ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové
Department of Pharmacology and Toxicology
Candidate **Mgr. Pavel Bárta**
Supervisor **Prof. PharmDr. Ing Milan Lázníček, CSc.**
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The targeting of receptor specific peptides or antibodies is one important diagnostic and therapeutic tool in the fight against cancer diseases.

Receptor specific peptides often have their origin from the human natural peptide hormones; hence they are commonly marked as peptide analogues. The peptide analogues contain in their amino acids sequence the binding site identical with the site of natural peptides, which they are originated from. This binding site is responsible for the interaction with the targeted receptor. The peptide analogues are employed instead of the natural peptides because their biological properties can be improved, for example facilitating distribution in the organism to their place of action, or protection from biological degradation. The peptide ligand binding itself on targeted receptors and its internalization into cells typically does not trigger a therapeutic effect. From this reason, peptide analogues carry active substances like cytotoxine or radioisotope; this has the therapeutic or diagnostic effect. The therapeutic effect of radioisotope includes cancer cell DNA damage that leads to the loss of ability to proliferate, which in turn causes cell death. The diagnostic purpose of radioisotopes is more common. The gamma radiation is used in imaging techniques to localize tumours.

Therapeutic monoclonal antibodies are able to prevent receptor specific signal transmission coming from the interaction of natural ligand with the targeted receptor. The mechanism of this protection typically comes from the blocking of the receptor binding sites. Commonly, the monoclonal antibody effect lies in the blockage of signal transmission from a receptor to effectors proteins situated inside a cell. In fact, monoclonal antibodies themselves often have a low therapeutic effect; hence it is beneficial to use them as transport ligands for cytotoxic substances and radionuclide, which are responsible for the cell death. The radiolabelling of monoclonal antibodies has the diagnostic effect too. The disadvantage of antibody is the size of its molecule. Whereas peptides of short peptide chain are easily distributed to the place of action, antibodies are distributed slowly and undergo faster biodegradation in the liver. The solution of this problem has its origin in the development of antibody fragments in the last years. The antibody fragments are gradually assembled on the targeted receptor protein structure. This method is termed the pre-targeting. The pre-targeting allows easy distribution in the body and decrease the risk of cytotoxic or radiological burden for patients.

The aim of the publication in chapter I was to study the potential influence of radiolabelling on the biological properties of minigastrin11 (MG11). This was performed with testing of its ability to interact with cholecystokinin receptor type 2 (CCK2r) and to internalize into pancreatic cancer cells AR42J. Minigastrin analogue was labelled with either 177Lu or 111In. The first mentioned isotope is used in therapeutic oncology and the second one for diagnostic purposes. The complex 111In-DOTA-MG11 binding on the receptor was slow and did not reach ligand – receptor association and dissociation equilibrium even after three hours of incubation. However, the complex 177Lu-DOTA-MG11 interaction on CCK2r reached the equilibrium after one hour incubation and the higher proportion of complex from the whole quantity added to cells was internalized than in the case of 111In-DOTA-MG11. The radiolabelled ligand 177Lu-DOTA-MG11 is able to effectively bind to the targeted CCK2r and has strong assumption to assert itself in Peptide receptor radionuclide therapy (PRRT). The radioligands 177Lu-DOTA-MG11 and 111In-DOTA-MG11 were investigated for their possible radionefrotoxicity, which often accompanies PRRT. The complication of damaged kidneys by radiolabelled peptides is caused with the rear tubular reabsorption, which cumulates radioactive isotopes in the proximal tubular cells. The cell line isolated from opossum kidney proximal tubular

cells (OK cells) was employed to test the possible accumulation of 111In- and 177Lu- labelled ligands. The results of the testing were satisfying, because they demonstrated no important accumulation of radioligands into kidney cells. The internalization of radiolabelled peptides reached the maximal values about 0.1 % of the total added amount.

Improving the method for the number of receptor per cell (NRPC) quantification was the target in the publication given in chapter II. The study compared the possibilities of NRPC calculation offered by the classical manual method with the possibility of NRPC quantification based on the employment of the automatic instrument LigandTracer®. This instrument allows real-time measurement of the interaction of radiolabelled ligand with targeted receptor on cell surface. The new kind of NRPC quantification was denoted as KEX method. The name comes from the basic principle of the method, which lies in kinetic extrapolation of the ligand – receptor interaction, which means the calculation of the theoretical value Bmax. The theoretical Bmax corresponds to the signal of completely saturated receptors on cell surface. The manual method belongs to the techniques of receptor quantification verified with many years of use and thus it is the golden standard of NRPC techniques. The measured values with the classical manual method and KEX technique demonstrated the compliance. The KEX technique sometimes showed slight overestimation of the calculated receptor number against the classical method. However, the KEX technique can be included among methods allowing cell receptor quantification and can be used in biological sciences or in nuclear medicine in the future. The rapid quantification of receptors and the ability binding of radiolabelled ligand to targeted cell surface protein structures can make the progress in the research of new potential therapeutic or diagnostic molecules for cancer treatment. The importance and validity of the KEX technique was repeatedly verified in another experimental study, which is summarized in the chapter V. The values of quantified number of receptors measured by the assays like the KEX technique, the classical manual method and western blotting on employed cell lines were compared in this study. The obtained results confirmed the accuracy of the KEX technique, so it can be counted in methods for NRPC quantification.

The scientific publication introduced in the chapter III was focused on the study of epidermal growth factor receptor (EGFR) affinity, which can be influenced in the present of gefitinib, the tyrosine kinase (TH) inhibitor. The finding of this study was that gefitinib could influence the formation of EGFR dimers. The formation of EGFR dimers can alter the interaction between EGFRr and its natural ligand epidermal growth factor (EGF). The prevalence of receptor monomers, homodimers and heterodimers differs among cell lines and depends on EGFR and HER2 receptor expression rate on a cell surface. EGF binds with different binding properties on monomer or dimer forms of EGFR. The conclusion of the study refers to the positive influence of gefitinib on EGFR dimers formation, which increases the receptor affinity to 125I-EGF.

The publication in the chapter IV attempted to prove the hypothesis that the receptor affinity and the kinetic reaction between ligand and its receptor differed across the cell lines. For example, the natural ligand EGF binds with different binding strength on the same receptor (EGFR) expressed in various cell lines. This finding was proved with the employment of the monoclonal antibody 131I-cetuximab attached to EGFR with different binding affinity on three used cell lines. The affinity difference factor among cell lines was about 10. The reason of the different receptor affinity to the ligand may lie in the various level of the receptor glycosylation, formation of homodimers or heterodimers and possible receptor mutation. The above mentioned options influencing the receptor affinity lead to the cases, when very good receptor binding ligand tested in vitro have very low affinity in vivo. The same findings can be in the use of therapeutic or diagnostic substances in a patient population. Some patients do not respond to a treatment. The results of successfully in vitro tested receptor ligands should not be generalized for every cell line carrying the same receptor. It should be taken into account the differentiation among cell lines. The same should be reconsidered for patient treatment, which ought to have an individual approach.

The last publication included in this thesis is in the chapter VI. It was aimed at the characterisation of radioligand properties in in vitro and in vivo conditions after its radiolabelling. The two factors influencing the radioligand character were studied like the choice of isotope and the manner of radiolabelling. The findings made for in vitro studies concluded that the chosen isotopes 131I and 177Lu and the methods of their labelling on the transportation monoclonal antibody nimotuzumab did not alter antibody internalization

characteristics into employed cell lines. However, the opposite was found for in vivo experiments which demonstrated the differences in distribution profiles depending on the methods for radiolabelling. 131I-nimotuzumab showed the lowest rate of accumulation in the liver and the longest time of blood elimination of all examined radiolabelled ligands. The antibody labelled with 177Lu via chelating compounds (DOTA, DTPA) was characterized with decreased elimination time and much more increased retention in the liver. The introduced in vivo results confirmed the influence of employed isotope and manner of its labelling on the radiolabelled antibody biological properties in the case of tested factors like blood clearance, the liver biodegradation and accumulation.