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Evaluation of radiotracers for beta cell imaging

Diabetes mellitus is characterized by hyperglycaemia (high blood glucose levels). In type 1 diabetes the insulin producing beta cells are destroyed by autoimmunity, whereas in type 2 diabetes the body does not respond properly to the produced insulin. In the end stages of both types of diabetes a significant amount of the insulin producing beta cells is lost. However, the exact relation between the number of beta cells, called the beta cell mass (BCM) and the onset and progression of diabetes is not known. The function of the beta cells can be measured with glucose, insulin and HbA_{1c} measurements, but these tests do not provide information on the actual BCM. The limited information available on BCM in diabetes is obtained from autopsy data, which are single time point evaluations.

Non-invasive imaging methods to monitor the BCM during the progression of the disease would not only provide crucial information about the role of BCM in diabetes but would also help to evaluate the effect of new therapies. A non-invasive imaging method could also be used to monitor islets after transplantation. Islet transplantation is a promising therapeutic option for type 1 diabetic patients with poor glycaemic control. This method is initially successful; however, long-term transplantation outcome is poor. Metabolic stress and recurrence of autoimmunity cause death of a large number of the transplanted islets. The most promising approach to visualize the beta cells non-invasively is with the highly sensitive radionuclide imaging methods, PET and SPECT.

One of the most promising tracers for the quantification of the BCM, both in the native pancreas and after islet transplantation, is radiolabelled exendin. Radiolabelled exendin binds to the glucagon like peptide-1 (GLP-1) receptor expressed on beta cells. To show the potential of indium-111 (¹¹¹In) labelled exendin to visualize transplanted islets, 400 and 800 islets were transplanted in the calf muscle of rats. Four weeks after transplantation animals were injected with ¹¹¹In-exendin and SPECT/CT images were acquired. The images showed a significant difference between 400 and 800 islets in SPECT signal. Subsequent histological analysis of the transplants showed excellent co-localization

between the ¹¹¹In-exendin uptake in the transplant and the GLP-1 receptor and insulin staining. Subsequently, the true potential of this tracer for BCM quantification with *in vivo* SPECT imaging was investigated in a mouse model. Mice were transplanted in the calf muscle with 50, 100, 200, 400 and 800 islets and again four weeks after transplantation animals were injected with ¹¹¹In-exendin and SPECT/CT images were acquired. In this study an excellent correlation between SPECT quantification of the ¹¹¹In-exendin uptake and the histologically determined transplant volume was observed (Pearson $r=0.87$), with visualization of as little as 50 transplanted islets. These results show that ¹¹¹In-exendin is not only a promising but also sensitive tracer for the quantification of the BCM of transplanted islets.

Subsequently, the potential of a different tracer, iodobenzamide (IBZM), for the quantification of the BCM was investigated. IBZM has a high specificity for the dopamine 2 receptor. Dopamine plays an important role in many brain functions and radiolabelled IBZM is nowadays used for imaging of neurodegenerative diseases like Parkinson's disease. Since the dopamine 2 receptor is also expressed in the islets of Langerhans, IBZM might also be a good candidate for visualization of the islets. Unfortunately, this tracer shows high unspecific uptake in the exocrine pancreas and is therefore not suited for visualization of islets in the native pancreas. However, this tracer might still be a good candidate for visualization of transplanted islets.

Initially, the specificity of IBZM was investigated *in vitro* both in an insulinoma cell line (INS-1 cells) and on isolated islets. Subsequently, an *in vivo* study was performed and 1000, 2000 and 3000 islets were transplanted in the calf muscle of rats. Six weeks after transplantation SPECT/CT images were acquired and the SPECT signal in the transplant was quantified. In this study we also observed a linear correlation between quantified SPECT signal and transplant volume (Pearson $r=0.73$). Furthermore, we were able to follow the transplant over time. These results suggest that IBZM can be successfully applied for non-invasive, longitudinal and quantitative monitoring of transplanted islets.

As shown in our previous study ^{111}In -exendin shows great potential for the quantification of the BCM of transplanted islets. However, BCM quantification in the native pancreas is more complicated due to dispersion of the signal over a larger area, especially in animals with diabetes (and thus a low BCM). Unfortunately, the pancreas of rodents is not visible on CT images and therefore it is difficult to delineate the pancreas based on CT images. A tracer accurately visualizing the entire pancreas would help to correctly delineate the pancreas and thereby improving BCM quantification. A potential exocrine tracer is technetium-99m ($^{99\text{m}}\text{Tc}$) demobesin. After injection of this tracer in both healthy and diabetic animals high accumulation in the pancreas was observed, while ^{111}In -exendin uptake was reduced by more than 50% in diabetic animals. Quantification of the ^{111}In -exendin signal in a volume of interest based on the $^{99\text{m}}\text{Tc}$ -demobesin uptake resulted in an excellent correlation (Pearson $r=0.92$) between ^{111}In -exendin uptake determined in the gamma counter and ^{111}In -exendin uptake determined from SPECT analysis. The use of $^{99\text{m}}\text{Tc}$ -demobesin allowed quantification of ^{111}In -exendin uptake in a larger region of the pancreas compared to studies where only ^{111}In -exendin was used, resulting in a more robust quantification of the BCM.

Since the use of radionuclides always raises questions about the potential radiation-induced damage to the islets, we investigated the potential damage to the islets as a result of the accumulation of the radiolabelled exendin. The radiolabelled exendin is internalized in the beta cells upon receptor binding and gets trapped in the cells, which results in high accumulation of the radionuclide in the islets of Langerhans. Therefore, the uptake in the islets is much higher as in the rest of the pancreatic tissue and thus the islet absorbed dose will be higher than the average pancreatic absorbed dose. Current models were not able to calculate the islet absorbed dose therefore, we developed a new model where we combined whole organ and small scale dosimetry to calculate the islet absorbed dose.

In our model the tissues with high accumulation of the radiolabelled exendin were included. These tissues are the kidneys, via which the radiolabelled exendin is cleared, the pancreas and the islets of Langerhans with the beta cells. As input for the model we used SPECT scans from a clinical study and biodistribution and autoradiography data from a preclinical study. Islet absorbed doses were calculated for male and female, healthy and diabetic patients, for patients with high or low kidney uptake and the use of different radionuclides was simulated (^{111}In and ^{68}Ga). The maximum calculated islet absorbed doses were small in all situations (^{68}Ga : 1.38mGy and ^{111}In : 66mGy) and indicates that even repeated exendin imaging will hardly increase the risk on diabetes.

Based on our studies we can conclude that both ^{111}In -exendin and ^{123}I -IBZM are promising tracers for the quantification of the BCM of transplanted islets. Furthermore the exocrine tracer $^{99\text{m}}\text{Tc}$ -demobesin can be used to visualize the whole pancreas and can help with the quantification of the radiolabelled exendin in the native pancreas. Finally our model showed that the increased risk on diabetes due to the ^{111}In -exendin islet absorbed dose is very limited.

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Islets transplanted in the right leg of a rat were clearly visible on the maximum intensity projection of a SPECT/CT image, acquired one hour after injection of 15 MBq ^{111}In -exendin-3 (red arrow). In addition to the signal from the transplant, radioactivity was observed in the bladder (black arrow).