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

Dorothea Moderegger$^1$, Mareli Allmeroth$^2$, Rudolf Zentel$^2$, Frank Roesch$^1$

$^1$Institute of Nuclear Chemistry, Johannes Gutenberg University of Mainz, D-55128 Mainz, Germany; 
$^2$Institute of Organic Chemistry, Johannes Gutenberg University of Mainz, D-55128 Mainz, Germany

Introduction: During the last decades polymer therapeutics became more and more an emerging field of interest.\textsuperscript{1} An example which has already been intensively studied in clinical trials is the biocompatible polymeric backbone N-(2-hydroxypropyl)-methacrylamide (HPMA).\textsuperscript{2} Nevertheless, detailed knowledge about the biodistribution of polymeric drug-delivery systems in living organisms is still lacking. Especially information about the tumor accumulation \textit{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 \textit{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\textsuperscript{3} were labeled with the positron emitting isotope fluorine-18 using the secondary labeling synthon 2-\textsuperscript{[18]}F-fluoroethyl-1-tosylate (\textsuperscript{[18]}F\textsuperscript{FETos}). For labeling purposes, the polymeric precursors were functionalized with ~ 4\% tyramine moieties thus offering a reactive site for the prosthetic labeling procedure using \textsuperscript{[18]}F\textsuperscript{FETos}. The radioactive coupling step was performed using a solution of 3 mg polymer, 1 \textmu L 5N NaOH and \textsuperscript{[18]}F\textsuperscript{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 \textsuperscript{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 \textsuperscript{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 \textsuperscript{18}F-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.\textsuperscript{4}

References


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Results: To understand how the molecular weight affects the tumor distribution, different HPMA-based polymeric systems were labeled successfully and evaluated \textit{in vivo} using \textmu PET 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 \textmu PET scans of the tumors over 60-120 min p.i. are shown in figure 2 (sagittal cross sections of the tumor tissue).

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

Figure 2. Dynamic \textmu PET scans over 60-120 min after injection of \textsuperscript{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.