Development of Radiotracers for the Determination of the Beta-Cell Mass *In Vivo*

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Abstract: The changes in beta-cell mass (BCM) during the course of diabetes are not yet well characterized. A non-invasive method to measure the BCM *in vivo* would allow us to study the BCM during the onset and progression of the diseases caused by beta-cell dysfunction. PET and SPECT imaging are attractive approaches to determine the BCM because of their high sensitivity and the possibility to quantitatively analyze the images. Several targets and their corresponding radiotracers have been examined for their ability to determine the BCM including radiolabeled antibodies, antibody fragments, peptides and small molecules. Although some of these tracers show promising results, there is still no reliable method to determine the beta-cell mass *in vivo*. In this review, the targets and the corresponding radiotracers evaluated so far for the determination of the BCM *in vivo* in humans will be discussed.

Keywords: Beta-cell mass, radiotracers, peptides, antibodies, nuclear medicine, imaging.

INTRODUCTION

Diabetes mellitus is characterized by a deficiency in control of glucose homeostasis. In type 1 diabetes (T1D), autoimmune destruction of pancreatic beta-cells leads to an absolute deficiency of insulin secretion with hyperglycemia as a consequence [1]. In type 2 diabetes (T2D), insulin resistance of the target cells is the cause of impaired glucose homeostasis as well as impaired beta-cell function [1-3] but other factors may also play a role [4]. The changes in beta-cell mass (BCM) during diabetes are not well understood nor well characterized. Various animal models have been used to study the effect of the BCM on insulin secretion and the development of diabetes. However, most information about the BCM in humans are obtained from biopsy studies of diabetic patients. Biopsy studies do not show the dynamics of the BCM at the onset and progression of the disease. Previous studies showed that there is a reserve capacity of beta-cells and T1D occurs when the BCM drops under a critical threshold (>80% loss of BCM) [5, 6]. Moreover, a low number of insulin-producing cells (approximately 10%) is still present in half of all insulin-dependent T1D patients [5]. A non-invasive method to measure the BCM *in vivo* would allow us to study the BCM during the onset and progression of diabetes.

An attractive approach to determine the BCM is PET and SPECT imaging after injection of a radiotracer. The major advantage of PET and SPECT imaging over other imaging modalities such as MRI and CT is their high sensitivity. Moreover, PET and SPECT imaging can give information about biochemical changes by quantitative determination of the uptake of a tracer in the target tissue. Potentially, PET and SPECT imaging would allow quantitative determination of the BCM noninvasively. Radiotracers, based on antibodies, peptides and small molecules, have been successfully used for the detection of tumors and inflammation. Several targets and their corresponding ligands for the determination of the BCM have been described and examined. There are several challenges that have to be encountered before a non-invasive method is feasible for the detection of the BCM. The BCM represents only 1-2% of the total pancreatic mass and the beta-cells are located in the islets of Langerhans scattered throughout the pancreas [7, 8]. The tracer has to be highly beta-cell specific; no or very low uptake of the tracer in the exocrine and the other endocrine cells is required, in order to measure significant changes in tracer uptake between healthy and diabetic subjects. The target has to be expressed on all beta-cells and the expression level should not be altered during development and during the course of diabetes. Moreover, a highly sensitive and quantitative imaging technique has to be used. Recently, an *in vitro* method to screen potential ligands for the determination of the beta-cell mass has been described [9]. The screening method is based on the binding capacity of the compounds to isolated islets, cell lines derived from beta-cell tumors (INS-1) [10] and cell lines from tumors of the exocrine pancreas (PANC-1) [11]. A compound is considered promising when it displays high binding to islets and beta-cell derived tumor cells and low binding to tumor cells of the exocrine pancreas. Multiple tracers were screened *in vitro* and only the most promising were tested *in vivo*. This method is currently considered the standard for screening of potential agents to determine the beta-cell mass.

Here, the targets and the corresponding radiotracers will be discussed with respect to their ability to determine the BCM including antibodies, antibody fragments, peptides and small molecules. Although some of them show promising results, there is no reliable method to determine the beta-cell mass *in vivo* yet. In this review the most promising targets and corresponding radiotracers evaluated so far for the determination of the BCM will be discussed.

RECEPTOR IMAGING

Peptide hormone receptor and neurotransmitter receptors are attractive targets for targeting of tumors and neurological and psychiatric disorders. The most successful example of peptide hormone receptor is targeting neuroendocrine tumors (NET) using radiolabeled somatostatin analogs. The somatostatin receptor is overexpressed on a wide variety of NET. The somatostatin analog DTPA-octreotide labeled with ^{111}In (Octreoscan) is the standard procedure for the diagnosis of NET [12, 13]. Several octreotide derivatives labeled with a variety of radionuclides have been successfully used for imaging and therapy of NET. The major advantage of peptides that are conjugated and labeled with a radiometal is the accumulation of the radiometal-complex in the target cells. When the peptide labeled with a radiometal is internalized, the radiolabeled metabolite is trapped in the lysosome and the radiometal will accumulate in the cell [14].

A successful example of neurotransmitter receptor targeting is the targeting of the dopamine receptor. Radiolabeled dopamine derivatives are commonly used to target the dopamine receptor expressing tissues. These tracers are clinically used to diagnose dementia and Parkinsons disease (PD). The tracers are also used to study dementia and drug addiction.

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Here, we discuss peptide hormone receptors and neurotransmitter receptor targeting and the corresponding tracers that can potentially be used for determination of the BCM.

Dopamine Receptor

Dopamine is a neurotransmitter that plays an important role in neurological and psychiatric disorders such as dementia, Parkinson's Disease and drug addiction [15]. Dopamine signaling is mediated by dopamine receptors. Five receptor types have been described (D1 – D5), classified in two groups: dopamine D1-like (D1 and D5) and dopamine D2-like (D2, D3 and D4) families [16, 17]. Dopamine receptors are mainly expressed in the central and peripheral nervous system. The effect of dopamine on the beta-cell function and insulin secretion is poorly understood. Recently, expression of dopamine receptor expression from both families have been detected by RT-PCR in beta-cells [18]. The expression of D2-like receptors was confirmed by immunostaining of INS-1E cells and rodent and human isolated islets [18]. These results suggest that targeting of the dopamine receptor may be a suitable approach for the determination of the beta-cell mass. ^{18}F-DOPA PET is used to differentiate between focal and diffuse beta-cell hyperplasia (nesidioblastosis), a neuroendocrine condition characterized by hyperinsulinemia in infant age [9, 19-23]. However, the high uptake in the exocrine pancreas (in relation to the islets) seems to limit the usefulness of ^{18}F-DOPA PET for the determination of the BCM. Moreover, besides the low target-to-background ratio, the tracer has more disadvantages, i.e. a complex preparation procedure and a low radiochemical yield.

VMAT2

The vesicular monoamine transporter (VMAT) is a transport protein located on the presynaptic cell. Two isoforms have been identified in rodent and human tissues; VMAT1 and VMAT2 [24-26]. VMAT2 is an integral membrane protein that acts to transport monoamines, particularly neurotransmitters such as dopamine, norepinephrine, serotonine and histamine. VMAT2 is expressed in the central and peripheral nervous system, the hematopoietic system and the neuroendocrine system [27-32]. In the neuroendocrine system, VMAT2 is expressed in chromaffin cells of the adrenal medulla, histamine-storing enterochromaffin-like cells of the stomach and pancreatic islet cells [27, 30, 32-35]. Isolated human islets show higher VMAT2 mRNA expression than cells in the exocrine pancreas [33]. The VMAT2 receptor is expressed on rodent and human beta-cells as determined immunohistochemically [27]. These findings suggest that the VMAT2 receptor is suitable for targeting the pancreatic beta-cells.

Tetrabenazine (TBZ) and dihydrotetrabenazine (DTBZ) specifically inhibit vesicular storage of monoamine uptake by high affinity binding to the synaptic VMAT2 [36]. Binding studies with ^{3}H-DTBZ showed high affinity for VMAT2 [37-40]. The VMAT2 ligand (+)-^{11}C-DTBZ was developed for VMAT2 targeting in the striatum and has successfully been used for PET studies in Parkinson's Disease and other neurodegenerative diseases [41, 42]. Recent studies showed reduced uptake of ^{11}C-DTBZ in the pancreas of streptozotocin-induced diabetic rats and rats that spontaneously develop diabetess (BB-DP rats) compared to non-diabetic rats [34, 43]. First clinical studies showed a reduction in pancreatic uptake of ^{11}C-DTBZ in long-standing T1D patients compared to the uptake in the pancreas of healthy control subjects [44, 45]. DTBZ compounds labeled with ^{18}F were developed to overcome the short half-life of ^{11}C-labeled DTBZ ($T_{1/2}$ = 20 min.). Preclinical studies showed high pancreatic uptake of ^{18}F-DTBZ in healthy rats with favorable biodistribution leading to improved target-to-background ratios [46, 47]. The pancreas was clearly visualized in rats by PET imaging after injection of ^{18}F-DTBZ . Recently an new DTBZ derivative was developed, ^{18}F-FP-(+)-DTBZ, with higher pancreatic uptake and lower uptake in the non-target tissues, especially the liver [48]. However, the correlation between the beta-cell mass and pancreatic uptake of ^{18}F-DTBZ was not examined in these studies [46-48].

A clinical study with ^{11}C-DTBZ showed that the pancreas was visible in PET images and the pancreatic uptake was reduced in diabetic patients [45, 49]. Although uptake of ^{11}C-DTBZ in the pancreata of diabetic patients was markedly reduced, significant uptake was still observed [45]. This residual uptake might be explained by VMAT2 expression in polypeptide (PP) cells in the islets [50] or binding of the tracer to other structures. A recent study showed VMAT2 expression on the sympathetic nerve endings in the pancreas [51]. The nerve endings in the islets, but not the nerve endings in the exocrine pancreas are degraded during the onset of T1D in a T1D rat model (BB-DP rats) [51]. It is unknown what the contribution of the VMAT2 expression in the nerve endings to the total VMAT2 expression in the pancreas is and if this might contribute to the residual uptake in diabetic subjects. Moreover, there is heterogeneous expression of VMAT2 in islets [50] which makes interpretation of DTBZ binding difficult. These findings do not exclude the use of VMAT2 as a target for beta-cell measurement, however the target VMAT2 and the tracer do not appear to be 100% specific for beta-cells.

GLP-1R

The glucagon-like peptide 1 receptor (GLP-1R) is expressed in rat, mouse and human pancreatic beta-cells and not in α, δ and pp cells [52]. There is high expression of the GLP-1R in the beta-cells and limited expression is found in the pancreatic duct and acini [53]. Furthermore, there is limited expression in other organs (duodenum, stomach, lung, heart and brain) [54]. This makes the GLP-1R a potential target for BCM imaging. The GLP-1R belongs to the family of the G-protein coupled receptors, in which internalization of the receptor-ligand complex is triggered after binding of the agonist to the receptor [55, 56]. GLP-1, the natural ligand of the GLP-1R, is not suitable for GLP-1R targeting due to the low stability in plasma resulting from rapid degradation by dipeptidyl peptidase IV (DPP IV) [57-60]. More stable agonists of the GLP-1 receptor are Exendin-3 and Exendin-4 [61, 62]. Exendin-3 is derived from Heloderma horridum, the Beaded Mexican Lizard (Mexico and southern Guatemala), and Exendin-4 from Heloderma Suspectum, the Gilamonster (a lizard living in the South West of the United States and northern Mexico). First preclinical studies with ^{123}I-labeled Exendin showed high uptake in pancreas and in subcutaneous insulinomas [63]. However, the highest uptake was observed 30 minutes after injection, probably due to the fast washout of the radionuclide after degradation of the internalized peptide. Introduction of a C-terminal Lysine enabled conjugation of a chelator (e.g. DTPA, DOTA) that facilitates labeling with radiometals. When the peptide labeled with a radiometal is internalized, the radiolabeled metabolite is trapped in the lysosome and the radiometal will accumulate in the cell [14]. ^{111}In-labeled Exendin showed a higher uptake and retention in the subcutaneous insulinomas compared to the ^{123}I-labeled compound [63]. Moreover, Wild *et al.* showed that ^{111}In-DTPA-Lys^{40}-Ahx-Exendin-4 showed high uptake in tumors in transgenic mice that spontaneously develop pancreatic insulinomas and in patients with insulinomas [64, 65]. In both studies the insulinomas could be clearly detected by SPECT imaging. Furthermore, it has been shown that the uptake of ^{111}In-DTPA-Lys^{40}-Exendin-3 correlates with the BCM in a linear manner in rats with alloxan-induced diabetes and that the pancreas can be visualized by SPECT imaging on a dedicated microSPECT scanner [66]. These studies suggest that Exendin labeled with ^{111}In is a promising tracer for the determination of the BCM. However, the pancreatic uptake of the tracer in rats was rather low, which might limit accurate quantification of the BCM *in vivo*. Conjugation of Exendin with DOTA or NOTA enables labeling with a positron emitter (eg ^{68}Ga, ^{64}Cu) for PET imaging. PET imaging is superior over SPECT in terms of sensitivity and resolution in humans [67].

However, the clinical feasibility of PET imaging with radiolabeled Exendin has yet to be determined.

SUR1 Receptors

ATP-sensitive K^+ channels (K_{ATP} channels) are thought to play a critical role in the insulin-dependent and sulfonylurea-dependent insulin secretion. K_{ATP} channels are composed of a small inwardly rectifying K^+ channel subunit (KIR6.1 or KIR6.2) and a sulfonylurea receptor (SUR1, SUR2A or SUR2B) belonging to the ATP-binding cassette superfamily [68]. The K_{ATP} channels of pancreatic beta-cells consist of a Kir6.2 and a SUR1 subunit. Sulfonylureas, such as glibenclamide and repaglinide are successfully used as blood glucose level lowering drugs in the treatment of T2D [69]. Several studies with radiolabeled glibenclamide and repaglinide analogs to target the SUR1 receptor have been conducted [70, 71]. ^{18}F-labeled glibenclamide and repaglinide analogs have been developed as tracers for the determination of the BCM by PET imaging [72, 73]. However, recent studies showed that these analogs are not suitable to determine the beta-cell mass, due to their low uptake in the pancreas that was not SUR1 receptor mediated and high uptake in adjacent non-target organs [73, 74]. It still has to be determined whether the pancreatic uptake is beta-cell specific. This raises the question whether the SUR1 receptor is useful as a target for the non-invasive determination of the BCM. SUR1 is expressed all over the islet as determined by immunostaining in cryosection of rat pancreas and co-localizes with glucagon, somatostatin and pancreatic polypeptide (pp) [75]. These data suggest that SUR1 is expressed in α, β as well as the pp cells, which might render the receptor less suitable as a target for BCM determination.

ANTIGENS

The invention of the hybridization technology to produce monoclonal antibodies (mAbs) in 1975 by Georges Köhler and César Milstein largely contributed to immune-based therapy of cancer, but also to nuclear imaging with monoclonal antibodies [76]. Since then, a large number of mAbs have been developed.

Monoclonal antibodies are potential nuclear imaging agents, as they very selectively recognize their specific epitopes. However, due to their long circulatory half-life together with their slow diffusion into tissue (caused by their relatively large size), new approaches are applied in order to increase the target-to-background ratios obtained with radiolabeled mAbs in radionuclide imaging. By enzymatic degradation or molecular engineering, antibodies can be modified to alter their pharmacokinetic properties to suit specific targeting requirements. For example, antibody fragments have more rapid target uptake and more rapid background clearance. There is a wide range of radioisotopes available for labeling of antibodies. The potential of using antibodies or their fragments in targeting a wide range of molecules presented on the cells of interest is very promising. However, the use of antibodies for beta-cell imaging is limited mainly due to unsatisfactory target-to-background ratios [14]. Here, we summarize the efforts undertaken up to date in antibody-based targeting strategies for radionuclide imaging of transplanted and native islets of Langerhans.

IC2

Anti-pancreatic autoantibodies have been found in most patients with Type I diabetic syndromes [77]. Islet cell surface antibodies (ICSA) have been shown to precede the onset of diabetes in the BB rat [78]. Therefore, if antipancreatic autoantibodies are involved in the pathogenesis of beta-cell destruction, ICSA are considered to be potential targets for BCM imaging.

IC2 is a beta-cell-specific monoclonal antibody against an ICSA [79, 80]. The IC2 monoclonal IgM antibody is produced in a rat-rat hybridoma and reacts with both normal and tumor cells derived from rat islets [79].

In 2001 Moore *et al*. presented promising results on the correlation between ^{111}In and ^{125}I-labeled IC2 antibody accumulation in the pancreas and the BCM [81]. In this work ^{111}In-labeled IC2 was characterized *in vitro* on isolated islets as well as *in vivo* in a diabetic mouse model. No binding to exocrine pancreas or stromal tissues was observed after intravenous administration of the tracer. It was also shown that IC2 did not react with insulin and therefore would not interfere with its secretion. After chemical modification, the antibody retained its specific affinity to beta-cells both *in vitro* and *in vivo*.

Biodistribution of radio-iodinated IC2 revealed a higher accumulation of radiotracer in normal pancreas compared to uptake in the pancreas of diabetic rats. A strong correlation of BCM with probe accumulation was observed in both STZ-diabetic and control animals. The authors concluded that the accumulation of the probe in the islet beta-cells has decreased with reduction of BCM and that it is feasible to detect this reduction by *ex vivo* nuclear imaging techniques after injection of IC2-DTPA-^{111}In. However, the absolute uptake of IC2 in the pancreas was rather low (approximately 0.5 %ID/g), which might hinder the detection of small differences in the BCM. Preclinical and clinical studies still have to be performed to examine the feasibility of IC2 to determine the BCM *in vivo*.

The target of the IC2 antibody is not yet defined. Definition of the target of IC2 will enable the development of new tracers that bind to the IC2 antibody target, which might lead to higher uptake in the beta-cells and in this way make it possible to determine small changes in BCM.

R2D6

The monoclonal antibody R2D6 recognizes a beta-cell specific plasma membrane ganglioside antigen in rats. R2D6 is a mouse monoclonal antibody that was generated against a beta-cell specific plasma membrane determinant that is not shared by other islet cell-types. R2D6 did not affect beta-cell physiology, when incubated with islets. Unfortunately R2D6 does not cross-react with the human beta-cell membrane antigen [82].

In 2001 Ladriene *et al.*, tested ^{125}I-labelled R2D6 in a rat model using radioimmunoscintigraphy [83]. *In vitro* experiments of radiolabeled R2D6 revealed a high specificity for beta-cells, but no binding differences between normal and STZ rat islets. Moreover, *in vivo* experiments in which rats were injected with ^{125}I-labeled R2D6 showed neither differences between probe accumulation in endocrine when compared to exocrine pancreas, nor did they show differences between diabetic and control animals. This specificity issue will probably hinder the development of such beta-cell specific radiolabelled mAbs [83].

Single Chain Antibodies (SCAs)

In order to overcome some of mAbs weaknesses as radiotracers (long circulatory half-life, low diffusion capacitiy, inadequate target-to-background ratios), single chain antibodies (SCAs) were developed as a promising option for immune-based diagnostics and therapy. It has been reported that removal of the Fc portion to produce an antibody fragment reduces non-specific binding and blood clearance time [84]. However, after testing the Fab fragment of one of beta-cell specific IgM antibodies (K14D10) by *in vitro* screening it appeared that its specificity (uptake in endocrine vs. exocrine pancreas) is far below that required for a reasonable signal-to-background ratio [84].

Recently, Ueberberg *et al*. demonstrated a new, promising approach for beta-cell imaging by generation of novel SCAs [85]. They screened a phage-library of more than 1.4×10^8 different human single chain variable fragments for SCAs presented on the islets with two different approaches of *in vivo* and *in vitro* screening. Phages that specifically bound to the islet cells were selected, amplified and purified. Importantly, no binding to

exocrine cells and several other control organs (liver, kidney, spleen, heart and lung) was detectable in any of the selected islet specific phage clones (ISPCs). Out of 6 ISPCs only two that selectively localized in the cytoplasm of beta and alpha cells were further evaluated (respectively: ISPC1 and 3). ISPC1 (SCA B1) that is beta-cell-specific and ISPC3 (SCA A1) that is alpha-cell-specific were labeled with ^{125}I. *In vitro* analysis of selected ^{125}I-labeled SCAs was performed in order to determine the respective binding properties in beta, alpha and exocrine cell lines. Binding of the beta-cell specific SCA B1 to INS-1 cells was rapid and specific. Also plasma clearance kinetics of selected SCAs were determined in rats *in vivo* and revealed a very rapid elimination of both SCA B1 and SCA A1 from the circulation ($t_{1/2}$ = 22.7 min and 19.2 min, respectively). SCA B1 co-localized with anti-insulin antibodies in human beta-cells and SCA A1 co-localized with anti-glucagone antibodies in human alpha-cells which confirmed its high specificity for human islets and its potential for use as a beta or alpha-cell marker. Also a strong positive correlation was found between probe accumulation in a specific cell-type and beta or alpha-cell mass (determined by quantitative morphometry). The specific molecular target of these SCAs remains to be elucidated [85].

Low toxicity, high specificity, rapid binding and clearance kinetics and direct correlation of SCAs accumulation in beta-cells with beta-cell mass could make these ISPCs interesting tracers for non invasive beta-cell imaging and quantification *in vivo* in rodents and humans.

SMALL MOLECULES

Dithizone

Dithizone is a well-known agent which chelates divalent heavy metal ions, for example Hg^{2+}, Pb^{2+}, Zn^{2+}, Pd^{2+} and Bi^{2+}. Of all cells, beta-cells have the highest zinc accumulation inside the cells. Zinc ions are involved in insulin metabolism and its secretion mechanisms by creating osmotically stable hexamers of insulin within secretory granules of beta-cells [86]. Dithizone has been especially useful for zinc assays and therefore serves as a specific vital dye to assess the purity and viability of isolated pancreatic islets [87, 88]. Because dithizone stains beta-cells *in vivo*, it could potentially be used as a marker of islet viability [89]. In the past, some attempts have been made to radiolabel dithizone [90], but radio-iodinated derivatives of dithizone were extremely unstable and readily released radioactive iodine in biological fluids [89]. Therefore a novel approach to develop a stable histamine conjugated dithizone has been tested and this radioiodinated dithizone-histamine has been evaluated in respect to preferential uptake in the pancreas [90, 91]. In a rat model of islet transplantation to the testes, specific uptake of the dithizone-[^{131}I]-histamine conjugate was demonstrated [91]. However, in normal and streptozotocin-treated rats, both the exocrine pancreas and liver showed significant uptake. Also in other transplantation models, the difference in uptake of radiolabeled dithizone-histamine between transplanted islets and an intact pancreas was very low (0.25% ± 0.12% vs. 0.20% ± 0.04%) [92]. Moreover, dithizone-[^{131}I]-histamine was described to exert toxic effects on beta-cells in higher concentrations [93, 94], therefore for *in vivo* imaging of islets with radiolabelled dithizone-histamine may only be of limited use.

Alloxan

Alloxan (2,4,5,6-tetraoxypyrimidine) is a toxic glucose analogue. When administrated to rodents, it selectively destroys beta-cells in the pancreas. Alloxan also selectively inhibits glucose-induced insulin secretion through its ability to inhibit the beta-cell glucose sensor glucokinase [95, 96]. It is widely used as a diabetogenic agent in animal models to mimic insulin-dependent diabetes mellitus as it accumulates in insulin-producing cells via the GLUT2 glucose transporter. Alloxan is therefore highly specific for beta-cells, but highly toxic at the same time. Autoradiography studies with ^{14}C-2-alloxan revealed that the radioactivity in the pancreatic islets was higher than in any other mouse tissue after intravenous injections [97]. In 2001 Malaisse *et al*., investigated the uptake of ^{14}C-2-alloxan in the pancreas in control and streptozotocin-induced diabetic (STZ) rats [98]. The activity concentration in the pancreas of diabetic rats was lower than in the pancreas of control rats. Because of high toxicity very low doses have to be administered, thus high specific activities are needed. Moreover, alloxan is very unstable and light-sensitive making the synthesis and transport of the tracer very cumbersome.

^{18}F-FDG

2-^{18}F-Fluoro-2-deoxy-glucose (^{18}F-FDG) is a radiolabeled glucose analog that is transported into the cell via glucose transporters followed by phosphorylation by hexokinases. Once phosphorylated, no further metabolic pathway exists and accumulation occurs ("metabolic trapping"). ^{18}F-FDG is a successful tool for the detection of tumors, since tumors have an enhanced glucose consumption. ^{18}F-FDG can also be used for the detection of inflammation because of the uptake of ^{18}F-FDG in most inflammatory cells. The onset of T1D is characterized by inflammation of the islets which is called insulitis. Therefore, it has been hypothesized that ^{18}F-FDG PET might be useful for the non-invasive detection of insulitis. A study in NOD mice, mice that develop auto-immune insulin-dependent diabetes resembling human T1D, showed increased uptake of ^{18}F-FDG in the pancreatic islets by autoradiography due to ^{18}F-FDG uptake in islet-infiltrating lymphocytes [99]. A clinical study showed higher uptake of ^{18}F-FDG in patients with newly diagnosed T1D compared to healthy subjects and patients with long-standing diabetes [100]. After the diagnosis of diabetes, the number of insulin-producing beta-cells and islet-infiltrating lymphocytes gradually decreases leading to similar uptake compared to healthy subjects. However, the ratio of uptake of ^{18}F-FDG between pancreatic islets and exocrine cells tested *in vitro* is considered insufficient for determination of the BCM [9]. Moreover, a study in streptozotocin-induced diabetic rats showed no significantly lower uptake of ^{18}F-FDG as compared to healthy rats [101]. Therefore, ^{18}F-FDG appears to be unsuitable for the quantification of the BCM

CONCLUSIONS

The determination of the BCM *in vivo* is a major challenge, because the BCM only represents 1-2% of the total pancreatic mass and the islets are spread throughout the pancreas. Therefore, a high specific and sensitive tracer is needed. The ideal tracer should preferably meet the following criteria:

1. The target of the tracer should be expressed specifically in the endocrine pancreas.
2. The target should be expressed on beta-cells only (and not on α, δ en PP cells).
3. High uptake in the endocrine pancreas and low uptake in the exocrine pancreas is needed.
4. The uptake of the tracer should correlate with the BCM .
5. The difference in uptake between diabetic and healthy subjects has to be large to enable detection of small differences in BCM.
6. high target-to-background-ratio, in terms of endocrine versus exocrine pancreas and also pancreas versus adjacent organs, is needed to quantify the uptake in a reliable manner.

To enable the determination of small changes in BCM, a very sensitive technique to determine the BCM has to be used. PET and SPECT are the most sensitive *in vivo* imaging methods available for clinical imaging at this time point, therefore they are promising tools for the determination of the BCM. In recent years several new tracers for beta-cell imaging with PET and SPECT have been developed showing promising results in animal models. The

monoclonal antibody IC2 shows beta-cell specific uptake in the pancreas and a strong correlation between tracer uptake and the BCM. However, the uptake in the pancreas is relatively low which hinders the determination of the BCM *in vivo*. DTBZ, labeled with ^{11}C and ^{18}F, shows high uptake in the pancreas where ^{18}F-FP-(+)-DTBZ shows the most promising results. However, the correlation with the beta-cell mass is still a matter of debate. Moreover, the difference in uptake between diabetic and healthy subjects might be too low to determine minor changes in BCM. The uptake of radiolabeled Exendin correlates with the BCM and is specific for beta-cells. The uptake of Exendin in the pancreas in rats appears to be relatively low which hampers determination of small differences in BCM.

The ideal tracer has not been found yet. Antibodies usually show low uptake and slow clearance resulting in low target-to-background ratio's. The size of an antibody limits tissue penetration which might hinder uptake in the beta-cells. Single chain antibodies and antibody fragments might overcome these problems. Peptide hormone receptor targeting has the advantage of internalization and metabolic trapping of the peptide, leading to high accumulation in the target cells. Moreover, there is fast clearance and fast and easy tissue penetration due to the small molecular size of such peptides. Small molecules (such as DTBZ) usually exhibit good tissue penetration and rapid clearance. The synthesis is more difficult and in some cases toxicity is a relevant issue.

Several tracers are close to become clinical applicable tracers for determination of the BCM but the search for improved tracers is still ongoing. The search for ligands of newly identified targets that might be useful to target beta-cells will continue.

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REFERENCES

References 102-104 are related articles recently published.

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2006; 29 (Suppl 1): S43-8.

[2] DeFronzo RA. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. Diabetes Rev 1997; 5: 177-269.

[3] Taylor SI. Deconstructing type 2 diabetes. Cell 1999; 97: 9-12.

[4] Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes 2004; 53 (Suppl 3): S16-21.

[5] Lohr M, Kloppel G. Residual insulin positivity and pancreatic atrophy in relation to duration of chronic type 1 (insulin-dependent) diabetes mellitus and microangiopathy. Diabetologia 1987; 30: 757-62.

[6] Steele C, Hagopian WA, Gitelman S, Masharani U, Cavaghan M, Rother KI, *et al*. Insulin secretion in type 1 diabetes. Diabetes 2004; 53: 426-33.

[7] Bonner-Weir S. Regulation of pancreatic beta-cell mass *in vivo*. Recent Prog Horm Res 1994; 49: 91-104.

[8] Weir GC, Bonner-Weir S, Leahy JL. Islet mass and function in diabetes and transplantation. Diabetes 1990; 39: 401-5.

[9] Sweet IR, Cook DL, Lernmark A, Greenbaum CJ, Krohn KA. Non-invasive imaging of beta cell mass: a quantitative analysis. Diabetes Technol Ther 2004; 6: 652-9.

[10] Asfari M, Janjic D, Meda P, Li G, Halban PA, Wollheim CB. Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. Endocrinology 1992; 130: 167-78.

[11] Lieber M, Mazzetta J, Nelson-Rees W, Kaplan M, Todaro G. Establishment of a continuous tumor-cell line (panc-1) from a human carcinoma of the exocrine pancreas. Int J Cancer 1975; 15: 741-7.

[12] Modlin IM, Tang LH. Approaches to the diagnosis of gut neuroendocrine tumors: the last word (today). Gastroenterology 1997; 112: 583-90.

[13] Krenning EP, Kwekkeboom DJ, Bakker WH, Breeman WA, Kooij PP, Oei HY, *et al*. Somatostatin receptor scintigraphy with [111In-DTPA-D-Phe1]- and [123I-Tyr3]-octreotide: the Rotterdam experience with more than 1000 patients. Eur J Nucl Med 1993; 20: 716-31.

[14] Gotthardt M, Boermann OC, Behr TM, Behe MP, Oyen WJ. Development and clinical application of peptide-based radiopharmaceuticals. Curr Pharm Des 2004; 10: 2951-63.

[15] Callier S, Snapyan M, Le Crom S, Prou D, Vincent JD, Vernier P. Evolution and cell biology of dopamine receptors in vertebrates. Biol Cell 2003; 95: 489-502.

[16] Civelli O, Bunzow JR, Grandy DK. Molecular diversity of the dopamine receptors. Annu Rev Pharmacol Toxicol 1993; 33: 281-307.

[17] Gingrich JA, Caron MG. Recent advances in the molecular biology of dopamine receptors. Annu Rev Neurosci 1993; 16: 299-321.

[18] Rubi B, Ljubicic S, Pournourmohammadi S, Carobbio S, Armanet M, Bartley C, *et al*. Dopamine D2-like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion. J Biol Chem 2005; 280: 36824-32.

[19] de Lonlay P, Simon-Carre A, Ribeiro MJ, Boddaert N, Giurgea I, Laborde K, *et al*. Congenital hyperinsulinism: pancreatic [18F]fluoro-L-dihydroxyphenylalanine (DOPA) positron emission tomography and immunohistochemistry study of DOPA decarboxylase and insulin secretion. J Clin Endocrinol Metab 2006; 91: 933-40.

[20] Otonkoski T, Nanto-Salonen K, Seppanen M, Veijola R, Huopio H, Hussain K, *et al*. Noninvasive diagnosis of focal hyperinsulinism of infancy with [18F]-DOPA positron emission tomography. Diabetes 2006; 55: 13-8.

[21] Ribeiro MJ, De Lonlay P, Delzescaux T, Boddaert N, Jaubert F, Bourgeois S, *et al*. Characterization of hyperinsulinism in infancy assessed with PET and 18F-fluoro-L-DOPA. J Nucl Med 2005; 46: 560-6.

[22] Mohnike K, Blankenstein O, Christesen HT, De Lonlay J, Hussain K, Koopmans KP, *et al*. Proposal for a standardized protocol for 18F-DOPA-PET (PET/CT) in congenital hyperinsulinism. Horm Res 2006; 66: 40-2.

[23] Hardy OT, Hernandez-Pampaloni M, Saffer JR, Suchi M, Ruchelli E, Zhuang H, *et al*. Diagnosis and localization of focal congenital hyperinsulinism by 18F-fluorodopa PET scan. J Pediatr 2007; 150: 140-5.

[24] Erickson JD, Eiden LE. Functional identification and molecular cloning of a human brain vesicle monoamine transporter. J Neurochem 1993; 61: 2314-7.

[25] Erickson JD, Eiden LE, Hoffman BJ. Expression cloning of a reserpine-sensitive vesicular monoamine transporter. Proc Natl Acad Sci USA 1992; 89: 10993-7.

[26] Liu Y, Peter D, Roghani A, Schuldiner S, Prive GG, Eisenberg D, *et al*. A cDNA that suppresses MPP+ toxicity encodes a vesicular amine transporter. Cell 1992; 70: 539-51.

[27] Anlauf M, Eissele R, Schafer MK, Eiden LE, Arnold R, Pauser U, *et al*. Expression of the two isoforms of the vesicular monoamine transporter (VMAT1 and VMAT2) in the endocrine pancreas and pancreatic endocrine tumors. J Histochem Cytochem 2003; 51: 1027-40.

[28] Anlauf M, Schafer MK, Schwark T, von Wurmb-Schwark N, Brand V, Sipos B, *et al*. Vesicular monoamine transporter 2 (VMAT2) expression in hematopoietic cells and in patients with systemic mastocytosis. J Histochem Cytochem 2006; 54: 201-13.

[29] Eiden LE. The vesicular neurotransmitter transporters: current perspectives and future prospects. FASEB J 2000; 14: 2396-400.

[30] Erickson JD, Schafer MK, Bonner TI, Eiden LE, Weihe E. Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. Proc Natl Acad Sci USA 1996; 93: 5166-71.

[31] Weihe E, Eiden LE. Chemical neuroanatomy of the vesicular amine transporters. FASEB J 2000; 14: 2435-49.

[32] Weihe E, Schafer MK, Erickson JD, Eiden LE. Localization of vesicular monoamine transporter isoforms (VMAT1 and VMAT2) to endocrine cells and neurons in rat. J Mol Neurosci 1994; 5: 149-64.

[33] Maffei A, Liu Z, Witkowski P, Moschella F, Del Pozzo G, Liu E, *et al*. Identification of tissue-restricted transcripts in human islets. Endocrinology 2004; 145: 4513-21.

[34] Simpson NR, Souza F, Witkowski P, Maffei A, Raffo A, Herron A, *et al*. Visualizing pancreatic beta-cell mass with [11C]DTBZ. Nucl Med Biol 2006; 33: 855-64.

[35] Peter D, Liu YJ, Sternini C, Degiorgio R, Brecha N, Edwards RH. Differential expression of 2 vesicular monoamine transporters. J Neurosci 1995; 15: 6179-88.

[36] Pettibone DJ, Totaro JA, Pflueger AB. Tetrabenazine-induced depletion of brain monoamines: characterization and interaction with selected antidepressants. Eur J Pharmacol 1984; 102: 425-30.

[37] Masuo Y, Pelaprat D, Scherman D, Rostene W. [3H]Dihydrotetrabenazine, a new marker for the visualization of dopaminergic denervation in the rat striatum. Neurosci Lett 1990; 114: 45-50.

[38] Scherman D, Jaudon P, Henry JP. Characterization of the monoamine carrier of chromaffin granule membrane by binding of [2-3H]dihydrotetrabenazine. Proc Natl Acad Sci USA 1983; 80: 584-8.

[39] Scherman D, Raisman R, Ploska A, Agid Y. [3H]dihydrotetrabenazine, a new *in vitro* monoaminergic probe for human brain. J Neurochem 1988; 50: 1131-6.

[40] Zucker M, Weizman A, Rehavi M. Characterization of high-affinity [3H]TBZOH binding to the human platelet vesicular monoamine transporter. Life Sci 2001; 69: 2311-7.

[41] Bohnen NI, Albin RL, Koeppe RA, Wernette KA, Kilbourn MR, Minoshima S, *et al.* Positron emission tomography of monoaminergic vesicular binding in aging and Parkinson disease. J Cereb Blood Flow Metab 2006; 26: 1198-212.

[42] Koeppe RA, Gilman S, Joshi A, Liu S, Little R, Junck L, *et al.* 11C-DTBZ and 18F-FDG PET measures in differentiating dementias. J Nucl Med 2005; 46: 936-44.

[43] Souza F, Simpson N, Raffo A, Saxena C, Maffei A, Hardy M, *et al*. Longitudinal noninvasive PET-based beta cell mass estimates in a spontaneous diabetes rat model. J Clin Invest 2006; 116: 1506-13.

[44] Freeby M, Goland R, Ichise M, Maffei A, Leibel R, Harris P. VMAT2 quantitation by PET as a biomarker for β-cell mass in health and disease. Diabetes Obes Metab 2008; 10 (Suppl 4): 98-108.

[45] Goland R, Freeby M, Parsey R, Saisho Y, Kumar D, Simpson N, *et al.* 11C-dihydrotetrabenazine PET of the pancreas in subjects with long-standing type 1 diabetes and in healthy controls. J Nucl Med 2009; 50: 382-9.

[46] Kung HF, Lieberman BP, Zhuang ZP, Oya S, Kung MP, Choi SR, *et al. In vivo* imaging of vesicular monoamine transporter 2 in pancreas using an (18)F epoxide derivative of tetrabenazine. Nucl Med Biol 2008; 35: 825-37.

[47] Zhu L, Liu J, Kung HF. Synthesis and evaluation of 2-amino-dihydrotetrabenzine derivatives as probes for imaging vesicular monoamine transporter-2. Bioorg Med Chem Lett 2009; 19: 5026-8.

[48] Kung MP, Hou C, Lieberman BP, Oya S, Ponde DE, Blankemeyer E, *et al. In vivo* imaging of β-cell mass in rats using 18F-FP-(+)-DTBZ: a potential PET ligand for studying diabetes mellitus. J Nucl Med 2008; 49: 1171-6.

[49] Freeby M, Simpson N, Saxena C, Dashnaw S, Hirsch J, Prince M, *et al.* Non-invasive pancreatic β-cell imaging using 11C dihydrotetra-benazine and positron emission tomography. Diabetes 2007; 56: A86 (Abstract).

[50] Saisho Y, Harris PE, Butler AE, Galasso R, Gurlo T, Rizza RA, *et al.* Relationship between pancreatic vesicular monoamine transporter 2 (VMAT2) and insulin expression in human pancreas. J Mol Histol 2008; 39: 543-51.

[51] Mei Q, Mundinger TO, Lernmark A, Taborsky GJ, Jr. Early, selective, and marked loss of sympathetic nerves from the islets of BioBreeder diabetic rats. Diabetes 2002; 51: 2997-3002.

[52] Tornehave D, Kristensen P, Romer J, Knudsen LB, Heller RS. Expression of the GLP-1 receptor in mouse, rat, and human pancreas. J Histochem Cytochem 2008; 56: 841-51.

[53] Korner M, Stockli M, Waser B, Reubi JC. GLP-1 receptor expression in human tumors and human normal tissues: potential for *in vivo* targeting. J Nucl Med 2007; 48: 736-43.

[54] Wei Y, Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. FEBS Lett 1995; 358: 219-24.

[55] Tulipano G, Stumm R, Pfeiffer M, Kreienkamp HJ, Hollt V, Schulz S. Differential beta-arrestin trafficking and endosomal sorting of somatostatin receptor subtypes. J Biol Chem 2004; 279: 21374-82.

[56] Hofland LJ, Lamberts SW. The pathophysiological consequences of somatostatin receptor internalization and resistance. Endocr Rev 2003; 24: 28-47.

[57] Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. Diabetes 1995; 44: 1126-31.

[58] Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. Endocrinology 1995; 136: 3585-96.

[59] Deacon CF, Hughes TE, Holst JJ. Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig. Diabetes 1998; 47: 764-9.

[60] Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem 1993; 214: 829-35.

[61] Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, *et al.* Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. Am J Physiol Endocrinol Metab 2001; 281: E155-61.

[62] Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem 1992; 267: 7402-5.

[63] Gotthardt M, Fischer M, Naeher I, Holz JB, Jungclas H, Fritsch HW, *et al*. Use of the incretin hormone glucagon-like peptide-1 (GLP-1) for the detection of insulinomas: initial experimental results. Eur J Nucl Med Mol Imaging 2002; 29: 597-606.

[64] Wild D, Behe M, Wicki A, Storch D, Waser B, Gotthardt M, *et al*. [Lys40(Ahx-DTPA-111In)NH2]exendin-4, a very promising ligand for glucagon-like peptide-1 (GLP-1) receptor targeting. J Nucl Med 2006; 47: 2025-33.

[65] Wild D, Macke H, Christ E, Gloor B, Reubi JC. Glucagon-like peptide 1-receptor scans to localize occult insulinomas. N Engl J Med 2008; 359: 766-8.

[66] Brom M, Baumeister P, Melis M, Laverman P, Joosten L, Behe M, *et al*. Determination of the beta-cell mass by SPECT imaging with In-111-DTPA-Exendin-3 in rats. J Nucl Med 2009; 50 (Suppl 2): 147.

[67] Rahmim A, Zaidi H. PET *versus* SPECT: strengths, limitations and challenges. Nucl Med Commun 2008; 29: 193-207.

[68] Aguilar-Bryan L, Clement JPT, Gonzalez G, Kunjilwar K, Babenko A, Bryan J. Toward understanding the assembly and structure of KATP channels. Physiol Rev 1998; 78: 227-45.

[69] Henquin JC. The fiftieth anniversary of hypoglycaemic sulphonamides. How did the mother compound work? Diabetologia 1992; 35: 907-12.

[70] Bernardi H, Fosset M, Lazdunski M. Characterization, purification, and affinity labeling of the brain [3H]glibenclamide-binding protein, a putative neuronal ATP-regulated K+ channel. Proc Natl Acad Sci USA 1988; 85: 9816-20.

[71] Wangler B, Beck C, Shiue CY, Schneider S, Schwanstecher C, Schwanstecher M, *et al*. Synthesis and *in vitro* evaluation of (S)-2-([11C]methoxy)-4-[3-methyl-1-(2-piperidine-1-yl-phenyl)-butyl-carbam oyl]-benzoic acid ([11C]methoxy-repaglinide): a potential beta-cell imaging agent. Bioorg Med Chem Lett 2004; 14: 5205-9.

[72] Shiue GG, Schirrmacher R, Shiue CY, Alavi AA. Synthesis of fluorine-18 labeled sulfonureas as beta-cell imaging agents. J Lab Comp Radiopharm 2001; 44: 127-39.

[73] Wangler B, Schneider S, Thews O, Schirrmacher E, Comagic S, Feilen P, *et al.* Synthesis and evaluation of (S)-2-(2-[F-18]fluoroethoxy)-4-([3-methyl-1-(2-piperidin-1-yl-phenyl)-butyl-carbamoyl]-methyl)-benzoic acid ([F-18]repaglinide): a promising radioligand for quantification of pancreatic beta-cell mass with positron emission tomography (PET). Nucl Med Biol 2004; 31: 639-47.

[74] Schmitz A, Shiue CY, Feng Q, Shiue GG, Deng S, Pourdehnad MT, *et al.* Synthesis and evaluation of fluorine-18 labeled glyburide analogs as beta-cell imaging agents. Nucl Med Biol 2004; 31: 483-91.

[75] Suzuki M, Fujikura K, Kotake K, Inagaki N, Seino S, Takata K. Immuno-localization of sulphonylurea receptor 1 in rat pancreas. Diabetologia 1999; 42: 1204-11.

[76] Dubowchik GM, Walker MA. Receptor-mediated and enzyme-dependent targeting of cytotoxic anticancer drugs. Pharmacol Ther 1999; 83: 67-123.

[77] Lernmark A, Freedman ZR, Hofmann C, Rubenstein AH, Steiner DF, Jackson RL, *et al.* Islet-cell-surface antibodies in juvenile diabetes mellitus. N Engl J Med 1978; 299: 375-80.

[78] Dyrberg T, Poussier P, Nakhooda F, Marliss EB, Lernmark A. Islet cell surface and lymphocyte antibodies often precede the spontaneous diabetes in the BB rat. Diabetologia 1984; 26: 159-65.

[79] Brogren CH, Hirsch F, Wood P, Druet P, Poussier P. Production and characterization of a monoclonal islet cell surface autoantibody from the BB rat. Diabetologia 1986; 29: 330-3.

[80] Aaen K, Rygaard J, Josefsen K, Petersen H, Brogren CH, Horn T, *et al.* Dependence of antigen expression on functional state of beta-cells. Diabetes 1990; 39: 697-701.

[81] Moore A, Bonner-Weir S, Weissleder R. Noninvasive *in vivo* measurement of beta-cell mass in mouse model of diabetes. Diabetes 2001; 50: 2231-6.

[82] Alejandro R, Shienvold FL, Hajek SA, Pierce M, Paul R, Mintz DH. A ganglioside antigen on the rat pancreatic B cell surface identified by monoclonal antibody R2D6. J Clin Invest 1984; 74: 25-38.

[83] Ladriere L, Malaisse-Lagae F, Alejandro R, Malaisse WJ. Pancreatic fate of a (125)I-labelled mouse monoclonal antibody directed against pancreatic B-cell surface ganglioside(s) in control and diabetic rats. Cell Biochem Funct 2001; 19: 107-15.

[84] Hampe CS, Wallen AR, Schlosser M, Ziegler M, Sweet IR. Quantitative evaluation of a monoclonal antibody and its fragment as potential markers for pancreatic beta cell mass. Exp Clin Endocrinol Diabetes 2005; 113: 381-7.

[85] Ueberberg S, Meier JJ, Waengler C, Schechinger W, Dietrich JW, Tannapfel A, *et al.* Generation of novel single-chain antibodies by phage-display technology to direct imaging agents highly selective to pancreatic beta- or alpha-cells *in vivo*. Diabetes 2009; 58: 2324-34.

[86] Sondergaard LG, Stoltenberg M, Flyvbjerg A, Brock B, Schmitz O, Danscher G, *et al.* Zinc ions in beta-cells of obese, insulin-resistant, and type 2 diabetic rats traced by autometallography. APMIS 2003; 111: 1147-54.

[87] Hansen WA, Christie MR, Kahn R, Norgaard A, Abel I, Petersen AM, *et al.* Supravital dithizone staining in the isolation of human and rat pancreatic islets. Diabetes Res 1989; 10: 53-7.

[88] Latif ZA, Noel J, Alejandro R. A simple method of staining fresh and cultured islets. Transplantation 1988; 45: 827-30.

[89] Fiedor P, Sitarek E, Tatarkiewicz K, Sabat M, Orlowski T, Rowinski W. *In vivo* visualization of rat pancreatic islets by intravenous injection of diphenyltiocarbazone (dithizone). Transplant Proc 1994; 26: 670-1.

[90] Sawada M, Nishikawa M, Adachi T, Midorikawa O, Hiai H. A Paneth cell specific zinc-binding protein in the rat. Purification and immunohistochemical localization. Lab Invest 1993; 68: 338-44.

[91] Garnuszek P, Licinska I, Fiedor P, Mazurek AP. The synthesis, radioiodination and preliminary biological study of the new carboxylic derivatives of dithizone. Appl Radiat Isot 1998; 49: 1563-71.

[92] Michalska W, Garnuszek P, Licinska I, Wilgus J, Szymanska K, Rowinski W, *et al.* Monitoring of pancreatic islets transplanted to colon mesentery. Transplant Proc 2002; 34: 653-4.

[93] Toroptsev IV, Eshchenko VA. Toxic action of dithizone on insulin-producing cells. Bull Exp Biol Med 1983; 96: 23-5.

[94] Clark SA, Borland KM, Sherman SD, Rusack TC, Chick WL. Staining and *in vitro* toxicity of dithizone with canine, porcine, and bovine islets. Cell Transplant 1994; 3: 299-306.

[95] Weaver DC, McDaniel ML, Lacy PE. Alloxan uptake by isolated rat islets of Langerhans. Endocrinology 1978; 102: 1847-55.

[96] Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008; 51: 216-26.

[97] Hammarstrom L, Hellman B, Ullberg S. On the accumulation of alloxan in the pancreatic beta-cells. Diabetologia 1967; 3: 340-4.

[98] Malaisse WJ, Doherty M, Ladriere L, Malaisse-Lagae F. Pancreatic uptake of [2-(14)C]alloxan. Int J Mol Med 2001; 7: 311-5.

[99] Kalliokoski T, Simell O, Haaparanta M, Viljanen T, Solin O, Knuuti J, *et al.* An autoradiographic study of [(18)F]FDG uptake to islets of Langerhans in NOD mouse. Diabetes Res Clin Pract 2005; 70: 217-24.

[100] Kalliokoski T, Nuutila P, Virtanen KA, Iozzo P, Bucci M, Svedstrom E, *et al.* Pancreatic glucose uptake *in vivo* in men with newly diagnosed type 1 diabetes. J Clin Endocrinol Metab 2008; 93: 1909-14.

[101] Malaisse WJ, Damhaut P, Malaisse-Lagae F, Ladriere L, Olivares E, Goldman S. Fate of 2-deoxy-2-[18F]fluoro-D-glucose in control and diabetic rats. Int J Mol Med 2000; 5: 525-32.

[102] Blankenberg FG. Monitoring of treatment-induced apoptosis in oncology with PET and SPECT. Curr Pharm Des 2008; 14(28): 2974-82.

[103] Larson SM. Cancer drug development with the help of radio-pharmaceuticals: academic experience. Curr Pharm Des 2009; 15(9): 950-6.

[104] Leitha T. Nuclear medicine: proof of principle for targeted drugs in diagnosis and therapy. Curr Pharm Des 2009; 15(2): 173-87.

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