Abstrakt v anglickém jazyce

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Title of rigorosum thesis: Study of the effect of the amount of chelating agent addition on the result of monoclonal antibody conjugation

Monoclonal antibodies have become the important tool not only for the therapy, but also for the diagnosis of the infectious, autoimmune or cancer diseases in the last few decades. In addition to their own therapeutic effect, monoclonal antibodies are used as vectors for biological substances with therapeutical and diagnostic importance. Besides bacterial and plant toxins, monoclonal antibodies are also effective in their capability to deliver radionuclides to the site of their specific binding, i.e. the antigen against which they were prepared. The so called radioimmunoconjugates are then successfully used in radiodiagnostic and radiotherapeutical field. Radionuclides can be bound to antibody molecule either directly in the case of halogenation or indirectly. The latter method is the most common one due to a greater stability and suitability for more radionuclides, which can be transported by antibodies. Substances that allow the radionuclide biding to the monoclonal antibody molecule are called bifunctional chelators (BFC). The degree of BFC conjugation to an antibody molecule depends on the properties of the chelator as well as the number and accessibility of binding sites on a monoclonal antibody structure. The aim of the present experimental study was the development of an experimental procedure for the quantification of the conjugation degree of the monoclonal antibody cetuximab with the selected chelating agents DTPA, DOTA and NOTA. Furthermore, the study also aimed at the determination of the optimal substance amount ratio for antibody:chelator to achieve optimal conjugation yield.

Monoclonal antibody cetuximab was conjugated with the subsequent chelating agents DTPA, DOTA and NOTA in the following ratios: 1:1, 1:5, 1:20, 1:50 and 1:100 (cetuximab:BFC). The conjugation proceeded in $NaHCO_3$ conjugation buffer (0,1 M) for 2,5 hours at 37 °C. The determination of the conjugated monoclonal antibody

concentration was made with the Bradford Protein Assay method and the amount of the conjugated chelator on cetuximab molecule was analysed with the Arsenazo III method.

The result of the experimental work comprised the successful application and validation of Arsenazo III method for the quantification of immunoconjugation degree. Furthermore, the found results determined the effect of the used chelator quantity on the resulting degree of monoclonal antibody conjugation by selected BFCs. There were found the numbers of conjugated BFC molecules per monoclonal antibody molecule for the tested ratios 1:1, 1:5, 1:20, 1:50 and 1:100 (cetuximab:chelator) as follows 14,8, 18,7, 15,3, 25,1 and 31,3 for DTPA; 3,3, 2,9, 3,3, 5,0 and 7,7 for DOTA and 1,2, 1,5, 12,6, 34,8 and 58,8 for NOTA. Moreover, there were found the optimal substance amount ratios 1:5, 1:50 and 1:20 (cetuximab:BFC) for chelators DTPA, DOTA and NOTA respectively.

In conclusion, there was the successful application of the Arsenazo III method for the determination of the monoclonal antibody conjugation degree with BFC. The method made in triplicates for selected chelators and cetuximab:chelator ratios reproducibly quantified the amount of conjugated chelator.