In Vitro Evaluation of Non-Specific Binding of the Candidate Beta Cell Mass PET Probe (+) 18F-FP-DTBZ

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Purpose: The vesicular monoamine transporter Type 2 (VMAT2) is a candidate beta cell mass (BCM) marker that can be evaluated non-invasively by PET using the radioligand (+) 18F-FP-DTBZ. We evaluated the utility of this BCM marker and radioligand in a cross sectional studies of healthy human volunteers and subjects with long standing type 1 diabetes, predicted to have little or no beta cell mass based on metabolic measurements. Using Binding Potential (BP) as the PET outcome measure, our in progress studies reveal that healthy controls (n=9) have an average BP = 2.15 compared to an average BP = 1.37 (63% of controls) found in T1D subjects (n=5). The significant PET signal in the T1D population is unexpected as histological evaluation of the amounts of VMAT2 in healthy controls and T1D pancreata correlate closely with insulin staining. One explanation for binding of the PET probe in T1D pancreata is that (+) 18F-FP-DTBZ binds not only specialty and displaceably to VMAT2 but non-specifically to pancreas membranes as well.

Methods: We evaluated non-specific binding of (+) 18F-FP-DTBZ in membrane preparations of human pancreata in vitro using a receptor binding assay as an estimate of the in vivo levels of non specific probe binding potentially observed in our clinical PET studies. Snap frozen pancreas tissue was received from nPOD and total pancreas membranes prepared in the presence of a protease inhibitor cocktail. Membrane protein concentrations were measured by the bicinchoninic acid method for normalization of the protein content of membranes samples obtained from different individuals. Aliquots of membranes were diluted in cold assay buffer [0.3 m sucrose, 25 mm HEPES (pH 7.5), and 5 mm MgCl2, containing a protease inhibitor mixture (100 µm PMSF, 100 µm benzamidine, 20 µg/ml leupeptin, and 10 µg/ml soybean trypsin inhibitor; final concentrations)]. 18F-FP-DTBZ (Specific Activity 900-1100 Ci/mmol) was added to 25 nM in the presence or absence of a 1000 molar excess of cold tetrabenazine in triplicate determinations. The reaction was terminated by the addition cold assay buffer and washing filters on a vacuum manifold. Radioactivity trapped in filters was counted in a gamma counter.

Summary of Results: The total and displaceable binding was measured in membrane samples obtained from eight individuals including one subject with long term T1D (<0.05 ng/ml c-peptide) and one subject with T2DM
(0.85 ng/ml c-peptide). The specific displaceable binding (total minus nondisplaceable binding) in healthy subjects with normal or near normal c-peptide averaged 40% of the total (range 22-56%). As predicted membranes prepared from the long term T1D pancreas showed no displaceable probe binding.

Conclusions: Our preliminary data suggest that there is non-displaceable non specific binding of (+) 18F-FP-DTBZ to pancreas membranes in vitro. Extrapolating the magnitude of the in vitro non specific binding of 18F-FP-DTBZ to our in vivo PET measurements of VMAT2 and BCM, suggests that the true in vivo measured BP (corrected for non-specific binding) for normal controls will be closer to 1 and that of individuals with no detectable BCM will be closer to 0 as predicted by metabolic measures. Our in vitro findings are currently being validated in an in vivo study using a complementary PET technique applying the non VMAT2 binding (-) stereoisomer of (+) 18F-FP-DTBZ.