111In-exendin Uptake in the Pancreas Correlates With the \(\beta\)-Cell Mass and Not With the \(\alpha\)-Cell Mass

Diabetes 2015;64:1324–1328 | DOI: 10.2337/db14-1212

Targeting of the GLP-1 receptor with 111In-labeled exendin is an attractive approach to determine the \(\beta\)-cell mass (BCM). Preclinical studies as well as a proof-of-concept study in type 1 diabetic patients and healthy subjects showed a direct correlation between BCM and radiotracer uptake. Despite these promising initial results, the influence of \(\alpha\)-cells on the uptake of the radiotracer remains a matter of debate. In this study, we determined the correlation between pancreatic tracer uptake and \(\beta\)- and \(\alpha\)-cell mass in a rat model for \(\beta\)-cell loss. The uptake of 111In-exendin (% ID/g) showed a strong positive linear correlation with the BCM (Pearson \(r = 0.82\)). The fraction of glucagon-positive cells in the total endocrine mass was increased after alloxan treatment (26% ± 4%, 43% ± 8%, and 69% ± 21% for 0, 45, and 60 mg/kg alloxan, respectively). The uptake of 111In-exendin showed a negative linear correlation with the \(\alpha\)-cell fraction (Pearson \(r = -0.76\)). These data clearly indicate toward specificity of 111In-exendin for \(\beta\)-cells and that the influence of the \(\alpha\)-cells on 111In-exendin uptake is negligible.

The role of the \(\beta\)-cell mass (BCM) in the development and progression of type 1 and type 2 diabetes remains poorly understood. Our knowledge is mainly based on autopsy studies and studies in pancreatic specimens obtained in patients undergoing pancreatectomy (1), representing information obtained at only one point in time during the course of the disease without further follow-up. Therefore, a method enabling longitudinal noninvasive determination of the BCM would represent a major breakthrough for diabetes research as it would allow better elucidation of the pathophysiology underlying the development of both types of diabetes (2). Such a noninvasive imaging technology for determination of the BCM in vivo has been developed based on a radiolabeled GLP-1 analog (111In-exendin). We have previously demonstrated that 111In-exendin uptake in the pancreas correlates linearly with the BCM in a rat model for \(\beta\)-cell loss, and the first clinical proof-of-principal revealed a clearly reduced uptake of the radiotracer in the pancreas of long-standing type 1 diabetic patients as compared with healthy volunteers (3). Despite these promising initial preclinical and clinical results, the specificity of radiolabeled exendin toward \(\beta\)-cells has been a matter of debate. Although relevant GLP-1 receptor (GLP-1R) expression in the exocrine pancreas was claimed (4–6), recent studies showed only low expression in acinar cells and no expression in ductal cells, whereas high GLP-1R expression could be demonstrated in the pancreatic islets, as determined by in vitro autoradiography with 125I-GLP-1 (7) and by immunohistochemistry with an extensively validated anti–GLP-1R antibody (8). We have recently shown by in vivo autoradiography that 111In-exendin specifically accumulates in the islets of Langerhans of rats and that GLP-1R mRNA expression is much higher in the islets compared with the exocrine pancreas. Moreover, the endocrine-to-exocrine ratio is even more favorable in humans than in rats in which the validity of the method for noninvasive determination of BCM has been demonstrated (3). Although these data clearly indicate toward specificity of 111In-exendin accumulation in islets, they do not rule out the potential influence of

Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Nijmegen, the Netherlands
Corresponding author: Maarten Brom, maarten.brom@radboudumc.nl.
Received 4 August 2014 and accepted 12 November 2014.
other endocrine cells on the accumulation of the tracer. One concern is the presence of GLP-1R on α-cells, reported in some studies (9–11), although other studies contradict these findings (12–14). The expression of GLP-1R on α-cells could potentially lead to accumulation of 111In-exendin in the α-cells and thus to an overestimation of the BCM as determined by this technique. To date, the influence of the presence of GLP-1R on α-cells on the uptake of 111In-exendin in the endocrine pancreas has not been studied. Therefore, we have examined the contribution of the α-cell mass on the accumulation of radiolabeled exendin in a rat model of alloxan-induced β-cell loss. The α-cell mass and BCM, determined by morphometric analysis, was compared with the uptake of 111In-exendin in healthy and alloxan-induced diabetic rats.

RESEARCH DESIGN AND METHODS

Radiolabeling
Radiolabeling and quality control of [Lys40(DTPA)]exendin-3 (Peptide Specialty Laboratories, Heidelberg, Germany) with 111InCl3 (Mallinkrodt Medical, Petten, the Netherlands) was performed as previously described (15).

Animals, Alloxan Treatment, and Biodistribution Studies
Female Brown Norway rats of 6–8 weeks were purchased from Harlan (Horst, the Netherlands). Animal experiments were performed after approval of the local ethical committee for animal experiments (RUDEC). Alloxan was injected intravenously (45 or 60 mg/kg, n = 4 per group) as previously described (3). A separate group was injected with vehicle only as a control (n = 4).

One week after alloxan injection, rats were injected intravenously with 15 MBq 111In-exendin (peptide dose 0.1 μg/rat). One hour after 111In-exendin administration, the rats were killed and the pancreas was dissected, weighed, and fixed in formalin. The radioactivity concentration in the pancreas was measured using an automated well-type gamma counter (Wallac 1480-Wizard; Perkin-Elmer, Boston, MA), and the uptake of 111In-exendin was calculated and expressed as the percentage of the administered dose per gram of tissue (% ID/g).

Histology and Determination of the BCM and α-Cell Mass
After 48 h fixation in formalin, pancreata were embedded in paraffin, and 4-μm sections were cut at three levels 100 μm apart. One section of each level was stained for insulin, and the BCM was determined by morphometric analysis as previously described (3). A consecutive section was stained for glucagon: the sections were rehydrated using xylene for 10 min and washed with xylene, washed twice with 100% ethanol, 75% ethanol, 50% ethanol, and water. Antigen retrieval was performed by a microwave treatment in 10 mmol/L sodium citrate buffer, pH 6.0, for 10 min. Endogenous peroxidase activity was blocked by 10 min incubation with 3% H2O2 in PBS. The sections were washed twice with demineralized water and three times with PBS and incubated with 5% normal goat serum (Bodinco, Alkmaar, the Netherlands) for 30 min. After removal of the goat serum, the sections were washed three times with PBS and incubated with 50 μL antiguacan antibody (1:500 diluted in PBS containing 1% BSA weight for volume) (catalog no. 2760; Cell Signaling, Leiden, the Netherlands) for 60 min. After washing three times with PBS, 50 μL goat anti-rabbit IgG-biotin conjugate (Vector, Burlingame, CA) diluted in PBS containing 1% BSA (1:200) was added and incubated for 30 min. The sections were washed three times with PBS, and the sections were incubated with 50 μL ABC complex (VECSTAIN Elite ABC kit; Vector Laboratories, Burlingame, CA) for 30 min. The bound antiguacan antibody was visualized using diaminobenzidine (Bright DAB; Sigma-Aldrich, St. Louis, MO) as a chromogen after washing three times with PBS. All slides were counterstained with hematoxylin and mounted with mounting fluid (Permout; Fisher Scientific, Waltham, MA). All steps were performed at room temperature in the dark.

The absolute α-cell mass was determined analogous with the BCM determination described above. The relative α-cell mass was calculated by dividing the α-cell mass by the total endocrine mass (BCM and α-cell mass).

Statistical Analysis
All mean values are expressed as mean ± SD. Statistical analysis was performed using unpaired, two-tailed Student t test using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). The level of significance was set at P < 0.05.

Correlation between the BCM and pancreatic uptake was determined by the Pearson correlation coefficient (r) using two-tailed ANOVA with GraphPad Prism. The level of significance was set at P < 0.05.

RESULTS

Histology
In the pancreas of healthy rats, scarce glucagon staining was observed at the periphery (Fig. 1A) and abundant insulin staining in the core of the islets (Fig. 1B). In alloxan-treated rats, the number of insulin-positive cells per islet was markedly reduced (Fig. 1D) and the islets mainly consist of glucagon-positive cells (Fig. 1C).

α-Cell Mass and BCM and 111In-exendin Uptake
The total mass of the endocrine pancreas was reduced in the alloxan-treated rats (8.5 ± 1.9, 5.5 ± 1.7, and 2.8 ± 0.8 mg for 0, 45, and 60 mg/kg alloxan, respectively). The loss of endocrine mass is due to β-cell loss in alloxan-treated rats (BCM 6.3 ± 1.7 mg in healthy rats and 3.2 ± 1.5 and 1.0 ± 0.9 in rats treated with 45 and 60 mg/kg, respectively) (Fig. 2A), since the absolute α-cell mass was not significantly different in rats treated with 45 or 60 mg/kg alloxan (2.2 ± 0.4, 2.3 ± 0.3, and 1.8 ± 0.3 mg for healthy and 45 and 60 mg/kg alloxan, respectively; P = 0.81 and P = 0.21, respectively) (Fig. 2B). Although the absolute α-cell mass did not change, the
relative \(\alpha\)-cell fraction (represented as the percentage of \(\alpha\)-cells of the total endocrine mass) was increased as a result of \(\beta\)-cell loss (and thereby loss of total endocrine mass) when rats were treated with 45 or 60 mg/kg alloxan (43% ± 8% and 69% ± 21%, respectively, vs. 26% ± 4% in healthy rats) (Fig. 2C). The pancreatic uptake of \(^{111}\text{In}-\text{exendin}\) showed a linear correlation with the BCM (Pearson \(r = 0.82, P < 0.005\)) (Fig. 3A). There was no significant correlation between the absolute \(\alpha\)-cell mass and \(^{111}\text{In}-\text{exendin}\) uptake in the pancreas (Pearson \(r = 0.18, P = 0.59\)) (Fig. 3B). The relative \(\alpha\)-cell fraction (fraction of \(\alpha\)-cell of the total endocrine mass) showed a negative linear correlation with pancreatic \(^{111}\text{In}-\text{exendin}\) uptake (Pearson \(r = -0.76, P < 0.01\)) (Fig. 3C).

**DISCUSSION**

In this study, we examined the contribution of the \(\alpha\)-cells to the uptake of \(^{111}\text{In}-\text{exendin}\), a radiotracer potentially enabling noninvasive determination of BCM, in a rat model of diabetes. We demonstrated that there is no correlation between the absolute \(\alpha\)-cell mass and pancreatic tracer uptake and a negative linear correlation between the relative \(\alpha\)-cell mass and \(^{111}\text{In}-\text{exendin}\) uptake, indicating a negligible influence of the \(\alpha\)-cells on tracer accumulation in the pancreas.

The expression of GLP-1R on \(\alpha\)-cells remains a matter of debate. Specific in vitro binding of \(^{125}\text{I}-\text{labeled GLP-1}\) to insulin and glucagon immunoreactive cells was found by combined autoradiography and immunohistochemical analysis of pancreatic tissue specimens of rats (10). A later study confirmed these data by showing that a small portion of the \(\alpha\)-cells express GLP-1R as determined by RT-PCR.

---

**Figure 1**—Immunohistochemical staining of pancreatic sections for glucagon (A and C) and insulin (B and D) of healthy rats (A and B) and rats treated with 60 mg/kg alloxan (C and D). In healthy rats, scarce glucagon staining in the periphery of the islet (A) and abundant insulin staining in the core of the islet (B) is observed. In severely diabetic rats, the islets consist predominantly of glucagon-producing cells (C), and only a few insulin-positive cells could be observed (D).

**Figure 2**—Absolute \(\beta\)-cell (A) and \(\alpha\)-cell (B) mass in control and alloxan-treated Brown Norway rats. The relative \(\alpha\)-cell fraction (C) was determined by dividing the absolute \(\alpha\)-cell mass by the sum of the absolute \(\alpha\)-cell mass and BCM.
and immunohistochemical analysis (9). However, several other studies showed that GLP-1R is specifically expressed in β-cells in mice, rats, and humans and not in α-cells (12–14). Another study showed that GLP-1 has a direct inhibitory effect on glucagon excretion by α-cells, but that GLP-1R is only expressed on ~1% of the α-cells (11). In a more recent study in which a highly specific anti–GLP-1R antibody was validated, the specificity of most (commercially) available GLP-1R antibodies was questioned (8). Therefore, based on the results presented in the literature, it remains a matter of debate whether GLP-1R is expressed on α-cells. It should be noted that in the case of peptide-based tracers, such as exendin, the tracer accumulation is dependent not only on the receptor expression density but also the internalization rate (16), meaning that if the expression of GLP-1R on α-cells or the internalization rate is low, the receptor expression itself might have a negligible influence on the accumulation of 111In-exendin in the islets.

The aim of our study was not to demonstrate the presence or absence of GLP-1R on α-cells but to evaluate the potential influence of the presence of α-cells in the islets of Langerhans on the accumulation of 111In-exendin in the pancreas. Our findings indicate that even if GLP-1Rs are present on α-cells, they only exert a minor influence on 111In-exendin uptake. This further supports the idea that measurement of the pancreatic uptake of 111In-exendin can indeed serve as an imaging biomarker for BCM.

Not only the BCM but also the number of other endocrine cells might change during the progression of diabetes. Noninvasive methods to longitudinally determine the endocrine cell mass and confirmation might provide important information on the role of these cells in the pathogenesis of diabetes. Recently, Eriksson et al. (17) showed that accumulation of the serotonin receptor tracer [11C]5-HTP is reduced in the pancreas of type 1 diabetic patients as compared with healthy volunteers, implying that this tracer could also be a useful noninvasive marker to determine the total mass of endocrine cells in the pancreas. Since the serotonin receptor is expressed in all endocrine cells, a combination of [11C]5-HTP imaging of serotonin activity and 111In-exendin imaging could provide useful complementary information about the β-cell and total endocrine mass as well as changes in endocrine cell conformation during the development of diabetes.

In conclusion, we have demonstrated that the uptake of 111In-exendin correlated with BCM and not with α-cell mass. The present data clearly indicate the specificity of

**Figure 3**—Correlation between the pancreatic uptake of 111In-exendin and the absolute BCM (A); uptake on y-axis in percentage of the injected dose per gram of tissue (% ID/g), BCM in milligrams on x-axis as determined by morphometric analysis after immunohistochemical staining with anti-insulin antibody. The correlation as determined by Pearson test is $r = 0.82$. No correlation between tracer uptake and absolute α-cell mass was observed (B); Pearson

$r = 0.18$. A negative correlation (Pearson $r = -0.76$) was shown between the pancreatic 111In-exendin uptake and the relative α-cell fraction calculated by dividing the α-cell mass by the total endocrine mass (C). Circles, control rats; squares, rats treated with 45 mg/kg alloxan; triangles, rats treated with 60 mg/kg alloxan.
111In-exendin for β-cells and a negligible influence of the α-cells on pancreatic 111In-exendin uptake. This observation further supports the idea that 111In-exendin uptake may indeed be a measure for BCM in vivo.

Funding. This work was supported by National Institutes of Health grant R01-AG-030328-01 and the European Community’s Seventh Framework Programme (FP7/2007-2013) project Betaimage, under grant agreement 222980.

Duality of Interest. M.G. is a consultant for Boehringer Ingelheim and is a patent holder in the field. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. M.B. researched data and wrote the manuscript. L.J. and C.F. researched data and reviewed and edited the manuscript. O.B. and M.G. contributed to the discussion and reviewed and edited the manuscript. M.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. This study was published in abstract form for the 50th Annual Meeting of the European Association for the Study of Diabetes, Vienna, Austria, 15–19 September 2014, and the Annual Congress of the European Association of Nuclear Medicine, Gothenburg, Sweden, 18–22 October 2014.

References

1. Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes 2004;53(Suppl. 3):S16–S21
2. Gotthardt M, Eizirik DL, Cnop M, Brom M. Beta cell imaging - a key tool in optimized diabetes prevention and treatment. Trends Endocrinol Metab 2014;25:375–377
3. Brom M, Woliner-van der Weg W, Joosten L, et al. Non-invasive quantification of the beta cell mass by SPECT with 111In-labelled exendin. Diabetologia 2014;57:950–959
4. Körner M, Stöckli 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;48:736–743
5. Xu G, Kaneto H, Lopez-Avalos MD, Weir GC, Bonner-Weir S. GLP-1/exendin-4 facilitates beta-cell neogenesis in rat and human pancreatic ducts. Diabetes Res Clin Pract 2006;73:107–110
6. Reubi JC, Perren A, Rehmann R, et al. Glucagon-like peptide-1 (GLP-1) receptors are not overexpressed in pancreatic islets from patients with severe hyperinsulinaemic hypoglycaemia following gastric bypass. Diabetologia 2010;53:2641–2645
7. Waser B, Reubi JC. Radiolabelled GLP-1 receptor antagonist binds to GLP-1 receptor-expressing human tissues. Eur J Nucl Med Mol Imaging 2014;41:1166–1171
8. Pyke C, Heller RS, Kirk RK, et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. Endocrinology 2014;155:1280–1290
9. Heller RS, Kieffer TJ, Habener JF. Insulinotropic glucagon-like peptide I receptor expression in glucagon-producing alpha-cells of the rat endocrine pancreas. Diabetes 1997;46:785–791
10. Orskov C, Poulsen SS. Glucagonlike peptide-1-(7-36)amide receptors only in islets of Langerhans. Autoradiographic survey of extracerebral tissues in rats. Diabetes 1991;40:1292–1296
11. De Marinis YZ, Salehi A, Ward CE, et al. GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca2+-channel-dependent exocytosis. Cell Metab 2010;11:543–553
12. Horsch D, Goke R, Eissele R, Michel B, Goke B. Reciprocal cellular distribution of glucagon-like peptide-1 (GLP-1) immunoreactivity and GLP-1 receptor mRNA in pancreatic islets of rat. Pancreas 1997;14:290–294
13. Moens K, Heimberg H, Flamez D, et al. Expression and functional activity of glucagon, glucagon-like peptide I, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. Diabetes 1996;45:257–261
14. Tornehave D, Kristensen P, Ramer J, Knudsen LB, Heller RS. Expression of the GLP-1 receptor in mouse, rat, and human pancreas. J Histochem Cytochem 2008;56:841–851
15. Brom M, Oyen WJ, Joosten L, Gotthardt M, Boerman OC. 68Ga-labelled exendin-3, a new agent for the detection of insulinomas with PET. Eur J Nucl Med Mol Imaging 2010;37:1345–1355
16. Zeglis BM, Holland JP, Lebedev AY, Cantorias MV, Lewis JS. Radiopharmaceuticals for Imaging in Oncology with Special Emphasis on Positron-Emitting Agents. In: Nuclear Oncology: Pathophysiology and Clinical Applications. H. Strauss W, Ed. New York, Springer, 2012
17. Eriksson O, Espes D, Selvaraju RK, et al. Positron emission tomography ligand [11C]5-hydroxy-tryptophan can be used as a surrogate marker for the human endocrine pancreas. Diabetes 2014;63:3428–3437