Towards an in vivo portrait of pancreatic beta cells: the bare essentials

VINET, Laurent, et al.

Abstract

Many questions about the natural history of both type 1 and type 2 diabetes remain unanswered, mostly because what we have experimentally learned has been derived from in vitro studies, which only partially mimic the complex situation of the living animal/human. Furthermore, the in vivo observations that can now be made in living individuals provide but indirect evaluations of beta cell mass and functions (in humans), or allow for testing only a single time point along the sequence of events that leads to the onset, maintenance and evolution of the diseases (in animal models). As a consequence, we lack a solid scientific basis to develop targeted therapies, and to monitor their actual effects, specifically in the human clinic. In view of the explosive diabetic epidemic, a method that could non invasively and repeatedly evaluate both beta cell mass and function, the two parameters whose alterations are key to most forms of diabetes, is now most needed.

Reference

TREATMENT STRATEGIES
DIABETES
Volume 3 Issue 2

• β-cell Imaging
• Diabetes, Obesity and Cancer
• Diabetes Prevention
• Glucose Monitoring
• Gynaecological Endocrinology
• Insulin Secretion
• New-onset Diabetes after Transplantation
• Paediatric Diabetes
• Patient Co-operation and Empowerment

Articles include:
Continuous Glucose Monitoring

Diabetes in Childhood: What’s New?

Diabetes Treatment and Cancer Risk: The Second Generation of Pharmaco-epidemiological Studies

Towards an In Vivo Portrait of Pancreatic β-cells: The Bare Essentials

Skeletal Muscle Insulin Resistance and Mitochondrial Dysfunction

Includes a review of the 16th FEND Annual Conference and the 47th Annual Meeting of the EASD
Towards an *In Vivo* Portrait of Pancreatic β-cells: The Bare Essentials

a report by Laurent Vinet, Smaragda Lamprianou, Joan Goulley, Christine Nabuurs, Riikka Immonen, Andrej Babic, Dhananjaya Sahoo, Norbert Lange, Xavier Montet and Paolo Meda

1. Department of Cell Physiology and Metabolism, University of Geneva; 2. Laboratory for Functional and Metabolic Imaging, EPFL; 3. Section of Pharmaceutical Sciences, Universities of Lausanne and Geneva; 4. Department of Radiology, University of Geneva

The Problem

Many questions about the natural history of both type 1 (T1D) and type 2 diabetes (T2D) remain unanswered, mostly because what we have experimentally learned has been derived from *in vitro* studies, which only partially mimic the complex situation of the living animal/human. Furthermore, the *in vivo* observations that can now be made in living individuals provide indirect evaluations of β-cell mass and functions (in humans), or allow for testing only a single time point along the sequence of events that leads to the onset, maintenance and evolution of the diseases (in animal models). As a consequence, we lack a solid scientific basis to develop targeted therapies, and to monitor their actual effects, specifically in the human clinic. In view of the explosive diabetic epidemic, a method that could non-invasively and repeatedly evaluate both β-cell mass and function, the two parameters whose alterations are key to most forms of diabetes, is now most needed.

The Dream

This dream could become a reality with the development of methods for the non-invasive imaging of pancreatic islets, and specifically their insulin-producing β-cells, would these methods be quantitative, and applicable to both the laboratory models (for research purposes), and humans (for monitoring the disease status). Obviously, such methods should be safe, both with regard to the individual and the β-cells, and allow for a repeated analysis of the very same individual at defined time intervals. Ideally, these methods should provide for a quantitative evaluation of both β-cell mass (largely affected in T1D, and more modestly decreased in T2D) and function (largely affected in T2D and in some forms of Maturity Onset Diabetes of the Young (MODY)). The present reality is still somewhat far from this dream, in spite of sustained effort and several promising directions.

The Difficulties

In a world in which imaging has pervaded most aspects of our life, including notably the entire bio-medical field, it may sound curious that we still face sizable difficulties to non-invasively image the native islets within the *in situ* pancreas. This is because the pancreatic islets of Langerhans are small (50-600 μm diameter), feature a heterogeneous cell composition (in humans about 60% of the islet cells are β-cells, intermixed with alpha, delta, epsilon and pancreatic polypeptide cells, as well as with endothelial cells, fibroblasts, cells of the immune system and some neurons), and are scattered throughout the exocrine pancreas (in the normal human pancreas, there are about 10⁷ islets, that altogether represent 1g wet weight tissue, or about 1% the volume of an adult pancreas). The gland itself is deeply located in the abdomen, where it is rhythmically displaced by respiratory, circulatory and intestinal movements. These factors combine to significantly complicate the image acquisition. Theoretically, such acquisition would require a highly resolutive and fast operating method, and a tissue penetration sufficient to reach the human pancreas. Furthermore, such a method would be required to provide 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 T2D and MODY patients).

The Tools

No existing method yet fulfills these minimal requirements. Thus, whereas several optical imaging methods are exquisitely resolutive and sensitive, all lack sufficient penetration capability, due to the light refracting and auto-fluorescence properties of most tissues (Figure 1). Conversely, several clinically relevant methods, which have such a penetration capacity, are either poorly resolutive (SPECT, PET), do not provide functional information (CT) or are modestly sensitive and not easily quantifiable (MRI) (Figure 1). Thus, it is likely that several methods would have to be combined, in a multimodal approach, to provide the anatomical and functional information that would be desirable.

Most of these methods, with the exception of optical coherence tomography, will further have to cope with the soft tissue nature of pancreatic islets, that provides them with a contrast similar to that of most other abdominal organs. Thus a β-cell- or islet-specific labelling will be required for differentiating the islets/β-cells from the surrounding organs. No tracer for such a specific labelling is yet available. Most current efforts are towards the development of ligands (small molecules, antibodies, antibody fragments, …) targeting membrane proteins deemed to be enriched, if not specific of the β-cell surface (SUR1, GLP-1r, VMAT2,…), which could be adapted for several
**Treatment Strategies - β-cell Imaging**

<table>
<thead>
<tr>
<th>Penetration Depth</th>
<th>optical</th>
<th>US</th>
<th>SPECT</th>
<th>PET</th>
<th>CT</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional Information</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quantifiable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Availability in Research</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Availability in the Clinics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figure 1.* Major characteristics of the methods most frequently considered for non-invasive imaging. No existing method features optimal performance (dark green boxes) for all the minimal characteristics which are required for an efficient imaging of the mass and function of pancreatic β-cells. In some cases, these characteristics are poor (light green boxes) or insufficient (red boxes). Optical = optical coherence tomography, optical projection microscopy, bioluminescence, fluorescence; US = ultrasound; SPECT = single-photon emission computed tomography; PET = positron emitting tomography; CT = computed tomography; MRI = magnetic resonance imaging.

![Figure 2](image-url) *Figure 2.* In vivo bioluminescence detects the experimental loss of pancreatic β-cells. Three normoglycemic mice, which were engineered to express firefly luciferase in pancreatic β-cells, were imaged for bioluminescence after injection of luciferin. Under these conditions, the photon emitted by beta cells (colour-coded areas) could be non-invasively recorded over the pancreatic region (left panel). The very same mice were then injected with streptozotocin, a drug that selectively kills beta cells. One day later, these mice, which had now become hyperglycemic, were imaged again (right panel). Comparison of the 2 images shows the marked decrease in the amount of photons in the pancreas region, which correlated with a major loss of beta cells.

of the methods mentioned above, after appropriate labelling with a reporter molecule (fluochromes for fluorescence microscopy, luciferase for bioluminescence, positron-emitting isotopes for PET, (super) paramagnetic nanoparticles for MRI...). In practice, however, these developments have been complicated by a variety of factors, including insufficient pancreas bioavailability after in vivo administration, presence of the target proteins also on other cell types (notably on neurons, which share many characteristics with pancreatic β-cells), and non specific binding. The situation may be improved by the simultaneous imaging of different targets. Other approaches, investigate manganese ions which modify MRI contrast. In β-cells, Mn²⁺ is handled as Ca⁺⁺, the prominent cation in the control of insulin secretion, raising the possibility that changes in the Mn²⁺-induced signal may provide information about β-cell function.

In all cases, the tracers and methods require careful evaluation in vitro, using different cell types as well as isolated islets, to evaluate binding specificity, binding affinity, retention at the β-cell surface and/or internalisation. The validation should then be extended to *in vivo* conditions, to evaluate the bioavailability of the tracer in different organs, its clearance from the vascular compartment, and the efficiency of the signal. A further step, is the assessment of the sensitivity of the method to detect changes in β-cell mass and function, like those anticipated under diabetic conditions. In the absence of specific cell tracers, the quantitative evaluation of β-cells within a diabetic pancreas may be complicated by the associated alterations in the vascular, immune and connective compartments of the gland. Alternative models, resulting in the targeted deletion of specific islet cell populations in the absence of major changes in other tissues, are now available to circumvent this complication.

**Some Research Methods**

Many methods have been tested for the experimental imaging of living β-cells within either isolated islets transplanted within the liver, under the kidney capsule, in muscles, in the anterior chamber of the eye, or within the native pancreas of laboratory animals. This section provides information about three of these methods, which offer unique advantages and have been recently reported.

*In vivo* bioluminescence (BLI) is the emission of light resulting from the catalysis of luciferin by a luciferase enzyme. Given that vertebrates do not express luciferase, the background levels are about nil and, thus, the sensitivity of the method is high, but the enzyme has to be expressed by transgenesis. The photon emission is made cell-specific by expressing a luciferase cDNA under control of a cell-specific promoter (the insulin promoter in the case of pancreatic β-cells; Figure 2). The method is truly non-invasive, since at least a portion of the emitted photons can be detected at the surface of the animals. The drawbacks are that the absolute amount of photons is low, so that the use of a high sensitive (thus expensive) camera is not dispensable, and is further significantly reduced as light crosses the tissues separating the cells of interest from the surface, a path along which the light is absorbed and refracted. As a result, the photons are projected over an area significantly larger than the source (this can now by partially corrected by algorithms that allow a better, 3D localisation of the source), providing for a limited spatial resolution. In spite of these limitations, the method is valuable since the photon emission is remarkably linear within a range of values of β-cell mass, providing a fast quantitative evaluation of the mass of cells emitting the photons. Thus, the method allows for an evaluation of the number of living (BLI is dependent on both the activity of the intracellular luciferase, and on the blood supply to bring luciferin to the cells expressing the enzyme) islets (whether of rodent or human source) after transplantation. It also allows to repeatedly monitor in the very same animal the loss of β-cells which is experimentally induced by chemicals activating beta cell apoptosis (Figure 2). The approach could also have a sufficient sensitivity to monitor the β-cell recovery.
which may occur under certain conditions, whether as a result of the growth of residual β-cells, the transdifferentiation of other pancreatic cell types or islet neogenesis.

Optical coherence tomography (OCT) is based on the holographic recording of light from unlabeled objects, that may be differentiated based on a natively different refractory index. The rather fast image acquisition times of the method provides unsurpassed time resolution (thus minimising the artifacts due to physiological movements). Fourier transform of the data allows to subsequently 3D reconstruct volumes of tissue, with remarkable definition. Indeed, the absence of labelling significantly increases the lateral resolution, providing for a variety of applications, ranging from the analysis of rapid cell functions (i.e. Ca²⁺ transients), sub-cellular organelles, molecules, and intact tissues in situ. Thus, the technology is now in routine use in the human clinic for the non-invasive imaging of the retina. In the context of pancreas, the method allowed to visualise label-free islets in the intact mouse pancreas, after the gland had been exposed by laparotomy. Unfortunately, islets deeply situated within the pancreas parenchyma, and the entire gland itself in its normal abdominal location, escape detection, as light cannot penetrate more than a few millimeters of intact tissue. This is a major limitation particularly for the quantitative evaluation of β-cell changes, that may not take place linearly in all islets and all pancreatic regions. Still, OCT allowed for the detection of changes resulting from streptozotocin-induced β-cell loss in limited volumes of the murine pancreas. The technique could also be useful to investigate the autoimmune attack of β-cells, provided that the refractory index of lymphocytes and macrophages differs from that of islet cells. The reason why the latter cells clearly stand out above the darker looking exocrine pancreas, remains to be determined. The lack of detection by OCT of islets in the pancreas of mice null for the ZnT8 transporter, suggests that this contrast may result from the islet cell handling of Zn²⁺, possibly because of altered insulin storage.

Magnetic resonance imaging (MRI) monitors the magnetisation resulting from the spin of certain atoms (mostly H⁺, which accounts for 63% of the body atoms) within a much larger, imposed magnetic field which is modulated by sequences of radio-frequencies. The spin orientation changes ("relaxation") when the radio-frequencies are interrupted. Relaxation is then converted to currents which, in turn, are translated into images. MRI can access all organs of humans and laboratory animals, provides exquisite anatomical detail, as well as functional information, and is essentially safe under the magnetic fields (1.5-7T) used in the clinics. However, under the latter conditions, the lateral resolution of the method (~200 - 500 μm) is not sufficient to visualise individual islets, and the contrast of the rodent pancreas is low and homogeneous. To improve these two parameters, we have used a much higher field (14.1T) MRI, in combination with an in vivo infusion of Mn²⁺. We have found that the method then allows to identify individual islets in the intact pancreas (due to a lateral resolution of ~30μm in 300-μm-thick "slices"), including under in vivo conditions (Figure 3), and to quantify their loss in an experimental model of T1D. However, our quantitative estimates somewhat undervalued the β-cell loss, since, under the conditions we used, MRI did not distinguish the islets that had lost β-cells from those that contained sizable numbers of glucagon-containing α-cells. It is anticipated that this problem will be solved with the development of β-cell-specific tracers.

Some Clinical Methods

The methods mentioned above cannot be translated to the clinic of the human pancreas, because they necessitate the transsection of an exogeneous gene (BLI), cannot reach the pancreas in situ (OCT) or require a strong magnetic field which raises serious technological challenges and some safety issues (MRI). Out of the several alternatives which could be envisaged, two major methods have been tested so far.

Positron emission tomography (PET) involves the coincident detection of the gamma rays emitted by trace dosis of a positron-emitting isotope (often ¹⁸F or ¹¹⁷In) labelling a biological (often glucose or, in the case of pancreas, exendin4) or synthetic molecule (in the case of pancreas, DTBZ, a ligand of the Vesicular Monoamine Transporter 2). The gamma photons, which travel across tissues along two
trajectories at 180° from each other, are detected by an external scintillator and the detected light amplified by a photomultiplier. The method can reach all human organs, is the most sensitive of the existing clinically-relevant imaging methods, easily provides quantitative data and is reasonably safe. The major drawbacks are the limited lateral resolution of the method (~1-2 mm), and the complexity of the synthesis of the isotope-labelled molecules, whose half-life (typically of only a few minutes) implies an almost immediate use of the newly synthesised molecules. Using [111In]–DTNB, the method has been reported to label the rodent pancreas in levels that decreased with the development of hyperglycaemia in the BB rat model of T1D. However, there is growing concern that the signal due to DTNB may not be only related to changes in β-cell mass. Using [111In]–exendin4, the method has been reported to also label rodent islets, as well as human islets autografted in striated muscles. However, controls in rodents have clearly shown that, beside the pancreatic islets, the latter tracer also strongly labels other abdominal organs, notably the kidney cortex. In spite of this partial β-cell specificity, both [111In]–DTNB and [111In]–exendin4 have now entered the initial phases of clinical evaluation.

Magnetic resonance imaging (MRI; see above for the basic principles of the method) is not complicated by the synthesis and use of the isotope-labelled ligands, has a quite good resolution (~200-500 μm in most clinical settings), and is safe. However, when compared to PET it is sizably less sensitive and less prone to an easy quantitative analysis. It has been largely used to image allografts of isolated human islets in type 1 diabetics. To this end, the islets were typically labelled with super-paramagnetic iron oxide, referred to as SPIOs, or paramagnetics gadolinium nanoparticles (20-50 nm in diameter), prior to transplantation in the host liver. The resolution and sensitivity of the method at the relatively low magnetic fields (1.5-9.4T) which are in clinical use does not allow to detect individual islets. However, hypo-(with iron oxide) or hyper-intensity areas (with gadolinium), thought to reflect intra- as well as extra-cellular aggregates of the labels/contrast agents, were detected and correlated with the survival of a secretory functioning graft. The imaging of the islets within the native pancreas is much more problematic, as labels/contrast agents are up taken by many cell types, and that no ligand is yet known to specifically target nanoparticles to β-cells. However, in vivo MRI of rodent pancreas using MR labels targeting CD8+ lymphocytes, has nicely revealed the accumulation of these cells around the islets ("peri-insults") of diabetes-prone NOD mice, a widely accepted model of T1D. The data suggests that, would a β-cell-specific tracer become available, the islet labelling could become detectable, even though the resolution of a clinical MRI is not sufficient to visualise individual islets, at variance with what can be achieved with the much higher magnetic fields mentioned above. Nevertheless, our preliminary data in rodents and humans, indicate that, when combined with a MR labelling that enhances the contrast of the pancreas image (Figure 4), a standard clinical MRI could provide an estimate that carefully reflects the mass of β-cells and/or their function. Further developments are now required to sort out the respective contribution of these two key β-cell parameters.

The Future

Several methods are already in use in the research laboratory that quantitatively image living β-cells of the native pancreas with acceptable precision and resolution. Further developments are now required to adapt these methods to a truly non-invasive in vivo imaging, that could be repeatable in the very same animal, at defined time intervals. This goal can now be achieved with only some of these methods, due to the deep abdominal location of pancreas, and the lack of specificity of β-cell tracers. If it is difficult to predict how rapidly these exciting basic research developments, which can already address a number of still unresolved questions about the patho-physiology of diabetes, may lead to developments translatable to the human clinic. A coordinated effort of biologists, chemists, physicists and medical imaging specialists is now required to develop the multimodal approaches that should allow monitoring of the large loss of β-cells in T1D. Whether the same approaches could also monitor the more modest β-cell loss of T2D, and the even smaller changes in β-cell mass, anticipated to be induced by therapeutic regimens expected to lower β-cell growth, remains to be determined. Under the latter two conditions, it also remains to be shown whether the imaging methods could provide some information on β-cell function, specifically with regard to insulin secretion. Without such information, the interpretation of any β-cell imaging would remain at best, ambiguous. The task to solve these questions is formidable. The challenge is exciting in this rapidly expanding field.

Acknowledgments

Our teams are supported by grants from the Swiss National Science Foundation (310000-122423, 310000-129402, CR32B3_129987), the Juvenile Diabetes Research Foundation (40-2011-11), and the European Union (BETAIMAGE 222980; MIDICA, C2008-T7).

References

10. Reiner T, Thorpe G, Gaglia J, Vieggeri C, Liew CW,


Reprints of all articles are available.
To order additional reprints:
Phone - 020 7953 8490 Fax - 020 7953 7709
Email - info@treatmentstrategies.co.uk