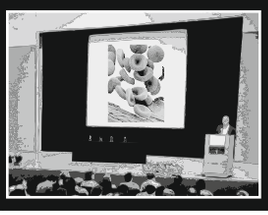
Conference Scene

Symposium on β-cell imaging at the 2011 EANM meeting



Annual Congress of the European Association of Nuclear Medicine
Birmingham, UK, 15–19 October 2011

At the Annual Congress of the European Association of Nuclear Medicine held on the 15–19 October 2011 in Birmingham, UK, Martin Béhé (Paul Scherrer Institute Würenlingen, Würenlingen, Switzerland) and Otto Boerman (Radboud University, Nijmegen, The Netherlands) organized a precongress symposium on the noninvasive imaging of the pancreatic β-cells. The symposium, which was sponsored by the EU 7FP framework program BetaImage, coordinated by Martin Gotthardt (Department of Nuclear Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands), took place on 15 October at the International Convention Centre, Birmingham, UK.

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Why β-cell imaging?

Diabetes, and especially the predominating Type 2 form, has reached epidemic proportions worldwide, with a predicted 30–50% increase in prevalence over the few next decades [101]. The pancreatic β-cells, which produce insulin in the pancreatic islets of Langerhans play a central role in the pathogenesis of most forms of the disease. Still, numerous questions about the natural history of diabetes remain unanswered, mostly due to the lack of noninvasive methods, which can be used to repeatedly monitor β-cell mass and function *in vivo*. Also, the available treatments are not curative, nor do they prevent the long-term clinical complications that result from the chronic hyperglycemia and metabolic alterations due to the altered balance between the islet hormones, notably insulin and glucagon. Presently, the efficacy of novel candidate therapies cannot be directly evaluated *in vivo* by repeated monitoring of the same animal and, *a fortiori,* the very same patient. Noninvasive imaging could be instrumental to address these key unanswered questions. Still, the imaging of native islets, and specifically of β-cells, within the *in situ* pancreas remains a sizable challenge, owing to a number of anatomical, cellular and physiological factors which converge to complicate image acquisition and analysis. Thus, several different methods are being investigated to determine which approach, or combination thereof, capable of sufficient tissue penetration to reach the human pancreas in the native abdominal location, could provide a sufficient resolution and fast operating acquisition to visualize individual islets of Langerhans. The ideal method(s) should provide for sound quantitative estimates of both the β-cell mass (presumably altered in most forms of diabetes) and function (largely affected in the residual β-cells of the most frequent Type 2 diabetes, and in the rare patients with a form of maturity onset diabetes of the young). Several American and European initiatives have been launched towards the development of such methods, including the Use of Innovative Strategies for β-cell Imaging in Diabetes Mellitus (BetaImage) project [102], which was initiated at the end of 2008 in the seventh Framework EU Program HEALTH. The EANM meeting [103] brought together four partners of this project and a collaborating clinician to discuss the advances and problems of the noninvasive imaging of pancreatic β-cells.

The lectures

Martin Béhé (Paul Scherrer Institute Würenlingen, Würenlingen, Switzerland) welcomed the speakers and attendees, and introduced the topic to discuss the current challenges and problems it raises. The essential problem is the dispersed and minute nature of the targeted pancreatic islets (in a 70 kg human, a 100 g pancreas contains approximately 10^6 pancreatic islets, where β-cells are located, which collectively represent no more than 1–2% of the volume of an adult pancreas, i.e., approximately 1 g wet weight tissue or ~10^9 cells). The islets are also of different sizes and shapes, and contain various types of endocrine (α-glucagon-producing cells,

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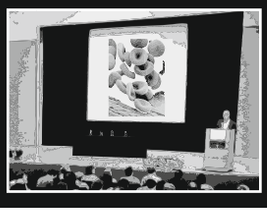
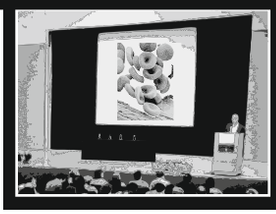


β-insulin-producing cells, δ-somatostatin-producing cells, ε-ghrelin-producing cells and pancreatic polypeptide-producing cells), and nonendocrine cells (endothelial cells, connective and immune cells). This complex microanatomical arrangement limits the standard imaging approaches because no single existing method features the spatial resolution, penetration depth, rapidity of image acquisition, safety, cost and widespread distribution that is much needed in research laboratories and clinics. Béhé also stressed that we still lack a probe to specifically target β-cells and enhance the imaging contrast in animals (for research purposes) and humans (for clinical monitoring). Ideally, such a probe should target an abundant protein of the β-cell surface, which would not be expressed in other cell types (at least within the abdominal organs that surround the pancreas), and whose expression would not be dramatically altered under diabetic conditions. Presently, the most studied candidates are the receptors of GLP-1 and the VMAT 2. The probes under current testing usually comprise a ligand and a signal molecule [1]. The ligand should be of small size to easily access the pancreatic islets and be rapidly cleared from the vasculature, and still have a sufficient affinity to strongly and specifically bind to the target protein. Its hydrophilic/lipophilic nature should ideally be modulated, depending on the location of the native/transplanted islets to be imaged. Indeed, hydrophilic molecules are usually eliminated by the renal route, whereas lipophilic molecules are usually excreted via the biliary route. Hence a lipophilic probe is unlikely to be convenient for imaging isolated islets transplanted in the liver, whereas a hydrophilic probe is likely to sizably label the kidneys, hence masking, or interfering, with the signals of native pancreatic islets transplanted into the kidney. Béhé further mentioned that the signal attached to the probe should also be carefully designed. Given the minute and spatially dispersed mass of β-cells, the signal intensity (e.g., the specific activity of the isotopes used in PET/SPECT) should be high, which raises both chemical synthesis and safety problems. It is obvious that the challenges are high and numerous; however, recent achievements provide at least a proof of principle that steady progress is being made, which presents exciting prospects for the years to come.

Decio Eizirik (Free University of Brussels, Brussels, Belgium) spoke about 'target definition for β-cell imaging'. He first recalled that if there is now good evidence that diabetes becomes clinically detectable when at least 50% (Type 2 diabetes) – 80% β-cells (Type 1 diabetes) are lost, the natural history of the disease remains uncertain. Specifically, we do not know whether the β-cell loss is linear or proceeds by discrete steps. *In vivo* imaging in both animal models and humans will be instrumental to answer this question. The difficulty in the implementation of such imaging lies in the small size and dispersion of pancreatic islets, which are formed from different types of cells, several of which share common proteins and mechanisms; hence, imaging probes of unique β-cell specificity will be required. To develop such probes, a systems biology approach is probably needed to identify mRNA and/or cognate proteins that are expressed in the pancreatic islets of Langerhans but not the surrounding pancreatic acini and other abdominal organs. The targets should also be highly expressed by β-cells and not the other islet cell types, and their expression should not be altered by inflammatory processes, notably Th1 cytokines, which are almost consistently associated with most forms of diabetes. Standard proteomic and RNA screenings were initially used to investigate rat and human β-cell preparations, before and after exposure to these cytokines, using a sequential selection process. Thus, candidate molecules were first allocated to known pathways then assessed for β-cell specificity and, eventually, for lack of alteration by Th1 cytokines. Out of 130 plasma membrane islet proteins, ten eventually passed all of the aforementioned screening steps and are being used to generate monoclonal antibodies for further characterization on sections of human tissues. As an example of such a candidate protein, Eizirik mentioned FXYD2β, a regulatory subunit of Na^+/K^+ ATPase. Three splice variants of the protein are known, of which only the form FXYD2βa is specific to pancreatic islets and has recently been shown to be downregulated in Type 1 diabetes.



This observation stimulated Eizirik to develop a microarray screening of alternative spliced transcripts, on the presumption that such a screening would identify more β-cell-specific proteins than other more standard approaches [2]. More than 90% of human genes are thought to be alternatively spliced and may explain the difference between the predicted number of mammalian genes (~25 × 10^3) and that of the cognate proteins (~10–20 × 10^4). In its present version, the approach screens for all transcribed miRNAs (splice variants of each gene), generating splice junction databases. Analysis of the transcripts that are not expressed in abdominal organs other than the pancreas identified 18,000 splice variants thought to be islet specific. The development of specific antibodies is now needed to validate the presence and distribution of the proteins encoded by these genes in β-cell membranes, using sections of multiple human tissues. It is noteworthy that more than 2000 of the candidate splice variants were either up- or down-regulated by the cytokines implicated in diabetes, including IL-1β and IL-2, suggesting some relationship with β-cell mass and/or function. Of the upregulated splice variants, 12 appear to code for β-cell targets that were unknown or had not yet been considered. The generation of specific antibodies is now required for the full validation of these candidates using immunostaining of human tissue arrays.

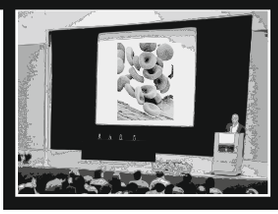
A question from the audience asked whether this approach could possibly differentiate changes in β-cell mass from those of β-cell function. Eizrik answered that an indirect indication about β-cell mass could be given by screening for only those splice variants which are not altered by diabetogenic cytokines.

Paolo Meda (Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland) spoke about 'state-of-the-art in β-cell imaging'. He first indicated that none of the available imaging approaches are ideal, and that if each features some advantage (e.g., high spatial resolution of optical methods, high sensitivity of PET/SPECT, unique anatomical detail of MRI), all are also plagued by serious limitations (e.g., poor tissue penetration of optical methods, insufficient resolution of PET/SPECT, limited sensitivity of MRI). Thus, a multimodal approach combining different imaging methods is likely to be needed in the future. Meda then illustrated what can currently be achieved in experimental models using *in vivo* bioluminescence (BLI). He reported on the production of mice whose β-cells were made double transgenic for the diphtheria toxin receptor and luciferase. In these animals, injection of diphteria toxin elicits the specific, rapid loss of β-cells, whose mass can be evaluated by BLI after injection of luciferin. The model allows for the quantification of graded losses of β-cells, and can be repeatedly applied to the very same animals to monitor the potential recovery of the native β-cell mass, which may occur with time. Meda further documented a multimodal approach (BLI/CT/PET), which allows investigators to refine the interpretation of image analysis. He illustrated that in the same animals in which BLI distinctly revealed a major loss of β-cells after diphtheria toxin injectiont, the ^{18}F-dihydrotetrabenazine (DTBZ) PET signal was unaffected in the pancreas [3]. Therefore, the approach should become useful for the validation of forthcoming new tracers expected to be β-cell specific. Meda then showed the results of a study [4] in which high magnetic field (14.1 T) MRI was used in combination with a prior infusion of manganese. This cation, which increases the contrast of MR images, is handled by β-cells such as calcium, and appears nontoxic. Pancreatic islets retain the cation for much longer periods of time than the other abdominal organs, resulting in a better contrast of the islets within the pancreatic lobes. This approach provides the first noninvasive visualization of individual pancreatic islets, the quantitative analysis of their number and relative volume, and monitoring of their quantitative loss after β-cells were killed with streptozotocin. However, analysis of the data showed that MRI somewhat undervalued these changes, presumably because it did not differentiate the islets that had been depleted of β-cells from those that contained increased numbers of glucagon-producing α-cells, owing to the nonspecific uptake of manganese. A solution to this problem requires the development of β-cell-specific probes. The Geneva team works on the idea that such probes could be constructed using biodegradable

polymers carrying different moieties (e.g., ligands, antibodies) to multiple β-cell targets (e.g., SUR-1, GLP-1R) and multiple labels (e.g., paramagnetic nanoparticles, positron emitting isotopes, fluorochromes) for imaging using multiple modalities. As a proof of principle, Meda illustrated the *in vivo* imaging obtained with furtive, gadolium nanoparticles tagged with a red fluorescent protein and a fluoresceinated form of the GLP-1 analog exendin 4. The probe enhanced the signal of pancreatic islets, as assessed by a correlative MRI/fluorescence analysis. Given that none of these approaches could be immediately taken into the human clinic, Meda showed the results of MRI performed with standard, clinical equipment working at 1.5 T. Using the diphtheria toxin receptor mouse model and the manganese enhancement approach he documented that the integrated pancreas signal correlated with the residual amount of β-cells and the insulin content of the pancreas. In a retrospective study performed on patients that underwent whole-body MRI in combination with a manganese infusion for the dynamic evaluation of heart dysfunctions, Meda's team found that the pancreas enhancement induced by manganese was significantly reduced in Type 2 diabetics, in agreement with the predicted loss of 30–50% β-cells.

A member of the audience commented that DTBZ was not adequate for the specific imaging of β-cells and that MRI could not be useful to quantify the signal generated by pancreatic islets, mostly because of the so-called partial volume effect. Meda recalled that in his presentation he showed that major changes in β-cell mass, easily detectable by BLI, were not revealed using PET and ^{18}F-DTBZ. With regard to the second comment, Meda mentioned that, in spite of obvious limitations, the MRI approach was the only one that could image individual islets, a parameter of importance given that increasing evidence shows that all islets are not similarly affected at the onset of diabetes. Asked about whether he thought that the approach could be sufficiently solid to allow for the characterization of different populations of patients, which are notoriously heterogeneous, Meda answered that, most likely, imaging will be more useful for the longitudinal monitoring of a given individual rather than for the average, statistical evaluation of a group. A further question raised the issue of safety regarding the use of manganese. Meda recalled that manganese is already in clinical MRI use and that the potential toxicity of the compound could be prevented by using low doses and influx rates. A final question raised the issue of whether the manganese-enhanced MRI reflected β-cell function more than β-cell mass. Meda mentioned that this possibility had motivated his choice of testing the cation. However, the present data do not allow us to unambiguously determine which fraction of the signal reflected function rather than mass. These discussions were continued between speakers and the audience during the coffee break.

After this break, Martin Brom (Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands) spoke about 'nuclear medicine tracers for β-cell imaging'. He first recalled several lines of evidence from the literature indicating that normoglycemic individuals largely differ in their mass of β-cells, and that the current clinical assays (e.g., monitoring of blood glucose) do not correlate with this mass. It follows that a method for the noninvasive, quantitative imaging of β-cell mass is now a major need. Research on this topic started approximately 10 years ago with the finding that the monoclonal antibodies IC2 selectively labeled β-cells on sections. However, once tested *in vivo*, these antibodies showed little access/binding to β-cells, which has since prompted attempts at developing smaller immunoglobulin forms (e.g., single chain antibodies, affibodies, camelid nanobodies). Brom then recalled that only 6 years ago, DTBZ, a ligand of the VMAT2, was labeled with ^{18}F for longitudinal PET monitoring of biobreeding rats undergoing spontaneous development of a Type 1 diabetes-like syndrome. The study provided evidence that the technique has the potential to quantitatively characterize a progressive loss of β-cells in a truly noninvasive way, using a technology that is already in use in many human clinics. However, there is increasing evidence to indicate that the PET signal obtained with DTBZ is not only due to β-cells, since VMAT2 is expressed by multiple cell types, notably many peripheral neurons. This finding has since stressed



the need for alternative and more specific β-cell probes. In this perspective, Broom illustrated the progress of the Nijmegen group to develop a PET probe targeting the GLP-1 receptor, which is well expressed at the β-cell surface [5]. Using ^{111}I-exendin 3, a GLP-1 analog with a significantly longer plasma half-life than the native hormone, he documented that, even though only a small proportion of the injected probe reached the pancreas (a sizable amount of the probe being incorporated by other organs, including the kidneys and the lungs), the pancreas labeling was sufficient to be detected and appeared specific, inasmuch as it was prevented by an excess of ^{40}Lys-exendin 3. In rodents treated with alloxan, a drug that specifically kills pancreatic β-cells, the PET signal was significantly reduced, and correlated with the residual mass of β-cells, as evaluated by a correlative PET/autoradiography/morphometry study. Brom further illustrated that the ^{111}I-exendin 3 probe may also be used to evaluate β-cell mass by SPECT, a technology that may be preferred to PET under certain conditions, for example, when the islet source can be concentrated in a small volume, which is typically the case with experimentally transplanted isolated islets [6]. Brom recalled that other PET/SPECT tracers have already been used to evaluate the islet mass after transplantation in the liver (the usual human situation) and under the kidney capsule (the most frequent transplantation site in experimental studies). He concluded that novel probe tracers need to be fully characterized *in vitro* as well as *in vivo*, given the unpredictable characteristics with regard to tissue bioavailability and binding.

A member of the audience queried the feasibility of obtaining a quantitative evaluation of an isotopic signal from islets transplanted into a striated muscle. Brom referred to a study by one of his team colleagues, to be later presented at the ENAM meeting, in which a significant correlation was found between a ^{111}I-exendin 3 SPECT signal and the mass of β-cells, as evaluated by morphometry of sections of the implanted site. Another question referred to the specificity of the exendin-mediated signal, given that GLP-1 receptors are also expressed in the stomach and the duodenum. Brom agreed that this could potentially interfere with the PET/SPECT signal, but indicated that the neighboring abdominal organs can be clearly distinguished from the pancreas using a correlative CT/PET (SPECT) approach. Thus, multimodal imaging is likely to be required to provide nonambiguous estimates of the pancreatic β-cell mass.

François Pattou (Lille University Hospital, Lille, France) spoke about the 'possible impact of β-cell imaging on clinic and research'. He reminded the audience about the normal pancreas anatomy, as experienced by an acting surgeon, and documented the use of multiple noninvasive (CT, MRI, endoscopic ultrasonography) and invasive (surgical sonography) imaging modalities for the pre- and peri-surgical localization of insulinomas. Usually these relatively rare tumors are benign and easy to detect, since they concentrate a large amount of insulin-producing β-cells in a small volume and often induce an increase in the regional vascularization. Pattou then stressed that, in comparison, the imaging of normal islets is much more complicated, owing to the dispersed distribution of the microorgans within the pancreas, their heterogeneous size, shape and cell composition (β-cells represent no more than 40% of the islet cells in humans). Still, the development of a method for their imaging is required for the development of innovative, targeted therapies. In this context, Pattou recalled that the current diabetes treatments are cumbersome, and in spite of extensive insulin supplementation do not significantly delay or decrease the long-term complications of diabetes. Also, many uncertainties remain about the clinical development of the syndrome (linear or step-wise loss of β-cells? Homogeneous or heterogeneous loss of islets in different pancreatic lobes? Why is loss of β-cell function significantly more important than that of β-cell number?) and others are raised by emerging clinical strategies (what is the mechanism responsible for the metabolic effects of gastroileal bypass? Do they involve changes in β-cells?). Answering these questions will require a method to noninvasively image the native β-cells *in situ*. Awaiting such a method, Pattou reviewed the studies that had imaged isolated human islets after transplantation in either liver or muscle. The liver transplanted islets have been

monitored by either PET (after *in vitro* labeling with [^{18}F]D-glucose) or MRI (after *in vitro* labeling with iron oxide nanoparticles), but questions remain as to whether these images accurately reflected the amount of the transplanted islets, their survival in the host and their secretory function. Pattou detailed the results of one of his studies, in which islets from a patient who underwent a subtotal pancreatectomy for the removal of an insulinoma were autologously transplanted into a muscle of one arm [7]. PET imaging after injection of ^{111}I-exendin 4 identified the site of islet implantation within the host muscle, which correlated with retained β-cell function, as determined by C-peptide release, and improved control of the patient blood glucose and insulin dependence.

The audience asked where the nanoparticles used for MRI were located. Meda, who contributed to this study, indicated that the nanoparticles were found by electron microscopy within the lysosomal compartments of several islet cell types, and were also abundant in the extracellular spaces of the islets. At this point, it is unclear whether the latter accumulations reflect the nonspecific trapping of the nanoparticles during the *in vitro* labeling, and/or their leakage from damaged/dead cells after transplantation. Another question posed to the speakers queried whether the blood glucose control could be achieved through the use of muscular implanted islets, given that, in contrast to native islets the transplanted islets are not vascularized by portal vessels. Pattou answered that, apparently, this does not impair the proper release of insulin nor the proper control of blood glucose. A last question queried whether the clinical conditions in which β-cell imaging could be predicted to have some utility, if not a major impact. Pattou answered that he could foresee at least three such conditions. First, the monitoring of diabetic patients receiving innovative drug or cellular treatments, expected to promote β-cell regeneration and/or function, since we have no data about the actual effects of these new treatments in humans. Second, the early post-transplantation monitoring of patients who received an islet transplant, given that the causes of the acute islet loss after transplantation remain to be determined (lack of vascularization? Immune attack? Other?). Third, the elucidation of emerging clinical situations. For example, the increasing request of gastroileal bypasses, which in some cases results in improved blood glucose and metabolic control requires that the underlying mechanism is thoroughly investigated (e.g., is the number of β-cells increased after the surgery?). Imaging of β-cells will at least determine whether the improved metabolic situation seen in some, but not all, patients correlates with different effects on β-cell mass and function.

Otto Boerman (Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands) closed the session by thanking the speakers for the state-of-the art presentations, and the audience (approximately 60 people) for the numerous questions and lively discussions during both sessions and the coffee break.

Conclusion

Several methods are already in use in the research laboratory that quantitatively image living β-cells of the native pancreas. Ongoing developments are aimed at improving the specificity of this imaging, via the development of cell-specific probes, and at devising multimodal approaches, which could provide parallel information about β-cell mass and function. Fewer methods are presently available for the clinical imaging of β-cells. An international, concerted effort from biologists, chemists, physicists, engineers, physicians and medical imaging specialists is certainly required to achieve this translation. The task remains formidable, but still the recent progresses are noteworthy.

Financial & competing interests disclosure

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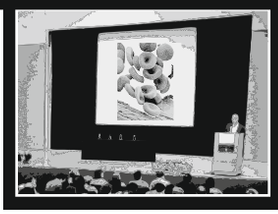

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