Quantitative and longitudinal imaging of intramuscular transplanted islets of Langerhans with SPECT using [^{123}I]IBZM

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Short running title: Quantitative islet imaging by [^{123}I]IBZM SPECT

Abstract

A non-invasive imaging method to monitor islet grafts could provide novel and improved insight in the fate of transplanted islets and potentially monitor the effect of therapeutic interventions. Therefore, such an imaging method could help improve long-term transplantation outcome. Here, we investigated the use of [^{123}I]IBZM for insulin positive graft volume quantification and longitudinal graft monitoring. SPECT images were acquired six weeks after islet transplantation in the calf muscle of rats. For longitudinal graft analysis, rats were monitored by SPECT for ten weeks. After animals were euthanized, graft containing muscles were dissected for ex vivo analysis and insulin positive graft volume determination. Six weeks after transplantation, a clear signal was observed in all grafts

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by SPECT imaging. Moreover, the intensity of the SPECT signal correlated linearly with insulin positive graft volume, as determined histologically. Longitudinal graft follow-up showed a clear SPECT signal of the transplant from three until ten weeks after transplantation. In this study, we demonstrate for the first time the successful application of a radiotracer, [^{123}I]IBZM, for non-invasive, in vivo graft volume quantification and longitudinal graft monitoring.

Introduction

Islet transplantation is a promising novel therapy for type 1 diabetic patients. Unfortunately, islet transplantation still results in only short term insulin independence while long term transplantation outcome remains poor. A reliable method to quantitatively monitor islet grafts could provide improved insight in islet loss after transplantation. Furthermore, it would grant the possibility to evaluate novel potential therapies that might help improve long term transplantation outcome.

During the last decade, extensive efforts have been made to develop a non-invasive imaging method to monitor islet grafts, resulting in a variety of graft imaging techniques. Several groups used pre-labeling methods to visualize the islet graft, while others exploited targets specifically expressed on beta cells, such as the glucagon-like peptide 1 receptor (GLP-1R) (1), the vesicular monoamine transporter 2 (VMAT2) (2) or serotonin biosynthesis (3), of which the tracers are currently under clinical investigation. Since combining different beta cell-specific radiotracers might reveal important complementary information on islet grafts (for example in case of receptor down regulation under certain conditions), the search for alternative imaging methods is ongoing.

Since the 1980's, D2 receptors have been used as a target for brain imaging (4). In view of the neuro-endocrine nature of islets, the hypothesis arose that beta cells express D2 receptors. In 2005, this

hypothesis was proven when Rubi et al. showed excellent co-localization of D2 receptor expression and insulin expression in human and rodent islets (5). Ever since, the presence of these receptors has been exploited for imaging of insulinomas (6), congenital hyperinsulinism (7) and islets, both in the pancreas and after transplantation, using [^{18}F]-L-3,4-dihydroxyphenylalanine ([^{18}F]-DOPA) and [^{18}F]fallypride Positron Emission Tomography (PET) (8, 9).

Another tracer displaying high affinity and specificity for D2 receptor is iodobenzamide (IBZM), a strong receptor antagonist (4, 10). Labeled with I-123 or I-125, it has been extensively used as a radiotracer for Single Photon Emission Computed Tomography (SPECT) in neurodegenerative diseases (11). Recently, in a proof-of-concepts study, we have demonstrated specific binding of [^{125}I]IBZM to isolated islets in vitro and showed the technical feasibility of non-invasive in vivo visualization of intramuscular islet grafts by D2 receptor targeting using [^{123}I]IBZM SPECT/CT (12). In the present study, we aimed to characterize the potential of [^{123}I]IBZM for longitudinal graft monitoring and in non-invasive functional graft volume quantification by determining the correlation between the SPECT signal in the graft and the insulin positive graft volume.

Methods

Animals

Twelve weeks old female WAG/Rij rats (Charles River Laboratories, Erkrath, Sulzfield, Germany) were used as islet donors as well as recipients. Animals had access to food and water ad libitum. Animal experiments were approved by the animal welfare committee of the Radboud University Nijmegen and carried out in accordance with their guidelines.

Islet isolation and transplantation

Islets of Langerhans were isolated and transplanted as described previously (12). Briefly, rats were suffocated using CO_2/O_2 and islets were isolated by collagenase digestion and purified on a

discontinuous Ficoll gradient (supplementary methods). After overnight recovery, islets showed normal morphology and, islet purity and viability exceeded 90%, as determined microscopically. Afterwards, islet were transplanted intramuscularly (supplementary methods) and no unexpected or adverse effects were observed in the recipient rats after transplantation.

SPECT imaging and quantification

To determine the correlation of SPECT signal with insulin positive graft volume, fifteen WAG/Rij rats (3 groups, n=5/group) were transplanted in the right calf muscle with 1,000; 2;000 or 3,000 islets as described above. SPECT/CT images were acquired (supplementary methods) six weeks after transplantation. Unfortunately, in vivo competition or displacement studies, using an excess of unlabeled compound, could not be performed due to the possible lethal effects of complete D2 receptor blocking. For longitudinal follow-up of transplanted islets, three WAG/RIJ rats were transplanted with 1,500 islets and SPECT/CT images were acquired in week 2, 3, 4, 5, 6, 8 and 10 after transplantation. SPECT scans were reconstructed using the U-SPECT-II reconstruction software (MILabs, Utrecht, The Netherlands) (supplementary methods). [^{123}I]IBZM uptake was quantified using Inveon Research Workplace (IRW) (Siemens Healthcare, Den Haag, The Netherlands). Total voxel intensity in the graft was corrected for background uptake in the contra-lateral muscle. The activity calculated in the grafts was corrected for injected activity in that animal.

Ex vivo analysis and insulin positive graft volume determination

After the last SPECT/CT scan, rats were euthanized and transplant containing muscles were collected for ex vivo SPECT (supplementary methods) and immunohistochemical analysis (supplemental methods). To determine the insulin positive graft volume, sections stained for insulin (supplementary methods) were analyzed using a DM5000 microscope (Leica, Solms, Germany) and images were obtained with a color camera (Evolution MP; Leica using Axio Vision 4.4 software). The insulin positive transplant area was drawn on each transplant containing section, at all levels. The insulin

positive transplant area was multiplied with 50 µm inter-slice distance to obtain insulin positive graft volumes.

Statistical analysis

All values are expressed as mean values ± standard deviation (SD). All correlations in this paper were calculated using the Pearson correlation coefficient (Pearson r) in GraphPad Prism v. 5.03 (GraphPad Software, Inc. San Diego, CA, USA). The level of significance was set at $p < 0.05$.

Results

Immunohistochemical graft analysis and volume assessment

All grafts could be detected in the calf muscle after HE staining. The grafts' locations, determined by HE staining, co-localized with insulin and D2 receptor staining, as determined on consecutive sections (supplementary figure 1). This observation indicates beta cell specific D2 receptor expression in the islet graft. The insulin positive graft volume, calculated histologically from the insulin-positive graft area in all graft containing slices , increased with the number of transplanted islets. Insulin positive graft volume ranged from 1.4×10^6 µm^3 to 1.2×10^7 µm^3. Two grafts were excluded from the volume assessment since no trustworthy graft volume could be calculated from the histological data due to technical errors during sectioning.

In vivo SPECT/CT imaging and correlation with graft volume

Six weeks after transplantation, all grafts (n=15) were visible on SPECT, one hour after [^{123}I]IBZM injection. For accurate image analysis, SPECT images were fused with the concomitant CT images for anatomical reference. Figure 1A clearly shows differences in [^{123}I]IBZM uptake in rats, transplanted with different amounts of islets and with differences in insulin positive graft volume, as determined by histology. Furthermore, the measured SPECT signal in the islet grafts, with an average target-to-

bhackground ratio of 12.5 ± 10.3 (range: 2.1 – 40.5) , showed a linear correlation (Pearson r = 0.73, p = 0.005; n=13) with insulin positive graft volume, as determined histologically as described above (Figure 1B left panel). Furthermore, the SPECT signal expressed as target-to-background signal showed a similar linear correlation (Pearson r = 0.73) with insulin positive graft volume (Figure 1B right panel). To our knowledge, this is the first study showing a high correlation (Pearson r=0.73) of functional islet graft volume and quantitative radiotracer uptake in the graft, indicating that radiotracer uptake is a representative measure for the amount of insulin producing beta cells in the graft. Although the liver (in humans and animal models), kidney and spleen (in animal models) are more commonly used sites for islet transplantation, their high background uptake renders these organs less suitable compared to the muscle for in vivo graft analysis using D2 receptor targeting. Furthermore, preclinical intramuscular islet transplantation offers the possibility to elegantly and accurately analyze the islet graft ex vivo since islets are not dispersed throughout the muscle, and clinically intramuscular islet transplantation is already being applied for islet auto-transplantation in diabetes patients rendering clinical translation of this technique feasible (13).

Ex vivo SPECT imaging

SPECT images of the dissected, transplant containing calf muscle samples showed a clear signal of the islet graft ex vivo. Furthermore, the SPECT signal of the islet graft observed ex vivo, corresponded nicely to the SPECT signal detected in the islet graft in vivo (Figure 1C), which confirms that the signal observed in vivo indeed originates from the islet graft in the muscle. Although skeletal muscle contains considerable dopamine levels, competition of tracer binding with endogenous dopamine will be negligible since the potency of dopamine to displace IBZM is very low (10).

Longitudinal transplant follow-up

Figure 2 shows the proof-of-concept longitudinal SPECT analysis of the intramuscular islet grafts. In all rats (n=3), islet grafts showed a clear SPECT signal from three until ten weeks (end of experiment)

after transplantation. All transplants (average insulin positive graft volume: 6.8 ± 2.1 x10^6 µm^3) were localized in the calf muscle, as determined by HE staining, and co-localized with insulin and D2 receptor expression, as determined immunohistochemically. Eter et al. have previously shown that the initial absence of detectable SPECT signal, also observed here, can be explained by ongoing revascularization of the islet graft (14). During islet isolation, islet vasculature is disrupted hampering sufficient tracer supply to the islet graft. However, in the first weeks after transplantation, functional blood supply in the islet graft is restored, which will allow reproducible [^{123}I]IBZM targeting of the islet graft at later time points after transplantation (14, 15).

Conclusions

In this study, non-invasive functional islet graft quantification and longitudinal graft monitoring using [^{123}I]IBZM SPECT was explored. We demonstrated, for the first time, a high linear correlation (Pearson r=0.73) between SPECT signal in the islet graft, obtained from a radiotracer, and insulin positive graft volume. Furthermore, we demonstrate the feasibility of islet graft follow-up from three until ten weeks after transplantation. These data go beyond previous work demonstrating the feasibility of nuclear islet graft imaging, since a linear correlation of functional islet graft volume and radiotracer uptake in vivo has never been demonstrated. In the future, [^{123}I]IBZM SPECT might provide an attractive tool to monitor islet graft survival since it allows longitudinal graft visualization and is able to detect differences in functional islet graft volume. The limited capacities of D2 receptor imaging for islets engrafted in the liver may no longer be a major drawback considering the increasing clinical interest in intramuscular islet transplantation (13, 15). In the end, [^{123}I]IBZM SPECT might provide important additional information on the islet graft and eventually, might help improve transplantation outcome.

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Declaration of interest

Non.

Contribution statement

SMAW and IK designed and performed experiments, acquired, analyzed and interpreted data, and drafted and critically revised the manuscript. DB, LJ, CF, OCB, MB and MG provided a substantial contribution to conception, design, data acquisition, analysis or interpretation. OCB, MB and MG revised the manuscript for intellectual content. All authors approved the final version. MG is responsible for the integrity of the manuscript as a whole.

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Legends to figures

Figure 1: A) In vivo SPECT/CT images of a small (left) (target-to-background ratio = 7.1), medium (middle) (target-to-background ratio = 16.9) and large (right) (target-to-background signal = 24.6) islet graft in the calf muscle, six weeks after transplantation, acquired 1 hour after injection of [^{123}I]IBZM. The red arrows indicate the transplant. B) Scatter plot of the correlation between the SPECT signal in the transplant expressed in arbitrary units per MBq (AU/MBq) (y-axis) (left panel) and in target-to-background ratios (y-axis) (right panel) and the viable graft volume (μm^3) as calculated histologically (x-axis) (Pearson r = 0.73, p = 0.005). C) In vivo SPECT/CT image (left) and ex vivo SPECT image (right) of a WAG/Rij calf muscle, transplanted with 3,000 islets. The red arrows indicate co-localization of the SPECT signal in the calf muscle detected in vivo and ex vivo.

Figure 2: In vivo SPECT/CT images of an islet graft in the calf muscle from three to ten weeks after transplantation. The red arrows indicate the transplant.

Figures