Radioactive labeling of HPMA-based polymeric systems with fluorine-18 for *in vivo* imaging and evaluation by positron emission tomography (PET)

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Introduction: During the last decades polymer therapeutics became more and more an emerging field of interest.[1] An example which has already been intensively studied in clinical trials is the biocompatible polymeric backbone N-(2-hydroxypropyl)-methacrylamide (HPMA).[2] Nevertheless, detailed knowledge about the biodistribution of polymeric drug-delivery systems in living organisms is still lacking. Especially information about the tumor accumulation *in vivo* due to the enhanced permeability and retention effect are of major interest. Here, positron emission tomography (PET) as non-invasive, molecular whole body imaging technique offers a great opportunity to visualize the *in vivo* behavior of radioactively labeled polymeric structures.

Experimental: Well defined HPMA-based random copolymers of different molecular weights (M_w=12 kDa and 77 kDa) synthesized via the RAFT polymerization technique[3] were labeled with the positron emitting isotope fluorine-18 using the secondary labeling synthon 2-[^{18}F]fluoroethyl-1-tosylate ([^{18}F]FETos). For labeling purposes, the polymeric precursors were functionalized with ~ 4% tyramine moieties thus offering a reactive site for the prosthetic labeling procedure using [^{18}F]FETos. The radioactive coupling step was performed using a solution of 3 mg polymer, 1 µL 5N NaOH and [^{18}F]FETos in 1 mL of DMSO (figure 1). The clear solution was kept at 120 °C for 15 min. The reaction mixture was purified using size exclusion chromatography (HiTrap Desalting Column, Sephadex G-25 Superfine, column volume 5 mL; flow: 1 mL/min physiological saline) leading to a pure solution of the ^{18}F-labeled polymer. For kinetic PET studies, the animals (tumor bearing Copenhagen rats, R3327-AT1 dunning prostate carcinoma) were anaesthetized with pentobarbital and a catheter was inserted into the left jugular vein for radiotracer application. Listmode acquisition was started with the injection of 25-35 MBq of the purified polymer solution. The tracer distribution was followed for 2h p.i. Thereafter, a whole body scan of the rat was performed. After the experiment, the rats were sacrificed and an urine sample was taken, and the radioactivity was measured with an γ-counter.

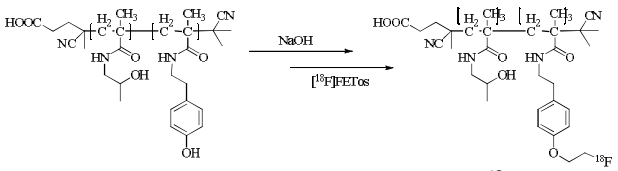

Figure 1. Radioactive labeling of polymers using [^{18}F]FETos.

Results: To understand how the molecular weight affects the tumor distribution, different HPMA-based polymeric systems were labeled successfully and evaluated *in vivo* using µPET imaging. The PET studies with tumor bearing rats showed relative activities compared to reference tissue (testes) of 250% for the higher MW polymer (77 kDa) and 200% for lower MW polymer (12 kDa). Dynamic µPET scans of the tumors over 60-120 min p.i. are shown in figure 2 (sagittal cross sections of the tumor tissue).

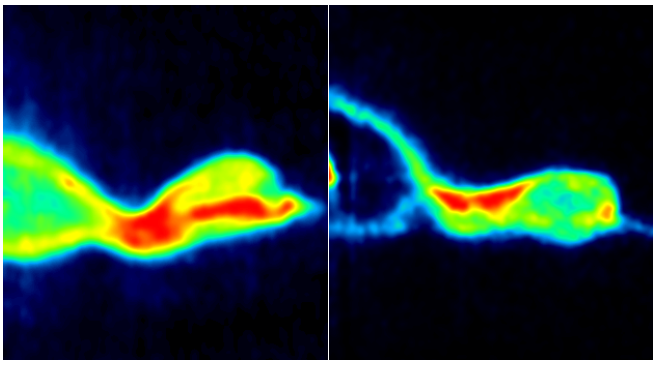

Figure 2. Dynamic µPET scans over 60-120 min after injection of ^{18}F-labeled HPMA-based polymeric systems: left: sagittal cross section of tumor tissue after administration of labeled polymer of MW = 12 kDa, right: 77 kDa labeled polymer.

Expectedly, analysis of urine probes taken after experiments clearly showed higher renal clearance of the lower MW polymer (69% ID/g) compared to the higher MW polymer (4% ID/g). These findings confirm the known renal excretion threshold for HPMA copolymers of 40 kDa. [4]

References

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