Abstrakt v anglickom jazyku

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Biophysics and Physical Chemistry

Student: František Lach

Supervisor: Mgr. Pavel Bárta, PhD

Consultant: Mgr. Lucie Hyršová

Title of diploma thesis: In vitro saturation study of ^{99m}Tc-HYNIC-ramucirumab on PC-3 cell line

The number of malignant tumours in the population has increased in recent years. Due to the frequent serious sides effects of chemotherapeutic drugs on the whole organism, targeted antitumor therapy is at the forefront. Due to its specific effect on the regulatory and signal pathways of protein structures, monoclonal antibodies are used for the target anti-tumour therapy. The basic properties of the growing tumour include vasculogenesis (the ability to build new blood vessels from the endothelial precursors) and angiogenesis (the process of self-inducing formation of blood vessels). Endothelial tumour progenitors include vascular endothelial growth factor (VEGF). VEGF activates its biological activity by binding to its transmembrane tyrosine-kinase receptors VEGFR. Indeed, the inhibition of the vascular endothelial factor receptors is the target of some monoclonal antibodies. Ramucirumab is a monoclonal antibody that selectively inhibits VEGF receptor type 2 (VEGFR-2) and thereby blocking the activation and signalizing pathways.

The aim of this diploma thesis was realised indirect radioactive labelling of the monoclonal antibody ramucirumab with the ^{99m}Tc radiodiagnostic nuclide using the succinimidyl-6-hydrazino-nicotinamide chelator (HYNIC). Radiochemical purity and stability of the prepared radiopharmaceuticals ^{99m}Tc-HYNIC-ramucirumab was verified for 24 hours. Radiochemical purity and stability was verified by HPLC and iTLC. Radiopharmaceuticals were also tested for their biological properties, therefore their ability to bind to the target receptor VEGFR-2, expressed on the tumour cell line of human prostate cancer. *In vitro* experiments were performed by using manual saturation technique and real-time radioimmunoassay. The aim was to find values of the equilibrium dissociation constant K_D, which determines the affinity of the tested substance to the target protein structure.

The result of radiolabelling of the monoclonal antibody ramucirumab was a preparation of a ^{99m}Tc-HYNIC-ramucirumab, of which prepared samples showed a radiochemical purity more than 95%, that is required in the European Pharmacopoeia for the application of radiopharmaceuticals to patients. No significant presence of free technetium was found to indicate the instability of the radiolabelled immunocomplex after 24 hours of the radioactive labelling. In *in vitro* experiments, the equilibrium

dissociation constant for the binding of ^{99m}Tc-HYNIC-ramucirumab to VEGFR-2 was determined in values K_D = 9.99 ± 2.06 nM for manual saturation technique and K_D = 1.54 ± 0.52 nM for automatic radioimmunoassay. The values obtained for *in vitro* studies confirmed the binding ability of the radiolabelled monoclonal antibody to VEGFR-2.

The result of this diploma thesis is the confirmation of the possibility of radiolabelling the monoclonal antibody ramucirumab with the diagnostic radionuclide ^{99m}Tc through the HYNIC chelating agent, with maintained binding abilities of the prepared radiopharmaceuticals. The prepared radiolabelled ^{99m}Tc-HYNIC-ramucirumab complex could become a promising ligand for visualizing VEGFR-2 of positive tumour diseases, if it also passed *in vivo* testing.