Evaluation of Peptide-Mediating Targeting Phage as Potential β-Cell Imaging Agents

Mai Lin[1], Michael McGuire[2], Angelo Lubag[3], Serguei Seliounine[1], Edward Tsyganov[1], A. Dean Sherry[3], Kathlynn Brown[2] and Xiankai Sun[1]

[1] Department of Radiology, [2] Department of Internal Medicine, [3] Advanced Imaging Research Center,

University of Texas Southwestern Medical Center at Dallas, Dallas, TX, United States

Introduction

The function of β-cells in islets of Langerhans is to adjust insulin secretion in response changes in blood glucose. β-cells are known to loose this function in both type 1 and type 2 diabetes, although for different reasons. Thus, development of β-cell targeting imaging probes would further facilitate diagnosis and monitoring of the progression of diabetes. Currently, the assessment of β-cell mass in the pancreas non-invasively is a challenge for any imaging modality due to the low population (2-3%) of Langerhans islets in the adult pancreas. By using phage display techniques, a rat islet peptide (RIP) phage clone has been isolated that targets and binds to islets in normal rats. To explore its potential as a nuclear imaging agent for imaging the β-cell, the RIP phage clone and a random control were radiolabeled with $^{124/125}$I to perform *in vitro* uptake assays, biodistribution studies, and non-invasive PET imaging in normal Sprague-Dawley rats.

Materials and Methods

Radiochemistry: Iodine-124/125, which was in the form of Na$^{124/125}$I and dissolved in 0.5 N NaOH, was purchased from Eastern Isotope and Perkin Elmer, respectively. Iodogen pre-coated tubes were purchased from Pierce. Before radiolabeling, centricon YM-30 filters (Millipore. MWCO: 30KDa) were used to eliminate *E.Coli* debris in the phage solution. The ^{125}I-iodination of random control and rat islets peptide-1 (RIP 1) phages was achieved by adding 1 μ L of ^{125}I solution (300-400 μ Ci) into iodogen pre-coated tube with 200 μ L of phage solution in 10 mM PBS (1 X 10^9 PFU, pH = 7.4). The reaction mixtures were then allowed to proceed at room temperature for 15 min and free ^{125}I was filtered off by centricon YM-30 tubes. As for the ^{124}I radiolabeling, the methods were similar as described above, except the activities used in the reaction were higher (1.5 mCi/ea). The radiochemical yields were determined by a radio-TLC system (Raytest, VA) with instant thin layer chromatography strips as the static phase and 10 mM PBS/methanol in 15/85 (v/v) as the mobile phase.

In Vitro Islet-bead and Insulinoma Uptake Studies: Before the experiments, islets were isolated from the pancreas of Sprague-Dawley rats in the weight of 150-175 g and embedded into alginate beads (ϕ 2.5 mm). The ^{125}I-labeled phages were then added (n = 5 for each phage) and the incubation was allowed at 37 °C. After 30 min, the beads were washed three times by cold PBS (10 mM) and then counted by γ-counter. As for uptake studies of insulinoma cells, 1 X 10^7 PFU of ^{125}I labeled phage clones (0.4-0.7 μ Ci) were incubated with 1 X 10^6 of INS 832/13 cells (n = 6 for each phage) in 12-well plate at 37°C. After 30 min, the cells were washed by cold PBS three times (10 mM) and imaged by a phosphor plate autoradiography system. The cells were then harvested and counted by γ-counter.

Small Animal PET Studies: All animal studies were performed in compliance with guidelines set by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center at Dallas. Two normal male Sprague-Dawley rats (Harlan, IN) at the age of 4-5 weeks weighing 150–175 g were injected with 640-683 μCi of the ^{124}I-labeled random control and RIP 1 phages (1 X 10^9 PFU/ea) via the tail vein in the volume of 250 μL, respectively. The rats were anesthetized prior to imaging at 4 and 24 h p.i.. The imaging acquisition time ranged from 70 to 150 mins. Post-PET biodistribution was performed to confirm the quantification results of the imaging studies.

Biodistribution Studies: The ^{125}I-labeled random control and RIP 1 phages were diluted with 10 mM PBS (pH 7.4). Normal male Sprague-Dawley rats (Harlan, IN) at the age of 4-5 weeks weighing 150–175 g (16 rats per phage, n = 4 at each time point) were injected with 5 μCi of the ^{125}I-labeled phages (5 X 107 PFU/ea) via the tail vein. The injected volume was 100 μL. The rats were anesthetized (3 % isoflrane) prior to be sacrificed at 1 h, 4 h, 24 h, and 48 h post injection (p.i.). Organs of interest were removed, weighed and counted. Standards were prepared and counted along with the samples to calculate the percent injected dose per gram (%ID/g).

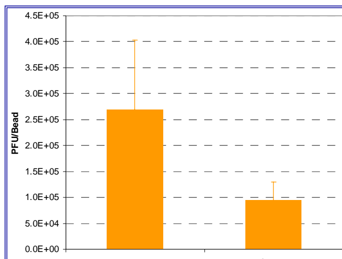

Figure 1. Islet-bead uptake studies of ^{125}I labeled RIP and control phage (n = 5 for each group; mean ± SD)

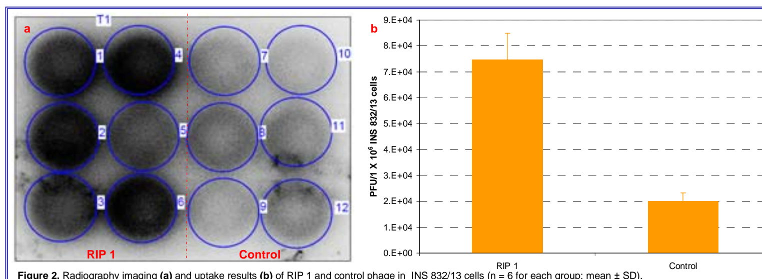

Figure 2. Radiography imaging **(a)** and uptake results **(b)** of RIP 1 and control phage in INS 832/13 cells (n = 6 for each group; mean ± SD).

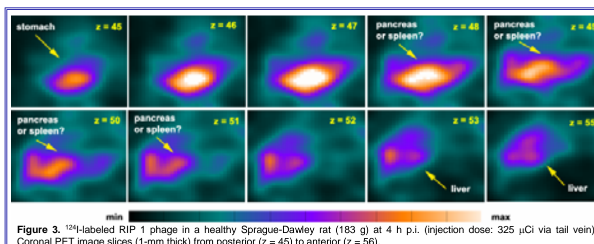

Figure 3. ^{124}I-labeled RIP 1 phage in a healthy Sprague-Dawley rat (183 g) at 4 h p.i. (injection dose: 325 μCi via tail vein): Coronal PET image slices (1-mm thick) from posterior (z = 45) to anterior (z = 56).

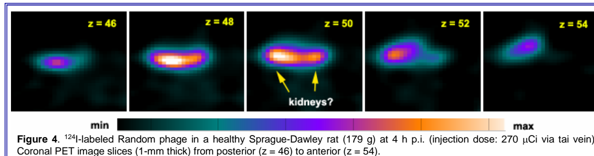

Figure 4. ^{124}I-labeled Random phage in a healthy Sprague-Dawley rat (179 g) at 4 h p.i. (injection dose: 270 μCi via tai vein): Coronal PET image slices (1-mm thick) from posterior (z = 46) to anterior (z = 54).

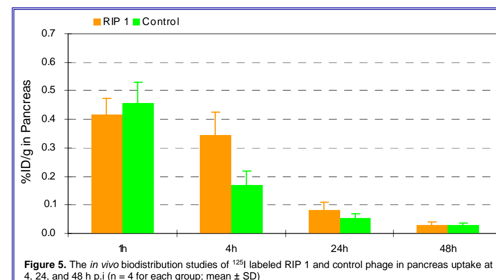

Figure 5. The *in vivo* biodistribution studies of ^{125}I labeled RIP 1 and control phage in pancreas uptake at 1, 4, 24, and 48 h p.i (n = 4 for each group; mean ± SD)

Table 1. Comparative uptake ratios of RIP 1 (~6 h p.i.) and random control (~6 d p.i.) phages in pancreas and other organs of interest.

	Pancreas/brain	Pancreas/fat	Pancreas/muscle	Pancreas/heart	Pancreas/kidneys	Pancreas/liver	Pancreas/stomach	Pancreas/spleen
RIP 1	16.21	6.31	5.85	5.15	1.43	1.26	0.60	0.30
Random Control	2.70	0.96	2.23	0.93	0.04	0.26	0.21	0.43

Results and Discussion

Radiochemistry: We have successfully labeled random control and RIP 1 phage with ^{124}I and ^{125}I at radiochemical yields of 30 – 40%. The highest achievable specific activity was 5 X 10^{-5} μCi/pfu. Analysis by radio-ITLC showed greater than 95% radiochemical purity.

In Vitro Uptake Studies: The binding of the random control and RIP 1 phage clones was tested on isolated islets. The number of RIP 1 phage attached to rat islets embedded beads was significantly higher than that of the control (Figure 1, RIP phage: 2.70 X 10^5 ± 1.33 X 10^5 PFU/bead; Control: 0.95 X 10^5 ± 0.35 X 10^5 PFU/bead. p < 0.01). Because β-cell mass in islets varies in each individual rat and islets represent a mixture of cells, the phages were further tested for binding to a β-cell line in cultured INS 832/13 cells, a robust glucose-stimulated insulin secretion line derived from INS-1 rat insulinoma cells. As shown in Figure 2, the RIP 1 labeled phage showed approximately three times greater binding to INS 1 cells compared to random control phage. These data suggest that the RIP 1 phage targets to the β cells in islets.

In Vivo PET Imaging and Biodistribution Studies: Two Sprague-Dawley rats injected with the radiolabeled phages (i.v.) were imaged by PET at 4, 24, 48 h, and 5 d p.i. The rat injected with ^{124}I-labeled RIP 1 phage died during the PET imaging data acquisition at the first time point for unknown reasons while the rat with ^{124}I-labeled random phage went through the imaging at all planned time points. Thus the only direct comparison of the two phages that can be made in this study is between the PET images at 4h p.i. As shown in Figures 3 and 4, both phages preferentially accumulated in the liver-stomach region, while the random phage showed somewhat less accumulation in this region. With ^{124}I-labeled RIP 1 phage, bright areas were seen between the stomach and the liver which might be identified as either spleen or pancreas, or both (Figure 3). In the PET images of ^{124}I-labeled random phage (Figure 4), the kidneys are visible. The high kidney uptake of random phage is not likely due to the deiodination of the radiolabeled phage, because the free ^{124}I should have preferentially accumulated in stomach, which has endogenous gene expression of sodium iodide symporter (NIS), rather than kidneys. Interestingly, histological examination also showed significant accumulation of phage in kidneys for unknown reasons. The elevated pancreas uptake of ^{124}I-labeled RIP 1 phage was also clearly exhibited in the post-PET biodistribution data (Table 1) as compared to uptake in liver, kidneys, heart, muscle, fat, and brain. This is consistent with the published observations (Samli, KN et al. Diabetes, 2005, 54, 2103-2108). In contrast, the random phage didn't show meaningful pancreas accumulation (Table 1). Both imaging and biodistribution results reveal high thyroid uptake (%ID/g) in the animals, however, considering its small size, the whole thyroid accumulation of ^{124}I radioactivity was just barely above one percent of the injection dose (RIP 1: 1.48 %ID/thyroid at the time when the rat died; Random phage: 1.03 %ID/thyroid at 6-d p.i.), indicative of the high in vivo stability of the two ^{124}I-labeled phages. The *in vivo* biodistribution studies in pancreas uptake (Figure 5) at 4 h p.i. (RIP phage: 0.35 ± 0.08 %ID/g; Control: 0.17 ± 0.05 %ID/g. p < 0.01) further indicates specific RIP 1 phage targeting. Considering the phage size, the biodistribution data surprisingly showed very low uptake of both phages in liver (RIP 1 phage: 2.11 ± 0.37 %ID/g; Random phage: 1.12 ± 0.10 %ID/g at 4 h p.i.) and rather efficient overall clearance (RIP1 and Control phage: > 80%ID was excreted at 24 h p.i.), which is consistent with the post-PET biodistribution results.

Conclusions

The phage containing RIP 1 peptides that target islets *in vivo* were successfully labeled with ^{124}I or ^{125}I and imaged in rats by PET. The labeled phage also bound to β-cell-derived INS1 cells with high specificity suggesting that the RIP 1 peptide may selectively bind to β-cells in islets. While further imaging studies are under way, our preliminary results showed the potential application of RIP 1 peptide mediated phage clones to PET imaging of β-cells.

Acknowledgements

The authors acknowledge support from the National Institutes of Health, NIDDK, via the program project grant, PO1 DK058398.