ABSTRACT

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Title of doctoral thesis:	The study of properties of radiolabelled monoclonal antibodies for imaging in oncology

Monoclonal antibodies belong to the group of targeted biological drugs that are currently successfully applied in various medical specialties, including oncology. The ability to specifically bind to antigens present in a tumour tissue is their significant attribute that can be used for imaging tumours in Nuclear Medicine. Receptors for vascular endothelial growth factor (VEGFR) and epidermal growth factor (EGFR) are possible tumour specific targets that are characteristic for some types of proliferating tumour tissue. The attachment of appropriate radionuclide is necessary for the potential radiodiagnostic use of monoclonal antibody. This task requires chemical modification of the antibody molecule that can damage its immunoreactivity and the binding to the target receptor.

The aim of the doctoral thesis was to study the influence of labelling conditions with selected diagnostic radionuclides on radiochemical characteristics, *in vitro* receptor specific binding and organ distribution of used monoclonal antibodies with the potential application in Oncology for radiodiagnostics and imaging of experimental tumours *in vivo.* Anti-EGFR monoclonal antibody cetuximab and anti-VEGFR2 monoclonal antibody ramucirumab were chosen for these purposes. In the case of cetuximab we studied the influence of the method during the labelling with iodine-131 and tested the possibility of CTK (Concentration Through Kinetics) method application with the use of target receptors expressed on the surface of living cells. In the case of ramucirumab (RAM) we studied the influence of labelling with selected radiometals on the radiochemical and biological characteristics – the antibody affinity to the target receptors *in vitro* and *in vivo.*

For the labelling of cetuximab with iodine-131 we used the chloramine T method. Monoclonal antibody ramucirumab was labelled using direct and indirect methods with

radionuclides suitable for radiodiagnostic imaging - technetium-99m (^{99m}Tc), gallium-67 (^{67}Ga) and zirconium-89 (^{89}Zr). The thin-layer and high-performance liquid chromatography were employed for the determination of radiochemical purity and stability. The affinity of labelled antibodies to their target receptor was characterized as the equilibrium dissociation constant K_D and was determined by the classic manual saturation technique and real-time automatized method. Human cancer cell lines expressing target receptors for the used antibodies were used as an experimental model in the *in vitro* experiments – A431 and HaCaT for cetuximab and PC3 and SKOV3 for ramucirumab. The biodistribution of ^{89}Zr-RAM was studied *ex vivo* after the application to the mice bearing PC3 and SKOV3 tumours. The same mice models were used to test the PET/CT imaging of tumours with ^{89}Zr-RAM.

The verification of radioiodination influence using the chloramine T method on the quality of cetuximab radiopreparations demonstrated that the shortening of reaction time does not affect their radiochemical purity, but it can influence their stability and affinity to EGFR. At the same time, the usage of ^{131}I-cetuximab demonstrated the potential and advantageous properties of CTK analysis with the living cells, especially at low antibody concentrations. The optimization of the RAM labelling method with selected radiometals resulted in the radiopreparations with high radiochemical purity, stability and minimal reduction of the antibody affinity to the cell surface VEGFR2 *in vitro*. The testing of the influence of different labelling methods with technetium-99m demonstrated less impact of indirect labelling with chelator than was observed using the direct method. These experiments subsequently enabled the preparation of RAM radiopreparation labelled with PET radionuclide ^{89}Zr using the chelating agent deferoxamine (DFO) with adequate quality for *in vivo* experiments. The application of ^{89}Zr-DFO-RAM to laboratory animals bearing VEGFR2-positive tumours demonstrated significant accumulation of the labelled antibody in tumours with the maximum 72 h after administration (9,4 ± 0,9 %ID/g in PC3 tumour and 13,4 ± 1,7 %ID/g in SKOV3 tumour). The accumulation in tumours was significant even 24 h post injection. The good tumour imaging using PET/CT technique 24 h after administration of ^{89}Zr-DFO-RAM was in accordance with *ex vivo* experiments. The gradual decrease of radioactive background in nontarget organs and retention of radioactivity in tumours resulted in relatively clear image of tumour lesion 144 h after administration.

The obtained experimental data confirmed that the labelling method affects the quality of radiopreparations of tested monoclonal antibodies. This can be reflected in the changes of radiochemical characteristics or the affinity reduction to target antigens. Results of *in vivo* experiments confirmed the importance of long physical half-life radionuclide for the labelling

of radiodiagnostic monoclonal antibodies because the imaging at longer time intervals post injection enabled clearer tumour imaging and better examination relevance.