Positron-Emission Tomography Imaging of Early Events after Transplantation of Islets of Langerhans

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The aim of our study was to assess cell trafficking and early events after intraportal islet transplantation. Sprague-Dawley rat islets were incubated for various times, with various concentrations of 2-[^{18}F]fluoro-2deoxy-D-glucose (FDG), and in presence of various glucose concentrations. FDG-labeled syngeneic islets or FDG alone were injected in rats. Radioactivity was measured in the liver and in various organs by positron-emission tomography for 6 hours. FDG uptake increased with incubation time or FDG concentration and decreased in presence of glucose. In vivo, all islets implanted in the liver, with an uptake 4.4 times higher than controls (44.2% vs. 10.1%, $P=0.02$). Radioactivity in the liver decreased at the same rate after injection of labeled-islets and FDG alone. Ex vivo labeling of islets and imaging of posttransplant early events were feasible. Islets engrafted exclusively in the liver. No islet loss could be demonstrated 6 hours after transplantation.

Keywords: Islet transplantation, Positron-emission tomography, Fluorodeoxyglucose.

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In recent years, results of pancreatic islet transplantation have improved significantly, with an insulin-independence rate of approximately 80% at 1 year (*1*). However, recipients of successful islet transplants have an islet function of only 20% compared with control individuals (*1*). After 3-year follow-up, half of them have been put back on insulin (*2*). This loss of endocrine cells is thought to already take place early after transplantation, as supported by a peak of C-peptide and a massive beta cell release in the circulation in the early hours after transplantation (*3*).

This loss is difficult to study because the amount of insulin produced by islets during a glucose challenge is the only way to approximate the amount of functioning insulin-producing cells (*4*), and no method can accurately assess islet loss after infusion. Moreover, there is no evidence that all islets injected into the portal vein implant in the liver or that none of them traffic to other organs with supposedly poor implantation rates.

Islet imaging is currently considered a potential method to assess posttransplant events, and considerable efforts are being devoted to develop efficient techniques aimed at visualizing transplanted islets (*5*). In spite of the lack of beta–cell-specific tracers (*6*), we have chosen to label islets ex vivo before intraportal transplantation to study early post-transplant events using positron-emission tomography (PET) imaging.

Male Sprague-Dawley rats (Janvier, Le Genest, France), weighing 350 to 400g, were used according to protocols approved by the institutional animal care and use committee. Islets were isolated under a protocol routinely used by our group (*7*). They were cultured overnight in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Basel, Switzerland) containing 11 mM glucose, 1 mM sodium pyruvate (Gibco), 10% fetal calf serum (Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin (hereafter referred as completed DMEM). After culture and dithizone staining, islet purity was 77.5±4.25%. Viability assessed by propidium iodide and fluorescein diacetate staining was 86.88±9.23%.

Clinical grade 2-[^{18}F]fluoro-2deoxy-D-glucose (FDG) was produced by the cyclone 18/9 cyclotron (IBA, Louvain-La-Neuve, Belgium) at Geneva University Hospitals. For labeling tests, 300 rat islet equivalents (IEq) were incubated at 37°C with FDG in 250 μL glucose-free completed DMEM. Duration of incubation, FDG activity, and glucose concentrations were modified as further indicated in Figure 1. At the end of the incubation period, islets were washed three times with glucose and FDG-free DMEM, and radioactivity was measured with an ISOMED 2000 ionization chamber (Nuklear Medizintechnik, Dresden GmbH, Germany). To express radioactivity caused by FDG uptake or outflow only, all measured values were corrected for the time at the end of incubation according to the half life of ^{18}F (110 min). FDG integration in the islets was expressed as "integrated radioactivity," defined as the measured radioactivity in the islets or as "percent integration," calculated as activity in islets/(activity in islets and supernatant).

Infusions were performed under anesthesia (16 mg/Kg intraperitoneally [IP] xylazine and 120 mg/Kg IP ketamine) 5 minutes before starting the first PET imaging. FDG alone (0.875±0.938 MBq) or 2,000 FDG-labeled IEq (0.596±0.316 MBq) were injected through a 22 G colonic venous catheter (3 animals per condition). A colonic catheter (Optiva 2, Johnson and Johnson, Spreitenbach, Switzerland) was selected because it allowed for injections in the portal tree without disturbing portal blood flow, which could potentially modify liver blood wash-out and PET imaging quality. At the end of

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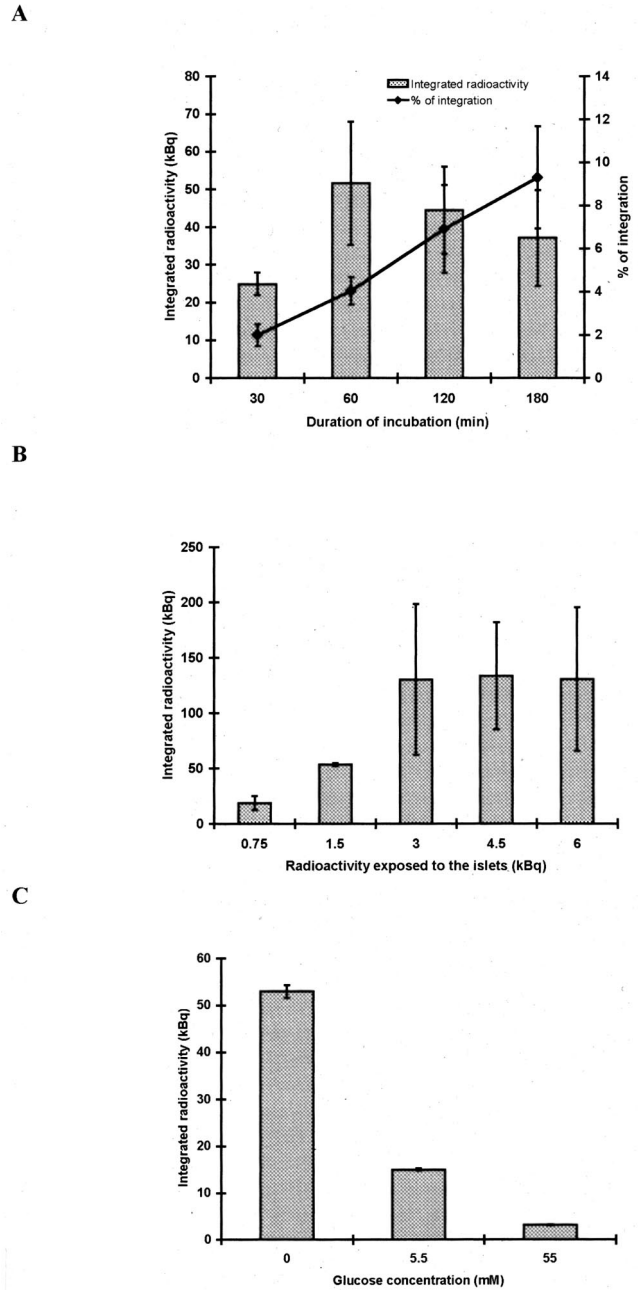

FIGURE 1. In vitro labeling of rat islets with 2-[^{18}F]fluoro-2deoxy-D-glucose (FDG). Results expressed as "integrated radioactivity" (measured radioactivity in the islets) and "percentage of integration" (activity in islets/[activity in islets and supernatant]±SD). All experiments were performed in triplicate. (A) After incubating 300 islet equivalents (IEQ) in 250 μL glucose-free Dulbecco's Modified Eagle Medium (DMEM) at 37°C with 1.5 MBq, the "percentage of integration" demonstrated an linear increase with the duration of incubation. Because of the short half-life of ^{18}F (110 min), the "integrated radioactivity" reached a peak at 60 minutes before decreasing again. (B) After incubating 300 IEQ in 250 μl glucose-free DMEM at 37°C for 60 minutes, labeling rate increased with the quantity of radioactivity exposed to the islets at the beginning of the experiment before reaching a plateau at 3 MBq. (C) Labeling was competitively inhibited by increasing concentrations of glucose in the incubation medium (incubation of 300 IEQ in 250 μL DMEM at 37°C for 60 min with 1.5 MBq).

the injection, the syringe and the catheter were flushed three times with 0.9% saline solution.

Scanning was performed on the whole body three dimensionally (3D) ECAT ART tomography (CTI/Siemens, Knoxville, TN). The 3D emission study lasted for 18 minutes before the postinjection transmission measurement (7 min). The default parameters used in clinical routine were applied (2 iterations, 8 subsets) followed by a postprocessing Gaussian filter (kernel FWHM=6.0 mm). The reconstructed images consist of 163 transaxial slices with 128×128 resolution and a voxel size set to 1.72×1.72×1.72 mm^3. For each studied organ, the region of interest was selected on the transaxial slices. The sum of radioactivity was determined for each organ and was corrected for the time of injection. Finally, the specific organ uptake was calculated by computing the ratio between the injected radioactivity and the specific organ radioactivity.

Statistical analysis was performed using the Statistica software (StatSoft, Tulsa, OK). Continuous variables were compared with the Student *t* test. Values of $P<0.05$ were considered as statistically significant.

In vitro labeling of rat islets with FDG demonstrated a linear increase in the percentage of integration according to the duration of incubation. Because of the short half life of ^{18}F (110 min), the integrated radioactivity reached a peak at 60 minutes before decreasing again (Fig. 1A). By incubating 300 IEQ islets in 250 μL glucose-free DMEM at 37°C, the labeling rate increased according to the radioactivity exposed to the islets at the beginning of the experiment before reaching a plateau at 3 MBq (Fig. 1B). Finally, labeling was competitively inhibited by an increasing concentration of glucose in the incubation medium (Fig. 1C). Islet viability before and after incubation were similar.

One hour after intraportal transplantation, liver radioactivity uptake increased 4.4-fold with FDG-labeled islets as compared with FDG alone (9.7±7 vs. 43.23±11.4, $P=0.02$). After injection, liver radioactivity decreased hourly by 44.68±15.11% for FDG alone and by 48.95±5.8% for FDG-labeled islets until 6 hours postinjection ($P=0.58$) (Fig. 2). In

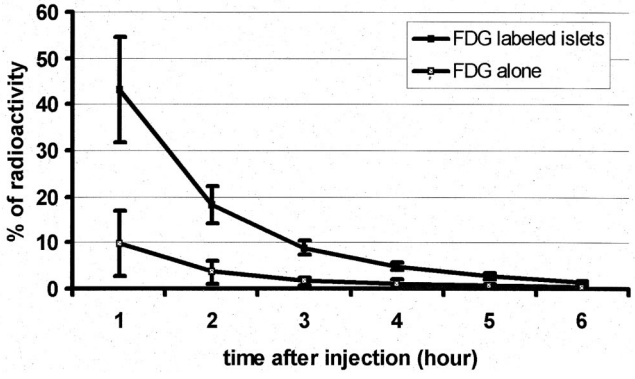

FIGURE 2. Liver uptake detected hourly by positron-emission tomography during the first 6 hours after injection. Uptake was significantly higher after FDG-labeled islet injection compared with FDG alone. Over time, liver radioactivity decreased hourly by 44.68±15.11% for FDG alone and by 48.95±5.8% for FDG-labeled islets ($P=0.58$, 3 animals per condition).

brain, lungs, heart, kidneys, and bladder, the signal was significantly stronger in cases of FDG-alone injection as compared with FDG-labeled islets (Table 1). Integration in the remaining organs was marginal.

In this study, we show that ex vivo labeling of islet of Langerhans with FDG is feasible, demonstrating an exclusive implantation in the liver after intraportal transplantation and PET imaging. Up to 6 hours after transplantation, labeled islets and FDG alone have a similar clearance of liver radioactivity, and no islet loss can be demonstrated.

We elected to use PET technology because of its high sensitivity, allowing detection and quantification of signals as small as 3 mm in size. FDG has been selected as the β^+-emitting marker. It is broadly used clinically and is easily available. Moreover, FDG exploits normal metabolic pathways, avoiding the risk of any functional alteration of the cells. It has been previously used with various cell types (*8–11*). To our knowledge, no study of FDG-radio labeled islet trafficking has been reported so far. In our model, a maximum of 4.9 MBq/Kg were used, whereas up to 250 MBq are used in humans, whatever their weight.

One hour after intraportal transplantation, FDG-labeled islets demonstrated a liver uptake 4.4 times higher than FDG alone. This site is the only one showing a stronger uptake with radio-labeled islets, showing no or only a nonclinically relevant amount of islets implants in other sites, such as the lungs or the brain.

As demonstrated by the in vitro FDG uptake experiments, FDG competes with glucose for uptake by the cell (Fig. 1C). It is phosphorylated into FDG-6-phosphate by hexokinase but cannot be further processed into fructose-6-phosphate because of the lack of a hydroxyl group on the second carbon. Part of the FDG-6-phosphatase is then dephosphorylated back to FDG. According to in vitro studies, 20%, 45%, and 65% of the initial FDG has left the cell 0.5, 2, and 4 hours after the end of the incubation (*6*, *8*, *10*, *11*). Our in vivo experiments (Fig. 2) demonstrated a faster decrease of organ radioactivity, reaching 40% to 50% hourly. This decrease was similar with FDG alone or FDG-labeled islets, demonstrating no significant islet loss during the 6-hour study period. This decrease of radioactivity was similar in cases of syngeneic (rat to rat) or xenogeneic transplantation (human islets in rats, data not shown) and was obviously related to the in vivo radioactivity washout by the hepatic blood flow.

TABLE 1. Specific organ tracer uptake

Organs	FDG-labeled islets	FDG alone	*P*
Brain	0.28±0.15	3.24±2	0.04
Lungs	0.39±0.22	2.37±0.37	<0.01
Heart	0.3±0.17	2.23±0.93	0.02
Liver	43.23±11.41	9.75±7	0.02
Kidneys	1.12±0.48	9.25±5.39	0.03
Bladder	0.99±0.46	13.05±10.13	0.09
Catheter	1.09±0.1	0.63±0.42	0.12

Specific organ uptake: uptake (% of injected radioactivity±SD) detected by positron-emission tomography imaging in specific organs 1 hour after intraportal injection of FDG-labeled islet or FDG alone.

FDG, Z-[^{18}F]fluoro-2deoxy-D-glucose.

Demonstrating islet graft loss would require a tracer with a longer half life than ^{18}F (110 min), which limited PET imaging to 6 hours after implantation. Most β^+-emitting radionucleotides, such as ^{11}C, ^{13}N, and ^{15}O, have similar half lives or have high rates of cellular outflow, as is the case for ^{64}Cu (*10*). Only a radionucleotide with both a longer half life and a high intracellular retention rate would improve results, but this is, as far as we know, currently not available. Its identification would be a prerequisite for further research using this approach. Iron labeling of the islets before magnetic resonance imaging has been recently proposed (*12*). This technique appears to label the islets in a more stable way, allowing imaging later after transplantation, but this technique is nonquantitative and might not be sensitive enough to detect small islet losses.

In summary, ex vivo labeling of islets with FDG and study of posttransplantation islet trafficking are feasible. In this rodent model, all infused islets implant in the liver after intraportal infusion. Up to 6 hours after transplantation, FDG-labeled islets and FDG alone demonstrate similar clearance of liver radioactivity, and no islet loss could be identified. Further studies of posttransplant events would require the design of a radiolabeling agent with higher intracellular stability and a longer half life.

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