Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Kralove

Department: Department of Pharmacology and Toxicology

Candidate: Zbynek Novy, MSc.

Supervisor: Assoc. prof. PharmDr. Frantisek Trejtnar, PhD.

Title of doctoral thesis: *In vitro* study of interactions of radiolabeled monoclonal antibodies with epidermal growth factor receptor

Various types of cancer are characterized by overexpression of epidermal growth factor receptor (EGFR) compared to normal tissue. Anti-EGFR antibodies utilize this phenomenon to treat certain oncological diseases. Intensively studied field is verifing of possibility to increase usability of these antibodies by incorporating suitable radionuclides into thiers structures, results can serve in radioimmunodiagnosis or radioimmunotherapy of mentioned malignities. Despite the fact that EGFR recepor and his signaling intracelluar pahtway is object of intense research, there is a lack of knowledge in the area of factors, which can significantly affect binding of radiolabeled antibodies to EGFR *in vitro*.

The aim of this doctoral thesis was to introduce method of radiolabeling anti-EGFR monoclonal antibodies (cetuximab, panitumumab) with iodine-131 and to use this radiolabeled antibodies to determine expression rate of EGFR in four selected cell lines (A431, HaCaT, HCT116 a HepG2). Furthermore, the second aim was to perform cell binding studies with lutetium-177 labeled cetuximab and panitumumab in those EGFR-defined cell models and to estimate effect of used radiometal chelator to cell binding in relation with employed cell line. Radiochemical characterisation was aslo the part of the study as well as prove of stability of prepared radiolabeled antibodies.

Determination of number of receptors per cell was performed by classical manual saturation technique and by new semi-automatized approach, i. e. kinetic extrapolation method (KEX). Receptor expression levels were also compared to the results of western blot analysis. The determination of EGFR expression level was performed in four selected cell lines. The modification of both monoclonal antibodies with three bifunctional chelators (DOTA, NOTA,

PCTA) was used for lutetium-177 radiolabeling. The optimization of anti-EGFR antibodies labeling with iodine-131 led to high yield and high radiochemical purity. The stability testing of prepared antibodies showed decrease in radiochemical purity after 24 hours in units of percents. Both methods determining number of receptors revealed results in accordance. Therefore KEX method could be considered as comparably useful as the classical saturation technique. The numbers of EGFR receptors per cell were as follows: 1.98×10^6 for A431 cell line, 0.91×10^6 for HaCaT cells, 0.15×10^6 in the case of HCT116 and 0.07×10^6 for HepG2. Overally, we can put studied cell lines in order according to decreasing number of expressed EGFR: A431 > HaCaT > HCT116 ≈ HepG2. The expression rate corelated with results from western blot analysis.

Protocols introduced for labeling of cetuximab and panitumumab with litetium-177 led to preparations with high radiochemical purity and high stability up to the period of one week. The cell binding studies showed, that the bound fraction of labeled antibody differs widely and is in relationship with used cell line and also is in accordance with its EGFR expression rate. The cell binding of lutetium-177 labeled antibodies was highly specific, whereas preincubation with cold antibody led to massive decrease of its binding. The evalution of effect of chelator type to cell binding *in vitro* showed no significant difference in uptake among antibodies modified with DOTA, NOTA or PCTA in appropriate cell lines. Acquired results demonstrate suitable radiochemical and biological properties of prepared ^{177}Lu-labeled antibodies and they appear as proper candidates for further research towards their potentional application in clinics.