Fluorine-18 click-labeling and evaluation of a folic acid derivative with increased polarity

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Aim: The folate receptor (FR) is (over)expressed on many human carcinomas and provides a perfect target for imaging and therapy approaches. Beside several folic acid based radiopharmaceuticals [1], efforts to develop a suitable fluorine-18-labeled folate for PET imaging were not very successful, so far. Either the radiolabeling/-chemistry was insufficient [2] or the pharmacological profile was unfavourable for clear-cut PET imaging [3]. Especially, an increased lipophilicity of the fluorine-18 click-folate led to pronounced hepatobiliary excretion [3]. Based on the fluorine-18 click-folate, we want to combine highly efficient flourine-18 click-chemistry with polar PEG-spacers to improve hydrophilicity and thus the *in vivo* behaviour.

Methods: The precursor was produced by coupling folic acid to an azido-PEG_4-amine at the γ-position of the glutamic acid residue. The non-radioactive reference compound was similarly synthesized using the corresponding fluorinated PEG_4-azide. The reference was used for binding affinity tests using KB cells. The flourine-18 labeling of the prosthetic group was carried out under various conditions, yielding ≥70% RCY (µW, 2 min). The clickable prosthetic group is obtained using a HPLC purification step and then reacted with the azido-folic acid in the µW to yield the final tracer. The lipophilicity of the final product was determined using the partition coefficient of *n*-octanol and Sörensen's buffer. Preliminary µPET-studies on KB tumor bearing mice, involving a control and a blocking group, were carried out. Additionally *ex vivo* biodistribution studies were performed.

Results: To ensure only the γ-position of the folic acid is functionalized, a regioselective build-up synthesis of the folic acid derivative was necessary. Flourine-18 labeling of the prosthetic group was successful in very high radiochemical yields. The flourine-18 click reaction was carried out under optimized conditions, which had been screened beforehand. We determined a logD-value of -0.9. Furthermore, we performed binding assays with the reference compound and [^{3}H]folic acid giving an IC_{50}-value of 3 nM. By using Cheng-Prusoff-equation we calculated a K_i-value of 1.6 nM. We tested the *in vitro* stability in FCS at several time points and showed that the tracer is stable for at least 90 min. As expected, preliminary µPET-studies showed a promising *in vivo* behavior with a specific uptake in the cortex of the tumors as well as high uptake in the kidneys. (Figure 1).

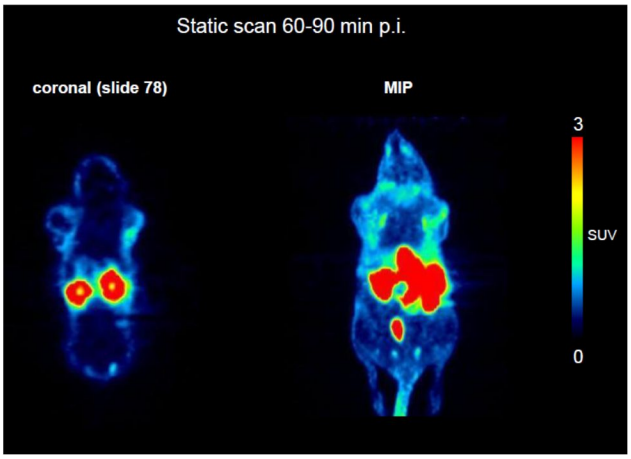

Figure 1: Coronal slice of a 60–90 min µPET scan p.i. (left); maximum intensity projection (right).

Conclusions: A new γ-azido-PEG_4-folate was successfully synthesized and "clicked" to a PEG-based ^{18}F-labeled prosthetic group. An excellent folate receptor affinity of the cold reference was determined and preliminary µPET-studies showed a high and specific uptake in the kidney cortex. Ultimately, the new flourine-18 click-PEG-folate will be applied to *in vivo* µPET imaging of tumor bearing rats and mice. We hope that this approach will combine efficient labeling chemistry with a desirable pharmacological profile to provide a flourine-18 folate suitable for high-quality PET imaging of the folate receptor. The *ex vivo* biodistribution showed that a reduction of the background signals occurred over time, due to the pegylation of the tracer.

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