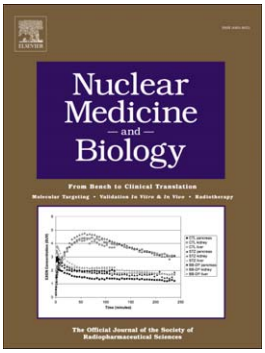

Pre-clinical Evaluation of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 For Imaging of Insulinoma

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Full Title: Pre-clinical Evaluation of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 For Imaging of Insulinoma.

Abbreviated Title: PET imaging of GLP-1 receptor in insulinoma

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ABSTRACT

Introduction: Insulinoma is the most common form of pancreatic endocrine tumors responsible for hyperinsulinism in adults. These tumors overexpress glucagon like peptide-1 (GLP-1) receptor, and biologically stable GLP-1 analogues have therefore been proposed as potential imaging agents. Here, we evaluate the potential of a positron emission tomography (PET) tracer, [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4, for imaging and quantification of GLP-1 receptors (GLP-1R) in insulinoma.

*Methods***:** [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 was evaluated for binding to GLP-1R by in vitro autoradiography binding studies in INS-1 tumor from xenografts. In vivo biodistribution was investigated in healthy control mice, INS-1 xenografted and PANC1 xenografted immunodeficient mice at two different doses of peptide: 2.5μg/kg (baseline) and 100μg/kg (block). In vivo imaging of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 in xenografted mice was evaluated by small animal PET/CT using a direct comparison with the clinically established insulinoma marker [^{11}C]5-hydroxy-tryptophan ([^{11}C]5-HTP).

*Results***:** GLP-1 receptor density could be quantified in INS-1 tumor biopsies. [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 showed significant uptake ($p \leq 0.05$) in GLP1-R positive tissues such as INS-1 tumor, lungs and pancreas upon comparison between baseline and blocking studies. In vivo imaging showed concordant results with higher tumor-to-muscle ratio in INS-1 xenografted mice compared with [^{11}C]5-HTP.

Conclusion: [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 has high affinity and specificity for GLP-1R expressed on insulinoma in vitro and in vivo.

1. Introduction

Insulinoma is the most common form of pancreatic neuroendocrine tumors (PNETs) of beta-cell origin. Although rare in the overall population, insulinomas are the most common cause of hyperinsulinemic hypoglycemia in the adult population [1-3]. The incidence has been reported as higher in autopsy studies (0.8% to 10%), suggesting that these tumors frequently remain undiagnosed [4-5]. Although insulinomas are neuroendocrine tumors, density of the somatostatin receptors are too low particularly in benign insulinomas for the adequate imaging with respective radioligands [6]. Whereas glucagon-like peptide 1 receptor (GLP-1R) is expressed with high incidence and density. Precise localization of lesions and staging are crucial for adequate patient management. Development of an imaging agent based on the ligand to GLP-1R such as Exendin-4 and positron emitting radionuclide would provide means for specific, sensitive, quantitative and non-invasive diagnosis using positron emission tomography (PET). The use of generator produced positron emitting ^{68}Ga [Physical half-life ($T_{1/2}$) = 68 min, 89% positron emission (β^+) and electron capture (EC) = 11%] radionuclide would make the tracer affordable and easy to access. GLP1-R is a well studied pancreatic beta-cell specific receptor, which is upregulated by up to 5 times in insulinomas compared to other GLP-1R positive tissues in the body such as lungs, gut region and normal beta-cells [7-9]. It is therefore an attractive target for insulinoma imaging.

Exendin-4 is a peptide of 39 amino acids which has been isolated from the venom of the lizard Heloderma suspectum (Gila monster) [10]. It is a naturally occurring analog of the glucagon like peptide-1 (GLP-1) which binds and activates the GLP-1 receptor with the same potency as GLP-1[11-12]. The GLP-1/GLP-1R system facilitates insulin release from pancreatic beta-cells to maintain glucose homeostasis [13]. Unlike GLP-1, which has a short biological half-life (≤2 min) due to cleavage by the circulating protease dipeptidyl peptidase 4 (DPPIV), Exendin-4 is resistant to cleavage and has a markedly increased biological half-life (≥ 20 min) in vivo [14-17]. It is currently approved for the treatment of Type 2 diabetes [18-19].

The aim of the presented study was the development of a PET imaging agent comprising ^{68}Ga and DO3A-VS-Cys^{40}-Exendin-4 with high specific radioactivity. The tracer was evaluated

preclinically in vitro and in vivo for the imaging and quantification of GLP-1R expressed in insulinomas, in a direct comparison with [^{11}C]5-HTP .

2. Materials and Methods

2.1. Radiochemistry

^{68}Ga was available from a ^{68}Ge/^{68}Ga generator system, where ^{68}Ge was attached to a column of an inorganic matrix based on titanium dioxide (1850 MBq, Eckert & Ziegler, Eurotope GmbH). The first fraction of 1.5 ml was discarded and the next 1.5 ml containing over 90% of the total radioactivity was collected and buffered with 200 μL of acetate buffer and 15 μL sodium hydroxide to provide pH of 4.6±0.4. In order to suppress radiolysis 200 μL of ethanol was added. Then the mixture was transferred to a glass vial containing 10.5 nanomoles of good manufacturing practice grade DO3A-VS-Cys^{40}-Exendin-4 (C S Bio Co., Menlo Park, CA, USA). The latter was incubated at 80-85 ºC for 15 min. The resulting product, [^{68}Ga]Ga- DO3A-VS-Cys^{40}-Exendin-4, was formulated in phosphate buffered saline to yield pH of 7.4. The stability of the tracer in the formulation mixture at room temperature was monitored by UV- and Radio-HPLC analysing the content directly after the synthesis and 1, 2, and 3 hours later.

The identity of the labelled product and radiochemical purity were determined with high-performance liquid chromatography (HPLC) on Elite LaChrom system consisting of an L-2130 pump, UV detector (L-2400) (Hitachi High Technologies America, Inc., USA), and a radiation flow detector (Bioscan) coupled in series. Separation of the analytes was accomplished using endcapped analytical column with stationary phase of covalently bonded pentylsilane (Discovery BIO Wide Pore C5; 5cm x 4.6 mm). The conditions were as followed: solvents; A=10 mM TFA; B=70% acetonitrile (MeCN), 30% H_2O, 10mM TFA with UV-detection at 220 nm; gradient elution: 0–2 min at 35% B, 2-9 min at 35 to 100% B, 9-12min at 100% B ; flow rate was 2.0 mL/min. Data acquisition and handling were performed using the EZChrom Elite Software Package. The recovery of the radioactivity from the HPLC column was determined by performing analysis with and without column and by collecting the fractions for the subsequent measurement of the radioactivity in a well-type NaI(Tl) scintillation counter. Data were corrected for dead-time and for decay.

The specific radioactivity of the product at the end of the synthesis was determined as a ratio of the ^{68}Ga radioactivity and total amount of the peptide present in the preparation. The radioactivity was measured in dose calibrator and corrected for the radioactivity fraction associated with the peptide as determined by radio-HPLC.

2.2. Cell Lines

The INS-1 cell line, derived from rat insulinoma was cultured in RPMI1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL penicillin/streptomycin (10.000 U/mL), 1 mM sodium pyruvate, 50μM β-mercaptoethanol and 10mM HEPES. The PANC1 cell line, derived from human pancreatic ductal cells was cultured in DMEN medium supplemented with 10% (v/v) heat-inactivated FBS and 100 units/mL penicillin/streptomycin (10.000 U/mL). Cells were incubated at 37 °C, 90% humidity and 5% CO_2.

2.3. Animal Model

Nu/nu Balb/c mice (n=31, Taconic M&B, Ry, Denmark), were housed under standard laboratory conditions with free access to laboratory animal food and water. Drinking water was supplemented with 50 mg/ml of glucose for mice bearing INS-1 xenografts, as inappropriate amount of insulin secreted into blood from tumor de-energizes the animal. Twenty four hours before any experiment, glucose supplemented water bottles were replaced with normal water. All animal handling and experiments were carried out in accordance with the guidelines of Uppsala University and were approved by the local animal ethics committee (ethical permit, C52/10).

Subcutaneous (s.c.) Xenograft model: INS-1 cells (10-15×10^6) mixed with RPMI1640 with supplements (n=16, 0.5 mL) or PANC1 cells (25-30×10^6) mixed with DMEM with supplements (n=5, 0.5 mL) were injected subcutaneously into right front leg of nu/nu Balb/c mice. After inoculation, the weight of the animal and size of the tumor was monitored on alternative days. Mice bearing tumors of 0.8 to 1 cm in diameter were used in the studies 7 days after injection for the PANC1 xenografted mice and 14-17 days after injection for INS-1 xenografted mice.

2.4. In Vitro Autoradiography

Biopsies from INS-1 xenografted mice were frozen to -80 °C and processed into 20 µm sections. To study tracer binding properties to the tissue, the sections were incubated in several concentrations (0.3-30 nM, distributed around the expected dissociation constant value) of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 in 200 mM TRIS + 1% BSA for 60 minutes at room temperature (RT). Non-displaceable binding was assessed by adding 200 nM of unlabelled Exendin4 (native peptide) or DO3A-VS-Cys^{40}-Exendin-4 (tracer precursor) to the incubation buffer 10 minutes before tracer administration. Tissue slices were then washed 3 times for 4 minutes in 150 mL 200mM TRIS at RT to remove excess tracer and then dried at 37 °C for 10 minutes. The sections were then exposed against a phosphor-imager screen (Amersham Biosciences, Uppsala, Sweden) for 2 hours, digitalized using a Phosphorimager SI (Molecular Dynamics, Sunnyvale, CA, USA) and analyzed using ImageQuant (Molecular Dynamics, Sunnyvale, CA, USA). The affinity (expressed as the dissociation constant K_d) and GLP-1R density (B_{max}), was determined by non-linear regression of total and non-specific binding using GraphPad Prism 5 (San Diego, CA, USA).

2.5. Biodistribution in Small-Animals

The biodistribution of [^{68}Ga]Ga- DO3A-VS-Cys^{40}-Exendin-4 was investigated in healthy control mice (20.45 ± 0.88; n=9), INS-1 tumor bearing (23.2 ± 2.17 g; n=10) and PANC1 tumor bearing nu/nu Balb/C mice (23 ± 1 g; n=5). Animals were administered either baseline (healthy control mice (n=5), INS-1 xenografted mice (n=5), and PANC1 xenografted mice (n=5)) or blocking (healthy control mice (n=4), INS-1xenografts (n=5)) doses of tracer intravenously through the tail vein under general anesthesia (isoflurane 3.0% in 50%/50% medical oxygen:air at 450 mL/min). The animals were allowed to wake up after tracer administration and organs were resected 80 minutes later, after euthanasia by CO_2. In the baseline study, radioactivity corresponding to a peptide dose of 2.5 µg/kg (0.6±0.1 MBq) was administered, while the radioactivity in the blocking study corresponded to 100 µg/kg (2.47±0.6 MBq). Organs were excised, weighed and radioactivity uptake was measured in a well-counter (Uppsala Imanet AB, GE Healthcare, Uppsala, Sweden). The tissue uptake was calculated as standardised uptake value (SUV), which relates the tissue uptake of

radiotracer (in Bq/g) to total injected dose (in Bq) and the whole body weight (kg). For result analysis, tissue uptake was compared with tracer in blood and presented as tissue-to-blood ratio.

2.6. Small-Animal PET-CT Imaging

INS-1 xenografts (21.7 ± 3.1 g; n=6) or PANC1 xenografts (22 ± 0 g; n=2) were administered baseline (n=5) or blocking (n=3) dose (same peptide amounts as in biodistribution study) intravenously in a maximum volume of 100 µl as single bolus injection via the tail vein under general anesthesia (isoflurane 3.0% in 50%/50% medical oxygen:air at 450 mL/min). The animals were allowed to wake up after tracer administration and were euthanized after 80 minutes by CO_2. Three INS-1 xenografts (21.3 ± 4.6 g), before being used for [^{68}Ga]Exendin-4 PET scans, were examined by [^{11}C]5-HTP (administered radioactivity = 10.8± 5.1, MBq) , 25 minutes post injection, under general isoflurane anaesthesia, for comparison with a currently clinically available neuroendocrine PET marker. [^{68}Ga]Exendin-4 PET scans and [^{11}C]5-HTP PET scans were conducted on different days. Each animal was placed in the gantry of the animal PET/CT scanner (Triumph^{TM} Trimodality System, TriFoil Imaging, Inc., Northridge, CA, USA) and examined by whole body PET for 60 minutes in list mode followed by a CT examination for 3 minutes (Field of View (FOV) = 8.0 cm). The PET data were reconstructed into a static image using a MLEM 2D algorithm (10 iterations). The CT raw files were reconstructed using Filter Back Projection (FBP).

PET and CT dicom files were analyzed using PMOD v3.13 (PMOD Technologies Ltd, Zurich, Switzerland). Volumes of Interest (VOI) were drawn manually on heart, liver, tumor, muscle and kidney. Tracer uptake in these organs from µPET images are expressed as tissue-to-reference tissue ratio. Muscle was used as reference tissue. .

2.7. Statistical Analysis

Data are expressed as mean ± standard deviation. Statistical significance was evaluated by unpaired student's t-test using GraphPad Prism version 5.04 (San Diego, CA, USA), where $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Radiochemistry

Buffers (pH: 4.2, 4.6 and 5.0), temperature (60, 75, and 94 ºC), and radical scavengers were optimised in ^{68}Ga-labelling of DO3A-VS-Cys^{40}-Exendin-4 in order to suppress radiolysis and ensure high radioactivity incorporation and specific radioactivity (SRA). In order to suppress the radiolysis, post labelling addition of ascorbic acid was investigated however addition of ethanol to the reaction mixture prior to the synthesis demonstrated more robust and higher yields. The non-decay-corrected radiochemical yield was 80±5%. [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 was produced with radiochemical purity of over 95% and SRA of 78±19 (n=19) MBq/nmol with variation dependent of the age of the generator. The tracer administration during animal studies was typically accomplished within 30±15 min thus corresponding to the SRA value of 60±16 MBq/nmol at the tracer administration time point. The tracer was stable in the formulation buffer for at least 3 hours at room temperature with radiochemical purity of >90%.

3.2. In Vitro Autoradiography

[^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 binding to INS-1 sections was displaceable by both native Exendin4 and tracer precursor DO3A-VS-Cys^{40}-Exendin-4 at nanomolar concentrations (Fig. 1). The affinity in INS-1 xenograft was 3.1 nM, and the specificity was >92% at concentrations below K_d. Saturable tracer binding was observed in the INS-1 xenograft samples with a K_d of 3.13 nM. GLP1-R density was estimated to be 175.8 pmol/ mg tissue (Fig. 2).

3.3. Biodistribution in Small Animals

In healthy control mice , baseline uptake of [^{68}Ga]Ga- DO3A-VS-Cys^{40}-Exendin-4 was highest in lungs, pancreas and kidneys (Fig. 3). At blocking levels of radioligand, uptake in lungs and pancreas decreased by 65% ($p \leq 0.05$) and 50% ($p \leq 0.05$) respectively. However uptake in liver and kidney increased by 56% and 80% (Fig. 3) and uptake in these tissues were likely related to excretion and non-specific accumulation of tracer at higher peptide dose.

Similar pattern of biodistribution of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 was observed in INS-1 xenografts with highest uptake in tumor at baseline level of the agent. Uptake in tumor, pancreas and lung decreased by 81% ($p \leq 0.01$), 80 % ($p \leq 0.05$) and 69% ($p=0.07$) respectively at blocking dose (Fig.

4). Uptake in PANC1 tumor was low at baseline dose, which is consistent with low or negligible expression of GLP1-R.

3.4. Small-Animal PET-CT Imaging

[^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 showed higher uptake in endocrine INS-1 tumors (tumor-to-muscle ratio= 44.8±14.5, $p \leq 0.05$) compared to exocrine PANC1 tumors (tumor-to-muscle ratio= 3± 2.6) as measured by animal PET-CT, 80 minutes post injection. The uptake in INS-1 tumors was almost completely displaced by co-injection of 100 µg/kg (Fig. 5). Quantification of fused PET-CT data showed that uptake in INS-1 tumor was reduced by approximately 92% ($p \leq 0.01$) . Apart from INS-1 tumor and kidneys, no other tissues showed marked uptake. Pancreas was difficult to visualize and delineate due to its proximity to kidney. Uptake in lungs is likely artificially low in the PET images due to the colour scale used for the images which is dominated by the kidneys and tumor.

In the INS-1 xenografts examined by [^{11}C]5-HTP, the image contrast was markedly lower (tumor-to-muscle ratio 4.2±0.8, $p \leq 0.05$) than that for [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 (tumor-to-muscle ratio 44.8±14.5),. This reflects not only the higher tumor uptake, but also the lower background levels for [^{68}Ga]Ga-DO3A-VS-Cys40-Exendin-4 (Fig. 5).

4. Discussion

The choice of ^{68}Ga was justified by its accessibility, straightforward and mild labeling chemistry as well as its favorable nuclide characteristics providing high quality images and quantification. The relatively low total and focal radiation dose as compared to that of Indium-111, Fluorine-18 and Copper-64 previously used in imaging studies of GLP-1R by radiolabeled GLP-1 analogues [20-22] was another factor taken into consideration. The generator based ^{68}Ga provides relatively cheaper production cost as compared to radionuclides produced in cyclotrons, e.g. Fluorine-18, Carbon-11, Copper-64,.Its shorter half-life as compared to Fluorine-18 and Copper-64 and high positron content allows for shorter acquisition time. Compared with the clinically available tracer for insulinomas, [^{11}C]5-HTP, [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 offers simpler labeling chemistry and lower cost of production. Another advantage was the possibility to obtain [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 with reproducible high specific radioactivity (compared to previously reported Ga-68 labeled exendin-4 derivative [23])which was required for the high affinity of the ligand to the target and its strong potency resulting in physiological response even at minute amounts. Finally, of importance is the potential quantification of tumor uptake and GLP-1R density by compartmental models or semi-quantitative measures such as SUV. Such quantification of GLP-1R in tumors may assist in better patient management according to the paradigm of personalized medicine, by allowing for patient stratification and improved staging of the disease.

The feasibility of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 for the visualization of GLP-1R in insulinoma was demonstrated in vitro, ex vivo, and in vivo. [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 was displaceable by both native Exendin-4 and precursor. This indicates that the introduction of the DO3A chelator has negligible effect on biocompatibility and GLP1-R affinity compared to the non-modified peptide. Estimated GLP-1R levels were high (B_{max}>100pmol/mg tissue) in insulinoma sections of rodent origins. The possibility of quantification of GLP1-R in biopsies by autoradiography or by in vivo Exendin-4-PET can potentially be used to stratify insulinoma malignancy, as it has been observed that GLP-1R is over-expressed in benign insulinomas, but reduced as malignancy develops [24].

Biodistribution studies in healthy control mice, INS-1 and PANC1 tumor carrying nu/nu mice showed high specific uptake of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 in INS-1 endocrine tumors,

pancreas and lungs. These tissues are known to be GLP-1R positive. On the other hand, uptake in GLP-1R negative tissues such as liver, muscles and exocrine PANC1tumors, was non-displaceable and low. The low hepatic background level is noteworthy since liver is a major site for potential metastasis. A high tumor to background contrast is therefore potentially achievable in these tissues. The substantial uptake in kidneys is likely dose limiting, similar to other ^{68}Ga-labeled peptides. Approaches for reducing the uptake and retention in kidney cortex are under development.

The imaging with animal PET-CT showed concordant results with the biodistribution studies. INS-1 tumors showed prominent uptake compared to the background, which is essential for detecting small lesions in vivo. Liver and muscle are sites of clinical importance for islet transplantation, and the low uptake in these organs suggest that visualization of insulinoma metastasis and islet grafts may be possible in these tissues.

Insulinomas as in most of PNETs are slow growing tumors. Hence the use of [^{18}F]FDG is limited for insulinoma because of the low glucose turnover [25-26].

4.1. Translation To The Clinic

Exendin-4 (Exenatide) is approved for clinical use as an antidiabetic drug for type 2 Diabetes, and its safety and tolerance in humans have been demonstrated. In this preclinical study, 2.5 µg/kg of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 was administered in the baseline studies. Due to the small size of the animals, it is difficult to reduce the dose even further while not compromising the administered amount of radioactivity below detection levels. There may be a concern of administering this dosage of peptide to human, as merely 5-10µg subcutaneous Exendin-4 (approximately 0.1µg/kg) is sufficient for eliciting a glucose lowering effect in diabetics. However, given the consistent production of tracer batches of >50 MBq/nmol in combination with the increase in body size from mouse to human, we estimate that the dosage given in the clinical setting will be approximately 0.01-0.05 µg/kg thereby falling under the microdosing concept.

The tumor-to-background ratio of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 was markedly improved in comparison with [^{11}C]5-HTP. The latter has been shown to have high sensitivity and specificity for the detection and staging of metastasized neuroendocrine tumors and is currently the state-of-the-art available in clinical routine (together with [^{18}F]DOPA/PET). In light of this, [^{68}Ga]Ga-

DO3A-VS-Cys^{40}-Exendin-4 may provide significant progress for the management of insulinomas, a rare yet crippling disease, and motivates further translation of this tracer to the clinical setting.

5. Conclusion

[^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 with reproducible high specific radioactivity was synthesized. It demonstrated high affinity and specificity for GLP-1R. It could distinguish between pancreatic endocrine tumor (INS-1) and pancreatic exocrine tumors (PANC1). The tracer uptake and the contrast of the tumor compared to background was improved compared to the clinically used insulinoma marker [^{11}C]5-HTP. Quantification of GLP-1R may provide the basis for the stratification of patients in planning of potential future radiotherapy targeted to GLP-1R.

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Conflict Of Interest

The authors declare that they have no conflict of interest.

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Figure Legends

Fig. 1. Autoradiographic study for the specificity of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 in an INS-1 xenograft. At tracer concentrations between 0.3-2.5nM, more than 90% of the baseline (total) uptake was displaceable by either 200nM non-modified Exendin-4 peptide or 200mM tracer precursor.

Fig. 2. Assessment of GLP-1R receptor density (B_{max}) and [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 affinity in INS-1 xenografts by an autoradiographic saturation binding study. Sections were incubated in 7 concentrations of radiotracers (0.3-23nM) either alone or together with 200nM Exendin4 peptide.

Fig. 3. Biodistribution of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 in healthy control mice Balb/c nu/nu mice, 80 minutes post injection. Significant uptake displaceable by excess unlabelled peptide precursor was observed in GLP1-R positive organs lungs and pancreas ($p \leq 0.05$). Asterisks indicate significance assessed by unpaired student's t-test. *$p \leq 0.05$.

Fig. 4. Biodistribution of [^{68}Ga]Ga-DO3A-VS-Cys^{40}-Exendin-4 in INS-1 tumor bearing mice 80 minutes post injection. The uptake of the tracer in PANC1 xenografted mice is shown for comparison. Significant uptake displaceable by unlabelled peptide precursor in excess was observed in INS-1 tumor ($p \leq 0.01$), pancreas ($p \leq 0.05$) and lung ($p=0.07$). Significantly low uptake was observed in PANC1 tumors at baseline dose ($p \leq 0.001$). Asterisks indicate significance assessed by unpaired student's t-test. *$p \leq 0.05$. **$p \leq 0.01$.***$p \leq 0.001$.

Fig. 5. Representative whole body PET/CT images of INS-1 xenografts, comparing the uptake of [**^{68}Ga**]GaDO3A-VS-Cys40-Exendin-4 at baseline dose (A) and after co-injection of excess precursor peptide (B). The animal PET/CT images show that the majority of the [^{68}Ga]GaDO3A-VS-Cys^{40}-Exendin-4 uptake in the tumor was displaceable. Additionally, [^{68}Ga]GaDO3A-VS-Cys^{40}-Exendin-4 has improved tumor to background ratio in a direct within individual comparison with [^{11}C]5-HTP (C). PANC1 tumors had significantly low tracer uptake at baseline dose, similar to in the ex vivo organ distribution study (D). T- Tumor, K- Kidney and L- Bladder.

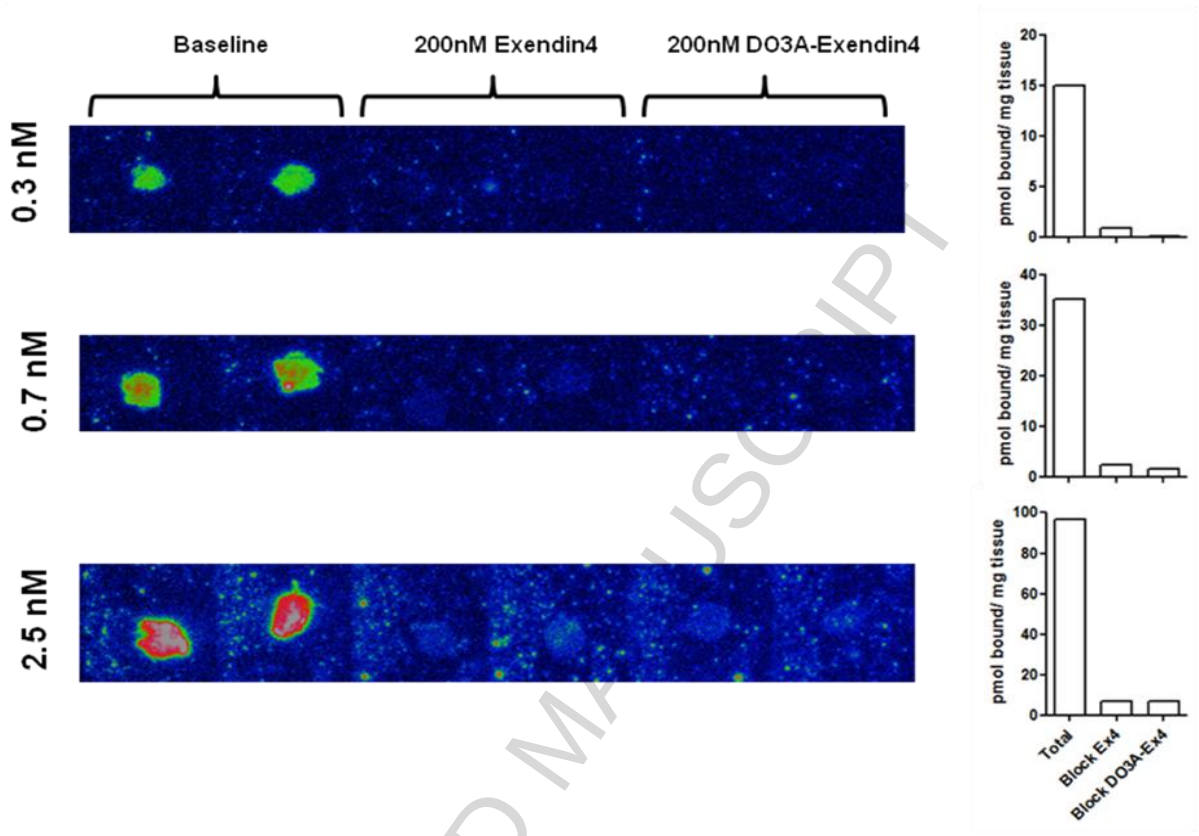

Fig. 1

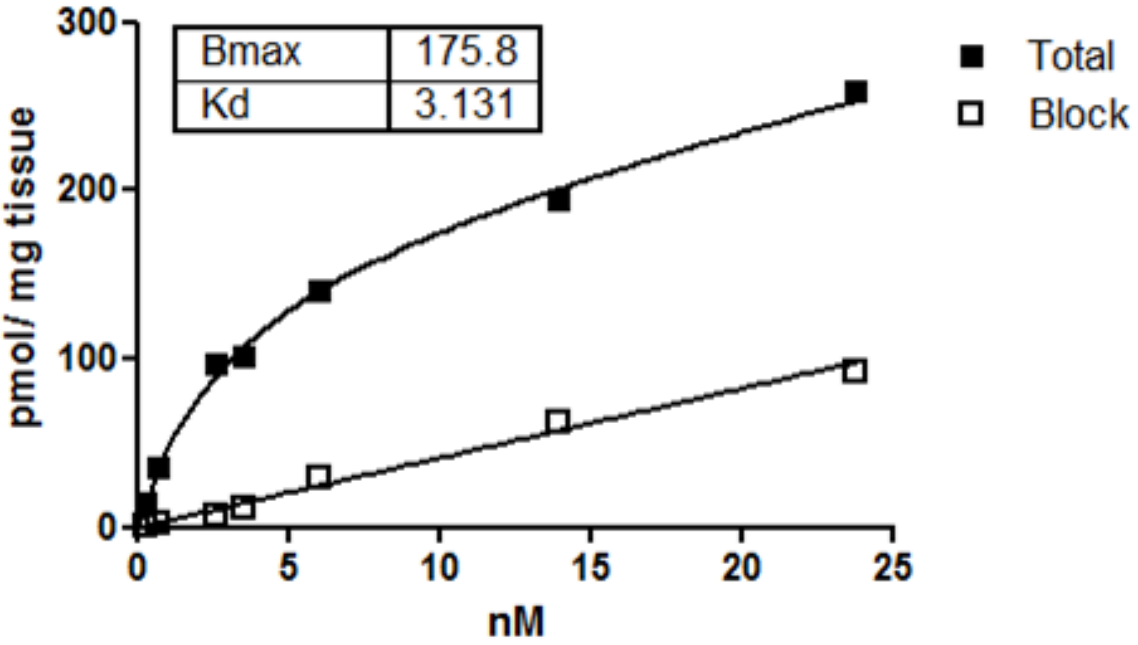

Fig. 2

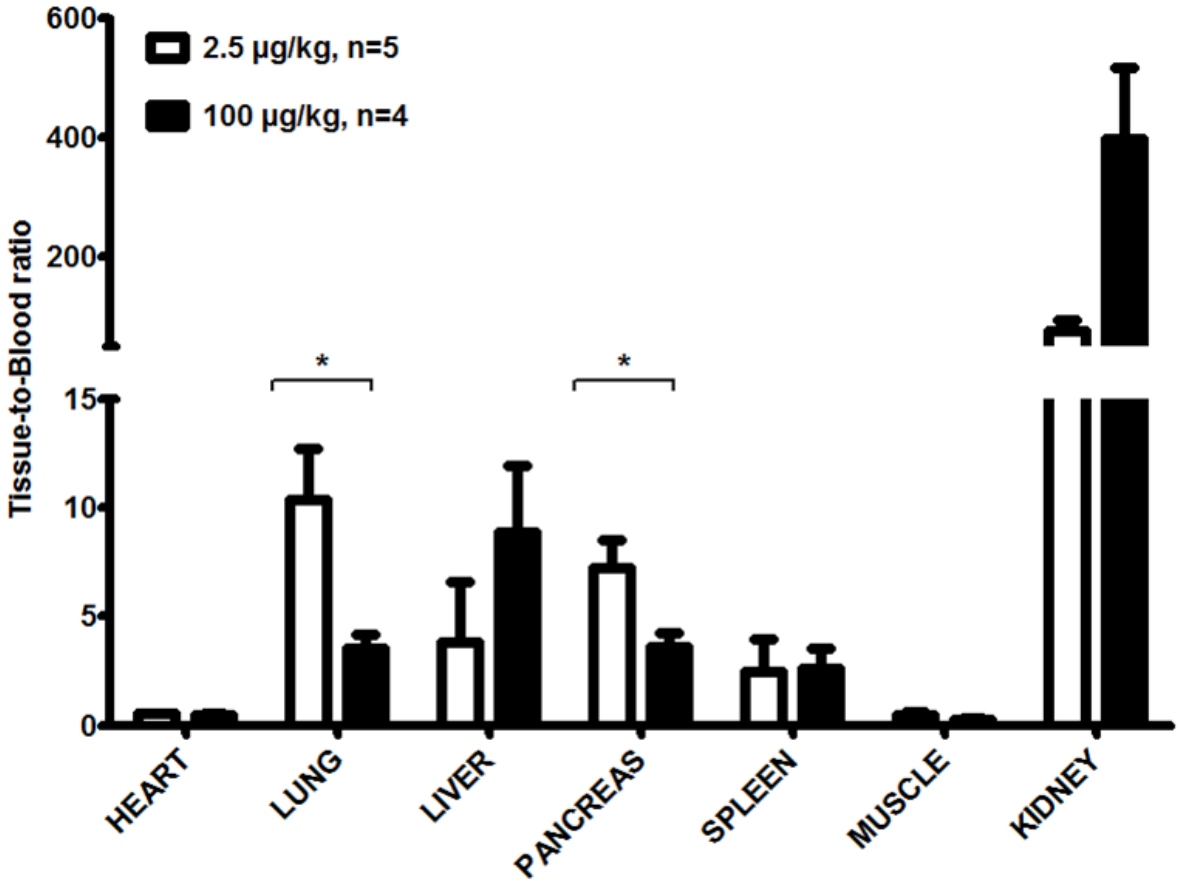

Fig. 3

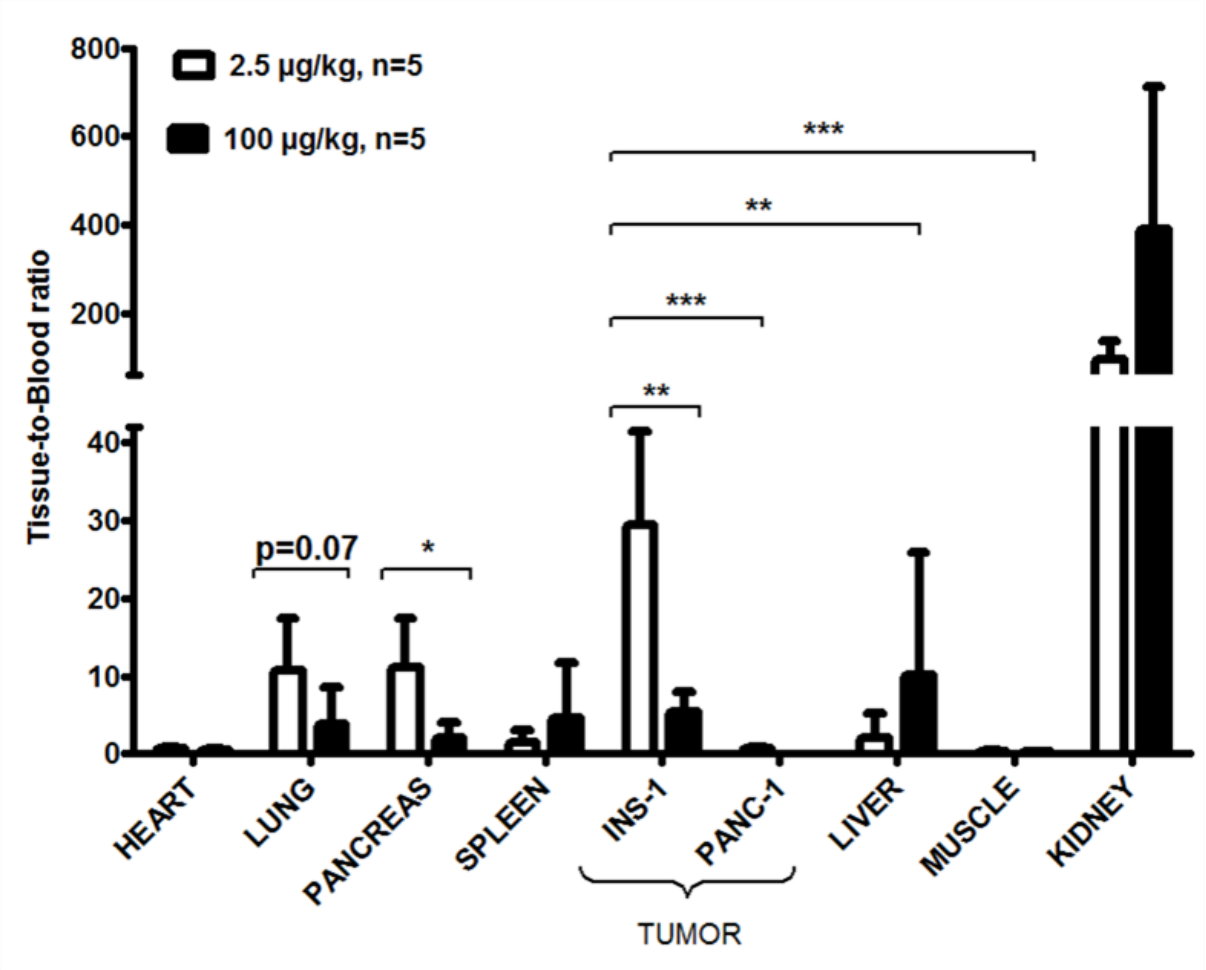

Fig. 4

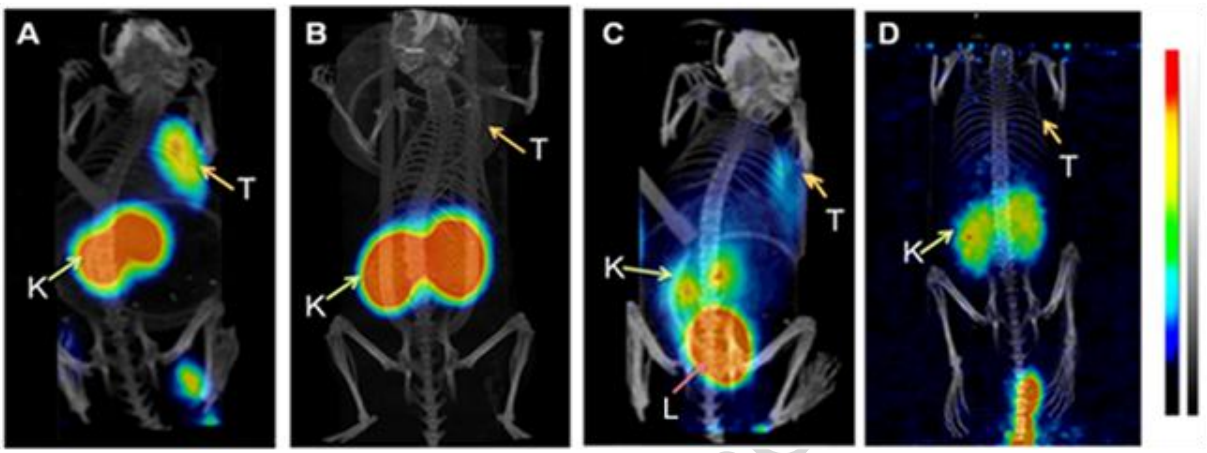

Fig. 5