Evaluation of 2-[^{18}F]Fluoroethoxy-5-bromoglibenclamide for the non-invasive visualization of the pancreatic islet cell mass in humans using PET

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Introduction: The non-invasive quantification of the pancreatic islet cell mass (PICM) *in vivo* is of great medical interest, but fails so far because of the unavailability of an applicable technique. In Type 1 diabetic patients the chronic and progressive loss of the insulin producing pancreatic islet cells due to autoimmune destruction has led to concerted efforts to prevent further loss of β-cells by autoantigen-specific immunotherapy of pre-diabetic patients. With the availability of a non-invasive technique, the effect of different intervention strategies could be easily monitored. Furthermore, the longitudinal monitoring of the PICM of patients with Type 2 diabetes mellitus under different antidiabetic therapies (e.g. GLP-agonists) may lead to the development of new strategies for treatment.

Aim: The intention of our present research activities is the development of an islet cell specific positron-emitter labelled tracer to image the insulin producing pancreatic islet cells non-invasively using positron emission tomography (PET).

Chemistry and Radiochemistry:
Our search for potential PET tracers for the visualization of the PICM led us to the development of 2-[^{18}F]fluoroethoxy-5-bromoglibenclamide (**2**). This compound was chosen from twenty newly synthesized fluorinated glibenclamide derivatives (Schirrmacher et al. unpublished results), due to its well preserved physiologic behaviour in vitro compared to the original glibenclamide. The radiolabeling was performed using 2-[^{18}F]fluoroethyltosylate as an intermediate in an overall radiochemical yield of 10% (referring to the starting activity of [^{18}F]fluoride). Briefly, no-carrier-added (nca) [^{18}F]fluoride prepared by the ^{18}O(p,n)^{18}F nuclear reaction on an enriched water (95 %+ ^{18}O) target was added to a solution of potassium carbonate/Kryptofix $_{2.2.2.}$ in a Pyrex vessel. The water was evaporated using a stream of nitrogen at 80°C and coevaporated to dryness with acetonitrile (2 x 1 ml). A solution of ethylene glycol di-*p*-tosylate (5 mg in 0.5 ml of acetonitrile) was added to the dried K[^{18}F] and the solution was heated at 80°C for 5 min. The crude 2-[^{18}F]fluoroethyltosylate was purified via HPLC (LiChrospher RP18 5EC, 250 x 10 mm, eluent: acetonitrile/water 1/1 (v/v), flow: 5ml/min retention time 10-12 min), loaded on a Sep-Pak C-18 solid phase cartridge and eluted with ether (2 ml). A solution of *N*-{4-[β-(2-hydroxy-5-bromobenzenecarboxamido)ethyl]-benzenesulfonyl}-*N'*-cyclohexylurea (**1**) (1 mg, 1.9 µmol) and 1 N NaOH (7 µl) in DMSO (250 µl) were added to the 2-[^{18}F]fluoroethyltosylate solution. The mixture was heated at 80^{0}C for 3 min and then at 120°C for additional 10 min.
The crude product **2** was injected into an isocratic HPLC system (LiChrospher RP18 5EC 250 x 10 mm, flow: 4ml/min, eluent: acetonitrile/0.5 M ammonia acetate buffer (52/48) (v/v)) and the fraction containing **2** (R_t = 21 min) was collected, diluted with water (20 ml) and loaded onto a Sep-Pac C-18 solid phase cartridge. The cartridge was dried in a stream of nitrogen, eluted with 1 ml of ethanol and diluted with 10 ml of physiological saline. After sterile filtration, the final sterile product was obtained as a 10 % ethanolic saline solution for injection in an overall radiochemical yield of 8-10 %.

Evaluations: For *in vitro* binding of the analogues, the non-radioactive ^{19}F-compound was synthesized and used to assess the binding affinity of the fluorinated glibenclamide analogues to human SUR1. Biological activity of the ^{19}F-compound was evaluated via insulin secretion experiments. The *in vivo* biodistribution of the ^{18}F-compound was analyzed in rats and humans via PET studies.
In vitro binding studies of the ^{19}F-compound revealed a complete monophasic inhibition curve with a Hill coefficient close to 1 (0.92), yielding a dissociation constant (K_D) of 0.22 nM. The insulin stimulating capacity of the ^{19}F-compound was also well preserved. The estimation of the lipophilicity revealed a Log P value of 1.69 for the ^{19}F-compound, which did not differ from that of glibenclamide itself. Analysis of the *in vivo* biodistribution in rats and humans showed a fast uptake and near constant retention of the ^{18}F-compound in the pancreatic tissue. However, due to resolution problems in the sequel of the high liver uptake of the tracer, a visualization of the PICM did not succeed.

Conclusions: The *in vitro* data clearly indicate that the newly synthesized fluorinated glibenclamide analogues bind with high affinity to the human SUR 1 and showed a physiologic insulin stimulating capacity. These data raise the anticipation that these analogues might serve as PET tracers for visualization of the PICM. However, *in vivo* studies in both rats and humans revealed that visualization of the endocrine pancreas was prevented by a high, most probably specific liver uptake of the tracer.

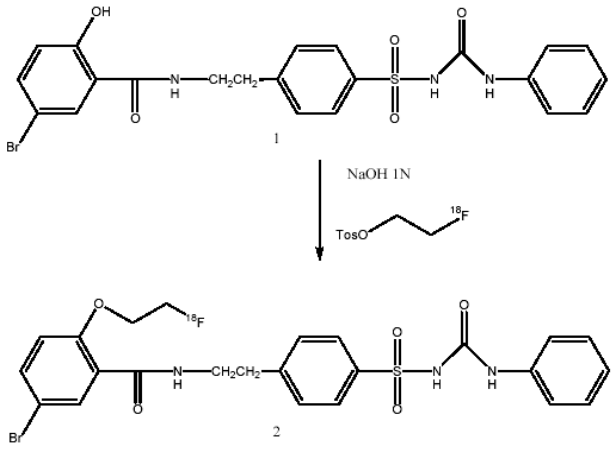

Fig.1:
synthesis of 2-[^{18}F]Fluoroethoxy-5-bromoglibenclamide