ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové
Department of Department of Pharmacology and Toxycology
Candidate **Adam Cepa, MSc.**
Supervisor **Prof. PharmDr. Ing. Milan Laznicek, CSc.**
Title of Doctoral Thesis **Modification of antibody fragments and their radiolabeling by unconventional positron emitters and biological testing for PET diagnostics**

Special oncology diagnostics require new types of selective radiopharmaceuticals, especially those that are suitable for molecular PET imaging and therapy, respectively, teranostics. The aim of this work is to present a new target radiopharmaceutical for immunoPET diagnosis and therapy based on the monoclonal antibody IgG M75 directed to human carbonic anhydrase IX, nimotuzumab (hR3) target epidermal growth factor receptor (EGFR) and one of the bombesin derivatives (BBN) repeats. These molecules were labeled with radionuclides 64Cu (t1/2 12.70 hours), 61Cu (t1/2 3.33 hours), 68Ga (t1/2 68 min), 177Lu (t1/2 6.71 days). These potential radiopharmaceuticals were evaluated in vitro and in vivo. The IgG M75 antibody and its scFv-Fc M75 fragment were prepared by the genetic modification were conjugated to a specific chelator specific phosphinate (PHS) and radiolabeled by the radionuclides 61,64Cu. The stability of the labeled conjugate was tested in human serum. Immunoreactivity of the labeled conjugate was evaluated in vitro on suitable colorectal carcinoma (HT-29) cell cultures and its imaging properties were estimated from in vivo and in vitro by HT-29 carcinoma experiments imaged by μPET/CT. The immunoconjugate was obtained at a specific activity of 0.7 to 0.1 MBq/μg. In vitro binding experiments revealed specific binding to HT-29 cells (45 ± 2.8 % of total activity added) and KD values measured at 9.2 nM. Imaging unambiguously demonstrated a significant distribution of labeled monoclonal antibodies in the tumor 18 hours after administration. The radioimmunoconjugate IgG M75-NCS-PHS-Cu-64 seems to be a suitable candidate for PET diagnosis of hypoxic tumors expressing human carbohydrate IX. hR3 was further conjugated to commercial DOTA chelator and unconventional chelator DOTAPO, these conjugates were labeled with radionuclides 64Cu and 177Lu with specific activities of 0.2 and 0.5 MBq/μg and subsequently evaluated in vitro on A431 cell cultures including in vivo imaging experiments on μSPECT/CT. The BBN peptide was conjugated with the DOTA chelator and labeled with 64Cu and 68Ga radionuclides with specific activities of 10 and 0.1 MBq/μg. Labeled conjugates were evaluated in vitro on PC-3 cell line models and imaged in vivo on μPET/CT.