Good, Better, Beta

Development and characterisation of novel beta cell tracers

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Diabetes is one of the leading causes of death worldwide, according to the WHO (1). Type 1 diabetes develops as a result of an autoimmune attack directed towards the insulin-producing beta cells. Type 2 diabetes is characterised by an ineffective use of insulin by the peripheral organs (2). Little is known about beta cell dynamics during the onset and progression of diabetes. A non-invasive imaging method would allow monitoring of the beta cell mass longitudinally and could identify changes in the beta cell mass during development of diabetes. Subsequently, it could provide information to individualise diabetes treatment. In the recent years, several tracers for beta cell imaging have been proposed and some have been tested in clinical trials. Yet, none of these tracers has been implemented for routinely visualsing beta cells in diabetic patients.

Preferably, a beta cell tracer should also be able to visualise insulinomas, tumours in the pancreas derived from insulin-producing beta cells (3). Currently, the glucagon-like peptide 1 receptor (GLP-1R) targeting peptide exendin is the most promising tracer for imaging beta cells. Peptides, targeting specific receptors on beta cells, best comply the requirements for a suitable tracer. Hence, the search for new beta cell imaging agents, as described in the first part of the thesis, have been focused on peptides.

Insulinomas have high expression of the pituitary adenylate cyclase-activating polypeptide type I (PAC1) receptor and the glucose-dependent insulinotropic polypeptide receptor (GIPR) (4,5). First, we have investigated the feasibility of radiolabelled maxadilan, a PAC1 receptor targeting agent, to detect insulinomas by SPECT imaging in a mouse model with subcutaneous insulinomas (INS-1 tumours). Maxadilan-DTPA-^{111}In[In] accumulated specifically in INS-1 tumours and in the pancreas. Furthermore, we were able to clearly visualise INS-1 tumours using SPECT/CT. Next, another promising tracer has been investigated for visualising neuroendocrine tumours. [Lys^{37}(^{111}In[In]-DTPA)]N-acetyl-GIP1-42 showed receptor-mediated binding to BHK-GIPR positive cells, NES2Y cells (human beta cell-like insulin secreting line) and isolated islets in vitro. Furthermore, the *in vivo* studies showed that GIP receptor transfected tumours and NES2Y tumours could be visualised with SPECT/CT using [Lys^{37}(^{111}In[In]-DTPA)]N-acetyl-GIP$_{1\text{-}42}$ as a tracer.

Exendin has been shown to specifically target the GLP-1R on beta cells in the pancreas and is able to visualise GLP-1R expressing insulinomas (6). Native beta cells, in contrast to tumour cells, display physiological levels of the receptor and are spread throughout the pancreas. As there are less receptors available for binding, a low peptide dose should be administered to avoid receptor saturation. Hence, to be able to be detected, a high molar activity is needed in order to get a sufficient amount of radioactive signal in the beta cells. To improve the molar activity of ^{111}In-labelled exendin, we have modified exendin by addition of six lysine residues and by conjugation of one, two or six DTPA moieties to these lysine residues, resulting in five new compounds. Addition of multiple DTPA molecules increased the molar activity, and was most pronounced for exendin with 6 DTPA moieties, named hexendin(40-45). As a result of the enhanced molar activity, the absolute pancreatic uptake of hexendin(40-45) in Brown Norway rats increased considerably, enabling an improved visualisation of the pancreas with SPECT/CT.

Next, we have investigated one of the main challenges when using radiolabelled exendin *in vivo*. Radiolabelled exendin accumulates at high concentrations in the kidneys, which could hamper the detection

of small insulinomas in the vicinity of the kidneys. Reduction of the accumulation in the kidney would greatly improve the visualisation of insulinomas. In this study, exendin-4 was extended with a Met-Ile-linker and subsequently conjugated with NOTA. Biodistribution studies in BALB/c nude mice bearing a subcutaneous INS-1 tumour showed stable tumour uptake over time for [^{68}Ga]Ga-NOTA-MI-exendin-4 and the INS-1 tumour was clearly visualised using SPECT/CT. Most importantly, kidney uptake was significantly lower for this extended exendin analogue compared to [^{68}Ga]Ga-NOTA-exendin-4 (figure 1).

In the second part of the thesis we have examined the clinical potential of radiolabelled exendin as a native beta cell imaging agent. First, we determined the most suitable animal model for non-invasive determination of the beta cell mass using [^{111}In]In-DTPA-exendin-3. The most important finding of this study was that rats seem to represent a more adequate model for beta cell mass assessment than mice. In rats, the uptake of exendin after alloxan treatment was similar to the uptake after a blocking dose, whereas in mice there was a considerable amount of exendin uptake after alloxan treatment. These results indicate that there was exendin uptake in the exocrine tissue as well. Considering the fact that exocrine GLP-1R expression measured on mRNA and protein level was very low, it is likely that [^{111}In]In-DTPA-exendin-3 binds to a binding site, other than the GLP-1R, in exocrine mouse pancreas. Therefore, determination of the beta cell mass using radiolabelled exendin may be more specific for the beta cells in rats than in mice.

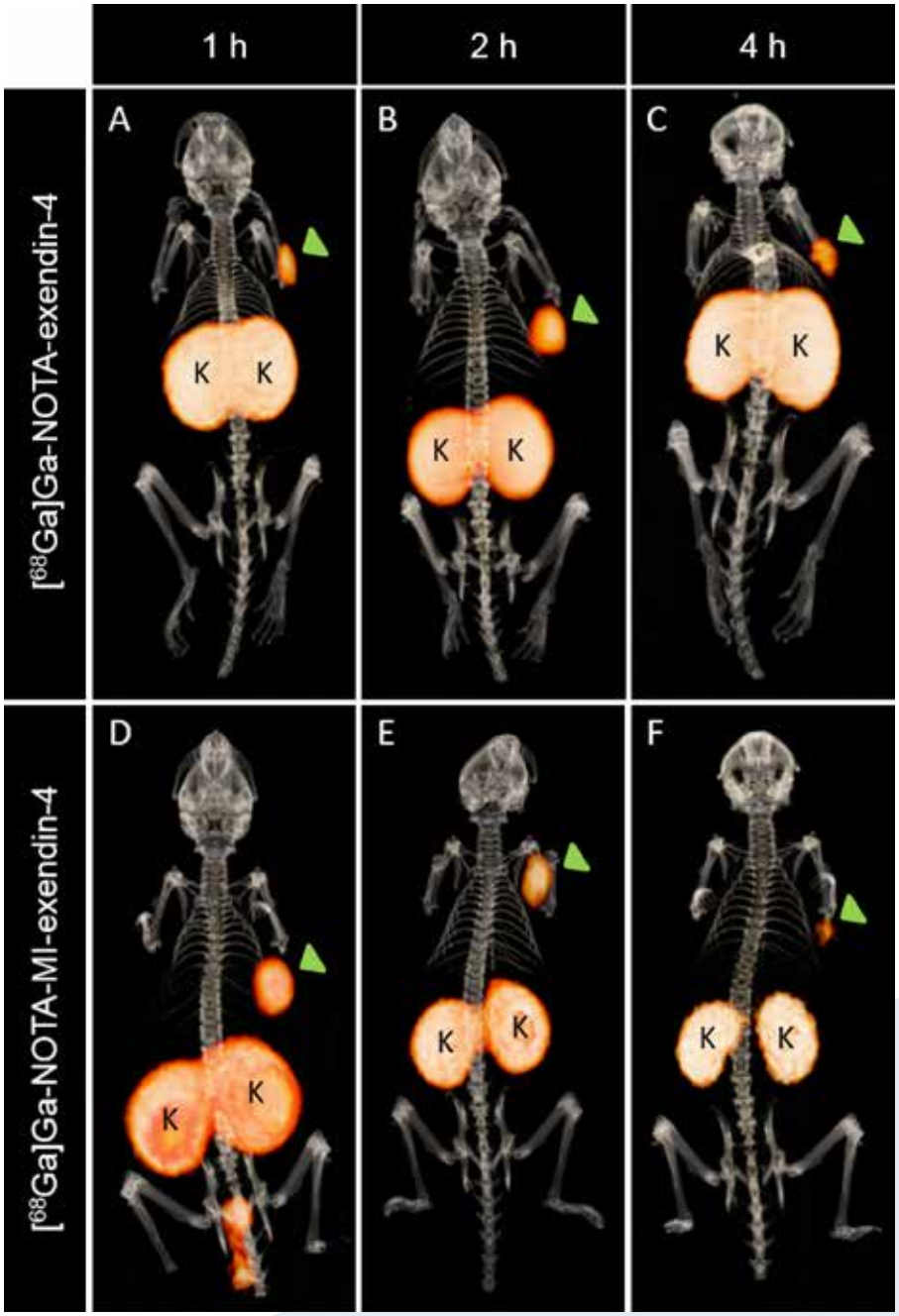

Figure 1. Fused PET/CT images of BALB/c nude mice bearing subcutaneous CHL-GLP-1R tumours (green arrow). Mice were injected with 1.6 MBq of either [^{68}Ga]Ga-NOTA-exendin-4 (A, B and C) or [^{68}Ga]Ga-NOTA-MI-exendin-4 (D, E and F). Images were obtained 1 h (A and D), 2 h (B and E) and 4 h (C and F) after injection. Kidneys are indicated with K.

Next, we have examined the use of [^{111}In]In-DTPA-exendin-3 for determining the beta cell mass in two rodent models for spontaneous type 1 diabetes. Nonobese diabetic (NOD) mice were monitored until 21 weeks of age by measuring blood glucose and performing SPECT/CT after injection of [^{111}In]In-DTPA-exendin-3. A linear correlation was found between the beta cell mass in these mice and the uptake of exendin in the pancreas, which was not affected by either insulitis or hyperglycemia. However, there was remaining [^{111}In]In-DTPA-

exendin-3 uptake in the pancreas, despite total ablation of the beta cells, rendering this a suboptimal animal model for exendin imaging as mentioned above. A more favourable correlation between exendin uptake and the beta cell mass was found in BioBreeding Diabetes Prone rats (figure 2). Also, in this model we have shown that the correlation between radiolabelled exendin uptake and beta cell mass was independent of insulitis and fluctuations in blood glucose levels. The results of this study clearly indicate that imaging with radiolabelled exendin represents a reliable and robust technology for non-invasive determination of the beta cell mass in type 1 diabetes.

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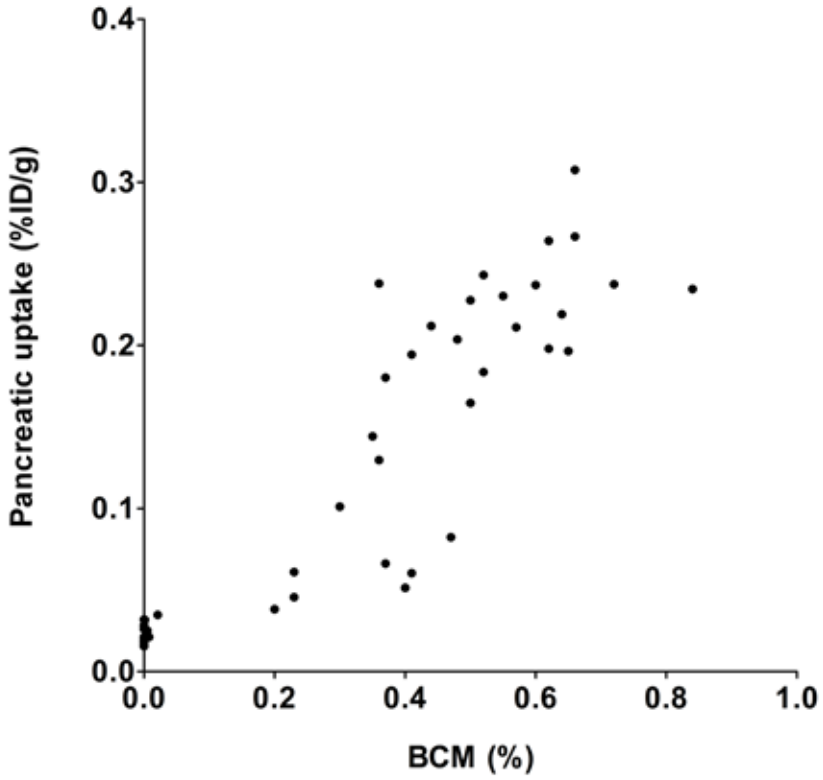

Figure 2. Correlation between pancreatic [^{111}In]In-DTPA-exendin accumulation and beta cell mass. The uptake of [^{111}In]In-DTPA-exendin in the pancreas (n=45), expressed as the percentage of the injected dose per gram of tissue (%ID/g, y-axis, determined by ex vivo counting of the entire pancreas) showed a strong correlation with the beta cell mass (expressed as percentage of the total pancreas, x-axis) determined by morphometric analysis (Pearson r = 0.89, p<0.0001).

References

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