases of suspected nesidioblastosis and post-gastric bypass hyperinsulinemia resulting in a sensitivity of 97.7% for insulinoma detection [7]. However, these radiopharmaceuticals are not yet commercially available and are used in clinical trials in few selected centers only. Although somatostatin receptors imaging has typically been associated with a low sensitivity for insulinoma detection, it has been recently showed that 68Ga-DOTATATE and DOTATOC PET/CT could successfully detect with high sensitivity the cause of endogenous pancreatic hypoglycemia including insulinoma and neurofibromatosis [8,9]. Moreover, 68Ga-DOTA-peptide PET/CT allows accurate patient selection for peptide receptor radionuclide therapy (PRRT) in case of malignant insulinoma. At the same time, current advances in cancer biology have underlined the critical role of amino acids to support the metabolic demands of tumor. Hence, radiolabeled amino acids that specifically target the up-regulated amino acid transporters in tumor cells have been considered as potential diagnostic agents [10–12]. 6-[18F]-fluoro-3,4-dihydroxy-L-phenylalanine (18F-FDOPA) is a commercially available radiolabeled amino acid currently proposed as PET/CT molecular imaging probe for NETs diagnosis, including insulinoma [13]. 18F-FDOPA is internalized in cells via the sodium-independent L-amino acids transporter system (LAT), decarboxylated and stored into neurosecretory granules [14]. The positive impact of 18F-FDOPA PET/CT for insulinoma diagnosis, particularly when combined with carbidopa premedication and early pancreatic images, has been recently demonstrated in preclinical studies based on insulinoma xenograft mouse model [15], and in clinical investigations involving adult patients with insulinoma-related hyperinsulinemic hypoglycemia [16,17]. However, although 18F-FDOPA PET/CT does function, it is far from ideal. Moreover, the electrophilic substitution, the conventional method for the production of 18F-FDOPA, suffers from low radiochemical yield and low specific activity [18].

A diagnostic algorithm has been recently proposed in order to localize potentially resectable insulinoma, to select patients with unresectable malignant tumors before PRRT treatment, and to exclude malignancy of insulinoma in cases of suspected neurofibromatosis and post-gastric bypass hypoglycemia. In patients with endogenous hyperinsulinemic hypoglycemia the molecular imaging is usually advocated to localize benign insulinoma after negative three phases contrast enhanced CT or MRI. 68Ga-NOTA-exendin-4 PET/CT represents the first choice. 68Ga-DOTATATE or carbidopa-assisted 18F-FDOPA PET/CT are recommended if 68Ga-NOTA-exendin-4 is not available. [2]

O-(2-[18F]-Fluoroethyl)-L-Tyrosine (18F-FET) is an artificial 18F-radiolabeled amino acid for PET/CT imaging. In analogy to 18F-FDOPA, the uptake of 18F-FET is due to its intracellular transport via the LAT system. However, after cellular internalization 18F-FET is not incorporated into proteins [20,21]. 18F-FET shows a significant uptake in brain tumors and plays a significant role in the diagnostic assessment of gliomas [22–24]. Moreover, a selective 18F-FET uptake was observed in squamous cell carcinoma despite disappointing results have been reported in most peripheral tumors [25]. 18F-FET can be produced with a high yield and easily distributed for clinical use. The in vivo stability, the high tumor accumulation and the low uptake in inflammatory tissue are some of the obvious advantages for 18F-FET utility in clinical setting. Interestingly, 18F-FET is known to have a low pancreatic uptake in humans providing a potential high contrast in a positive detection of insulinoma [26]. However, to the best of our knowledge, no evidences exist about the utilization of 18F-FET as a diagnostic PET agent for insulinoma neither in animal model nor in human.

In view of the above, the primary aim of the present study is to evaluate the feasibility of 18F-FET PET for insulinoma detection. With this as our starting point we have provided first data from a murine xenograft model of insulinoma investigated by 18F-FET and 18F-FDOPA PET/CT. Moreover, as a proof-of-concept study, we have head-to-head compared 18F-FET and 18F-FDOPA PET/CT in one patient with clinical suspicion of insulinoma and afterwards histologically proved after surgical excision.

2. Methods

2.1. Radiotracers

18F-FET for preclinical imaging was synthesized according to the method of Bourdier et al. using a Raytest Synchrom R&D module [27] at the Institut Pluridisciplinaire Hubert Curien (IPHC), Strasbourg, France. 18F-FET was obtained with a yield of 54–64% decay corrected. OMA cartridges, TET precursor (2S)-(2-tosyloxyethyl)-(N-trityl-tyrosine-tert-butyl ester) and authentic reference (non-radioactive FET) were obtained from ABX (Germany) and used as received. Solvent for reaction and HPLC purification were obtained from Aldrich (France) and used as received. 18F-FET (700 MBq/mL in phosphate buffer) was diluted with 0.9% NaCl for intra venous injection.

18F-FDOPA for preclinical imaging was obtained from CISBIO International (DOPACIS, Nancy-France) and was synthesized by electrophilic substitution following the method reported by de Vries et al. [28]. 18F-FDOPA doses were prepared by dilution of the radiopharmaceutical (220 MBq in 2.2 mL) in the appropriate volume of 0.9% NaCl (BBRAUN, veterinary grade) to reach an injection volume of 150 µL.

Both 18F-FET and 18F-FDOPA pharmaceutical grade for PET/CT clinical investigations were obtained from IASON GmbH (Graz, Austria).

2.2. Cell culture

The beta cell line RIN-m5F (ATCC CRL-11605TM) was used to develop the xenograft mice model of insulinoma [29]. RIN-m5F cells secrete insulin, and induce AADC. Cells were cultured in 75 cm² culture flasks containing 20 mL of RPMI 1640 medium without N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid, and glutamine (Invitrogen, US). Medium was supplemented with 10% foetal bovine serum (Gibco, USA) and 100 µg/mL of Gentamycine (Panpharma, France). Cells were grown at 37°C in a humidified atmosphere containing 5% CO₂ and subcultured twice a week. The viability and number of cells were determined with the trypan blue exclusion technique using a Kova Clastic Slide 10 (Hycor Biomedical, Kassel, Germany).

2.3. Animals and tumor xenograft model

Female athymic nude mice (Charles Rivers) were kept at constant temperature (22°C) and humidity (40%) with 12-h light/dark cycles and were allowed free access to forage and water until the beginning of each imaging procedure. Animals were housed in individually ventilated cages with HEPA filters in the dedicated IPHC facility in Strasbourg.
A cell suspension of six million RIN-m5F cells in 100 μL of PBS was injected subcutaneously in the mice right hind leg under inhalation anesthesia (1.5–2% of isoflurane). Tumor width and length were measured (mm) with a caliper in order to calculate the tumor volume using the approximated formula: Volume = length × width^2/2 (mm^3). To evaluate tumor functionality (i.e., insulin secretion), morning serum glycemia levels in the xenograft mice were assessed from blood samples collected from the tail vein of non-fasted mice using a commercial blood glucose monitoring system (Contour TS, Bayer). To reduce the occurrence of lethal hypoglycemia, water was replaced by a 20% glucose solution 15 days after the subcutaneous injection of RIN5-mf cells suspension [15].

All animal experiments were performed in accordance with the European Institutes of Health Guidelines regarding the care and use of animals for experimental procedures. The study protocol was approved by the Alsace Regional Ethics Committee for Animal Experimentation (Approval ID: APARI#2944).

2.4. μPET Imaging

Animals were scanned when the tumor reached the minimum dimension of 2.5 × 2.5 mm^2 or a volume of greater than 20 mm^3. Animals underwent 20 min of dynamic 3D PET acquisitions starting 2 min after 18F-FET (8.8 ± 1.7 MBq in 150 μL) or 18F-FDOPA (5.1 ± 0.9 MBq in 150 μL) intravenous injection through the jugular vein. 18F-FDOPA PET was performed after caridopina administration via a gavage needle 1 hour before the intravenous radiotracer injection. All μPET studies were performed on a dedicated small-animal PET scanner (Iris, Inviscan) at IPHC’s small animal imaging facilities. The Iris μPET system consists in 16 block detectors arranged in two octagonal rings. Each module comprises a LYSO:Ce matrix of 702 crystals of 1.6 mm × 1.6 mm × 12 mm, with a pitch of about 1.7 mm, coupled to a 64 anodes PMT (Hamamatsu H8500). This geometrical configuration leads to an axial coverage of 95 mm and a 80 mm transverse field of view. The overall spatial resolution is less than 1.5 mm with a sensitivity of 8.5 % according to the NEMA standards.

Complementary CT was performed after 18F-FET μPET on a cone beam mCT system developed in our Institution [30,31]. Each CT examination (2 FOV, 42 sec/FOV, 40 kV, 75 μA) was performed right after μPET examination without changing the animal position using an interchangeable bed and used only as anatomical reference for PET images.

Throughout the whole examinations, mice were held at a constant temperature of 37 °C. During both μPET investigations, animals temperature and respiration rate were monitored (Minerve) to assess the depth of anesthesia. As a preventive measure of hypoglycemic crisis, 300 μL of 20% glucose solution were administrated orally to each animal using a gavage needle thirty minutes before imaging procedure. For μPET imaging, ketamine and xylazine anesthesia was induced by an intraperitoneal injection of 50 mg/kg ketamine, 6 mg/kg xylazine in a volume of 100 μL/30 g body weight, and maintained with 1.5% isoflurane in medical air during the entire procedure using a calibrated vaporizer.

Acquired list-mode PET data were binned into twenty frames of 1-min each and four frames of 5-min each. Data were reconstructed using the iterative 3D OSEM algorithm (6 iterations, 8 subsets) into a 201 × 201 × 120 3D volume. The resulting 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, radioactivity decay and dead time. No attenuation and scatter corrections were applied. CT data were reconstructed using the Feldkamp algorithm into a 256 × 192 × 596 3D volume with 0.2 × 0.2 × 0.2 mm^3 voxels. Hybrid PET/CT images were obtained by matching PET and CT data using a semi-automated volume based registration algorithm.

PET data analyses were performed using the AMIDE software package (http://amide.sourceforge.net) as previously detailed [15]. Briefly, a focal non-physiological increase in radiotracer uptake in the area of tumor development was considered as positive PET result. An elliptical volume of interest (VOI) was drawn on the tumor in order to assess the 18F-FET uptake. Standardized Uptake Value (SUV) was calculated using the mean voxel value within the VOIs and then used for generating time-activity curves over the whole dynamic PET acquisition. VOI was drawn on the tumor using the image resultant from the first 5 min PET acquisition. Tumoral VOI activity was first corrected by the mean voxel value measured in the contralateral thigh muscle. Afterwards, only voxels higher than 40% of the maximum voxel value in the tumor were considered for further analysis. Finally, time-activity curves were extracted from VOIs over the whole dynamic PET acquisition. Standardized Uptake Values (SUVs) were calculated using the mean voxel value within the VOIs. SUVs were recorded and used for generating time-activity curves.

2.5. Clinical 18F-FET and 18F-FDOPA PET/CT

In attempt to evaluate insulinoma 18F-FET uptake and to compare it with 18F-FDOPA uptake, one patient with endogenous hyperinsulinemic hypoglycemia underwent both PET/CT investigations (Biograph mCT TOF, Siemens Healthcare). 18F-FET and 18F-FDOPA PET/CT acquisition protocols were strictly the same including: [1] a dynamic PET acquisition centered over the upper abdomen (12 frames of 10 seconds, 3 frames of 60 seconds, and 1 frames of 15 min) after the iv injection of 270 MBq of 18F-FET or 226 MBq/kg of 18F-FDOPA, [2] a delayed whole body acquisition starting immediately after the end of the dynamic phase (3 min/step), CT was performed without contrast enhancement in any case. PET data were reconstructed iteratively (OSEM algorithm) using CT data for attenuation correction. Co-registered images were displayed on a workstation and interpreted by one experienced nuclear medicine physician who was not aware of pancreatic tumor location. A positive pancreatic abnormality was defined as a focal area of increased radiotracer uptake compared to the surrounding tissue. For quantitative assessment of 18F-FET and 18F-FDOPA uptake, maximum standardized uptake values (SUVmax) were measured. Tumoral SUVmax was defined within a spherical VOI centered on the tumor and including it completely.

In keeping with local institutional guidelines, patient gave written informed consent to perform 18F-FDOPA and 18F-FET PET/CT, as well as to use anonymized personal data extracted from his medical records for scientific purposes.

2.6. Data analysis

Differences between animal groups were tested for statistical significance using the non-parametric Mann-Whitney U-test for independent samples. Wilcoxon matched-pairs signed rank test was used to compare results between early and delayed PET acquisitions in the same animal group. For convenient purpose, results are represented as median and interquartile range. p<0.05 was considered as significant. Statistical analysis was performed using GraphPad, Prism 7 software package.

3. Results

3.1. 18F-FET μPET results in RIN-m5F-related xenograft mouse model

RIN-m5F insulinoma xenograft was developed in ten athymic nude mice (23–28 g, 8–10 weeks-old). Three out of ten animals died of lethal hypoglycemnic crisis before 18F-FET μPET. Hence, seven mice were scanned 3–4 weeks after the subcutaneous inoculation of RIN-m5F cells suspension. In these cases, the tumor xenograft mean volume was 31 mm^3 ranging from 20 mm^3 to 42 mm^3. Serial measurements of morning serum glycemia levels performed before and after the subcutaneous cellular injection underlined a progressive decrease of blood glucose concentration in mice, confirming the secretory character of the tumor xenograft. Glycemia mean value was 175 mg/dl (range: 306–299).
Insulinoma xenograft was clearly detected in all the seven imaged mice during the whole μPET dynamic examination. Representative 18F-FET μPET images are shown in Fig. 1. The mean time-activity curves resulting from the tumor xenograft, muscle and liver are reported in Fig. 2A. The tumor was characterized by an early increased 18F-FET uptake followed by SUV decline over time until the end of the 20 min dynamic PET acquisition of about 23% (Fig. 2B). Pancreas, liver and kidneys (main 18F-FET urinary excretion) showed intense radiotracer uptake. A moderate but progressive and homogeneous radiotracer uptake characterized skeletal muscles over the dynamic PET acquisition. Tumoral radiotracer peak intensity and the best tumor-to-background contrast were reached about 5 minutes after the 18F-FET iv injection. The mean SUV of 18F-FET assessed on the insulinoma xenograft was 1.21 ± 0.10 at the early phase (0–5 min post injection) and 0.93 ± 0.06 at the delayed phase (15–20 min post injection) of the dynamic PET acquisition (p = 0.016).

3.2. 18F-FET and 18F-FDOPA microPET comparison in xenograft mouse model

An independent series of three athymic nude mice (females, 24–28 g, 8–10 weeks-old) bearing a RIN-m5F insulinoma xenograft was investigated by 18F-FDOPA μPET after carbidopa premedication (20 mg in 100 μL of NaCl per os), μPET results from these animals were detailed by our team elsewhere [12]. Briefly, the mean SUV of 18F-FDOPA assessed on the insulinoma xenograft was 1.37 ± 0.19 at the early phase (0–5 min post injection) and 0.89 ± 0.09 at the delayed phase (15–20 min post injection) of the dynamic PET acquisition. Comparing 18F-FET to 18F-FDOPA activity/time curves, no significant differences were observed over the dynamic PET acquisitions (Fig. 2C). In particular, tumoral SUV values measured at both early and delayed PET acquisition after 18F-FET iv injection were comparable to those obtained after 18F-FDOPA administration (p = 0.31 and p = 0.72, respectively). Moreover, tumoral SUV measured during both 18F-FET and 18F-FDOPA PET/CT was always lower than hepatic SUV.

3.3. 18F-FET and 18F-FDOPA PET/CT comparison in one patient

A 30-year-old man with type-1 multiple endocrine neoplasia (MEN-1) syndrome was referred to the Internal Medicine of our Institution for further evaluation of hyperinsulinemic hypoglycemia defined by a positive fasting test. Contrast-enhanced MRI revealed the presence of four nodules ranging from 5 to about 20 mm in the body and tail of the pancreas. Lesions were hyperintense and hypointense on T2- and T1-weighted sequences, respectively, without typical arterial hyper enhancement after gadolinium injection preventing a final lesion characterization (Fig. 3). Endoscopic ultrasound-guided fine needle aspiration was not conclusive. Carbidopa-assisted 18F-FDOPA and 18F-FET PET/CT showed one concordant focal area of moderate but well-defined increased uptake in the pancreatic tail, visualized until about 20 min after radiotracer injection (Fig. 4). The SUVmax tumor to liver ratio was 1.5 and 1.1 for 18F-FDOPA, and 1.1 and 1 for 18F-FET at early (0–5 min post injection) and delayed (5–20 min post injection) PET/CT acquisition, respectively. Moreover, no 18F-FDOPA or 18F-FET uptake abnormalities were detectable on whole-body PET/CT images performed about 30 min after radiotracer iv injection. Pathological examination after surgery confirmed the presence of two insulinomas of 7 mm in the body (ki67%: 1, grade 1) and 11 mm in the tail (ki67%: 3, grade 2), respectively, without lymphatic spread.

4. Discussion

To the best of our knowledge, this is the first study that evaluates the accumulation of 18F-FET in insulinoma and compares both kinetic and intensity of 18F-FET uptake to that of 18F-FDOPA in a xenograft mice model. Moreover, as a proof-of-concept study we have provided the head-to-head comparison between 18F-FET and 18F-FDOPA PET/CT in one patient. As a whole, large similarity between 18F-FET and 18F-FDOPA behaviors has been pointed out in xenograft animal model and human insulinoma.
The development of different strategies for targeting amino acid metabolism represents a growing research direction towards innovative approaches for tumor diagnosis. Accordingly, in the past years, radiolabeled amino acids have been extensively proposed for PET/CT imaging in oncology mainly due to the overexpression of membrane amino acid transporters in several tumor cell lines and consequent tumoral amino acid avidity [32]. Among the proposed radiolabeled amino acids, 18F-FDOPA quickly found its own place for clinical investigations of patients with neuroendocrine tumors. The synthesis of 18F-FDOPA is commonly based on electrophilic fluorination but alternative procedures have been investigated and thus proposed to afford faster and cheaper radiotracer production [18]. Moreover, besides the research of a more efficient method for 18F-FDOPA production, considerable efforts have been devoted to the development of surrogate radiopharmaceuticals that could replace or complement 18F-FDOPA (Fig. 5). In this respect, 3-O-methyl-FDOPA (18F-OMFD) in 1991 [33] and more recently 18F-3-[2-18F]fluoroethyl-L-tyrosine (18F-OFED) were synthesized [34]. According to preliminary data, 18F-OFED shows the same bio distribution pattern of 18F-FDOPA but benefits of a more efficient nucleophilic synthesis, resulting in higher specific activity. Thus, 18F-OFED might be proposed as a potential alternative to 18F-FDOPA.

18F-FET is a further tyrosine/phenylalanine analog. 18F-FET synthesis is based on a nucleophilic process and therefore profits of the same production advantages of 18F-OFED. Moreover, like the other tyrosine derivatives, it shares the tyrosine skeleton and the same stereoisomer of 18F-FDOPA (Fig. 5). Some of the advantages of 18F-FET utilization in clinical setting are the in vivo stability, the high tumor accumulation and the low uptake in inflammatory tissue. Contrarily to glioma, inadequate results have been reported in most peripheral tumors and inflammatory process [36–40]. However, it has to be considered, that PET images in such studies were obtained at 30-60 min after radiotracer iv. injection. Based on our results obtained with insulinoma, the timing of images acquisition may play a role allowing increase of 18F-FET PET sensitivity. The evaluation of early PET images performed 5-20 min after radiotracer iv. injection could represent an interesting clinical research direction in patients with peripheral tumors and inflammatory process.

A comparison of the diagnostic performance of 18F-FET and 18F-FDOPA PET/CT for gliomas detection has been recently reported suggesting that both radiotracers could be used equally in this clinical setting [18]. Thus, the structural analogies between 18F-FET and 18F-FDOPA as well as the limited pancreatic physiological uptake of 18F-FET in human, encouraged us to evaluate 18F-FET as diagnostic radiotracer for insulinoma detection. Obviously, the RIN-m5f xenograft preclinical model used in our experimental protocol significantly differs compared to benign human insulinoma in term of tumoral growing pattern, insulin secretion, and anatomic localization, representing a limitation of our study. Anyhow the RIN-m5f model in rodents compared...
perfectly with some atypical cases of human insulinomas showing a prompt uptake of $^{18}$F-FDOPA without sustained tumoral retention [13,14]. This delicate model enables us to compare $^{18}$F-FET versus $^{18}$F-FDOPA on a rodent model before further evaluation in human. In our preclinical model and in one patient with endogenous hyperinsulinemic hypoglycemia, the dynamic uptake pattern and the uptake intensity of $^{18}$F-FET and $^{18}$F-FDOPA were almost the same in the tumor (Figs. 3 and 4). In animal xenograft model as well as in human, both radiotracers showed early insulinoma visualization and a tumoral washout within 20 minutes. This feature was previously reported and discussed in the case of $^{18}$F-FDOPA in both RIN-m5f xenograft-based preclinical model [12] and in some patients with insulinoma [13,14].

Contrary to $^{18}$F-FDOPA, $^{18}$F-FET exhibits a delayed clearance from the blood pool and radioactivity in the blood is rather high up to 20 min post-injection [36]. This is visible in the early $^{18}$F-FET PET images of mice showing high radioactivity in the heart (Fig. 1). Similarly, the aorta is visible in the early $^{18}$F-FET PET images of the patient with insulinoma (Fig. 4B) but not in the corresponding $^{18}$F-FDOPA images (Fig. 4A). Thus, the high blood pool retention could be considered as a reason for $^{18}$F-FET uptake in insulinoma.

At present, no definitive conclusions can be made explaining the absence of durable retention of both $^{18}$F-FET and $^{18}$F-FDOPA in selected human benign insulinoma. In animal, the rapid growing pattern of the xenograft possibly translates an increased malignant potential eventually related to the athymic status of the nude mice and to the several cellular subcultures before subcutaneous injection, thus introducing a bias in image interpretation. Moreover, some crucial biological properties of $^{18}$F-FET are largely unknown in particular its metabolic pathway after cellular internalization by the LAT system, which works exclusively as an exchanger of amino acids [35,36]. Although speculative, two hypotheses might be considered:

1. $^{18}$F-FET undertakes no metabolic processes so it just as rapidly goes out of cells again defending the positivity of early PET images only. However, Habermaier et al. [21] have recently revealed that intracellular $^{18}$F-FET trapping may be caused by the asymmetry of its intracellular and extracellular recognition by LAT1 and that $^{18}$F-FET is an influx substrate for LAT1 but is not a good efflux substrate. In view of the above, insulinoma visualization could mainly depend from increased vascularization. Nevertheless, the insulinomas developed in our patient were not hyper vascularized as suggested by the lack of typical contrast enhancement on the arterial phase of MRI investigation (Fig. 3). Interestingly, the $^{18}$F-FDOPA showed the same uptake profile while its intracellular trapping machinery is well admitted. Thus, the existence of more complex mechanisms explaining the tumoral $^{18}$F-FET accumulation, eventually associated to a variable vascular contribution, is plausible.

2. $^{18}$F-FET could be rapidly converted in $^{18}$F-OFED or $^{18}$F-fluoroethyltyramine and metabolized by intracellular catecholamine-O-methyl transferase (COMT) or the monoamine oxidase (MAO), preventing any prolonged cellular retention.

Unfortunately, no data are still available to validate the previous assumptions and further investigations are needed.

In conclusion, in the considered preclinical and clinical insulinoma model, $^{18}$F-FET compares equally to $^{18}$F-FDOPA. Moreover, the same uptake pattern was observed on the xenograft mice model and in one patient with insulinoma. However, although $^{18}$F-FET appears potentially...
Fig. 4. Carbidopa-assisted $^{18}$F-FDOPA (A) and $^{18}$F-FET PET/CT results (B) in a 30-year-old patient with hyperinsulinemic hypoglycemia in the setting of MEN1 syndrome. $^{18}$F-FDOPA and $^{18}$F-FET PET/CT showed a concordant focal area of increased radiotracer uptake corresponding to a 11 mm nodule of the pancreatic tail. Lesion was visualized during the first twenty minutes of $^{18}$F-FDOPA and $^{18}$F-FET PET acquisition.

Fig. 5. Chemical structures of selected tyrosine based $^{18}$F-radiolabeled radiotracers.

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attractive for clinical PET/CT investigation due to its clinical availability at pharmaceutical grade, its structural analogues with \( ^{18} \text{F}-\text{FDOPA} \), and its limited pancreatic physiological uptake, it seems suboptimal in patients with endogenous hyperinsulinemic hypoglycemia particularly in those cases related to small size insulinomas and relative amino acid metabolism. Thus, if available, radiolabeled GAL-1 analogues based imaging remains the most appropriate molecular diagnostic choice in challenging clinical situation when conventional imaging is negative or inconclusive. Nevertheless, we think that the clinical role of \( ^{18} \text{F}-\text{PET/CT} \) for insulinoma detection should be further explored in prospective studies involving large cohorts of patients.

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