AN APPROACH FOR CHEMICAL EVALUATION OF IMMUNOCONJUGATES OF "COLD" ^{177}LUTETIUM-RITUXIMAB

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Introduction

Various radiolabeled monoclonal antibodies have been developed for the treatment and diagnosis of malignancies. Rituximab is a chimeric mouse-human monoclonal antibody. Rituximab selectively binds with high affinity to the CD20 antigen (human B-lymphocyte restricted differentiation antigen, Bp35), a hydrophobic transmembrane protein, which is expressed on B-lymphocytes and on >90% of B cell non-Hodgkin's lymphomas. These properties make the CD20 receptor a suitable target for radioactive therapy. ^{177}Lu has been considered as a promising radionuclide for developing therapeutic radiopharmaceuticals, esp. radiolabeled monoclonal antibodies, owing to its suitable decay characteristics, as well as the feasibility of large-scale production in adequate specific activity and radionuclidic purity.

Methods

In order to obtain ^{177}Lu anti-CD20 radioimmunoconjugates for using in therapeutic studies, different bifunctional chelating agents-antiCD20 (rituximab) (BFCA-rituximab) were labeled by "cold" ^{177}Lu chloride for preliminary chemical characterization (Figure 1).

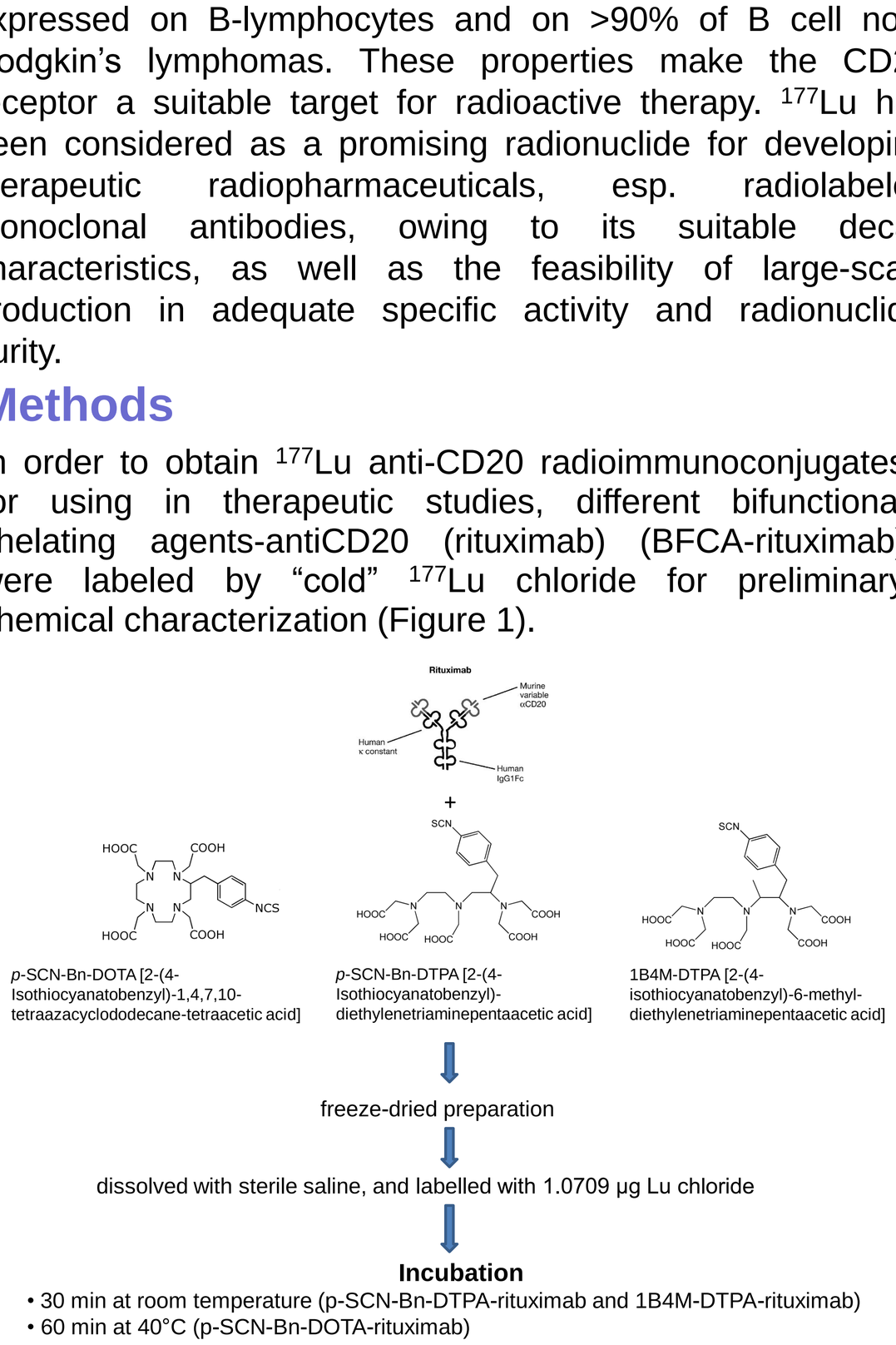
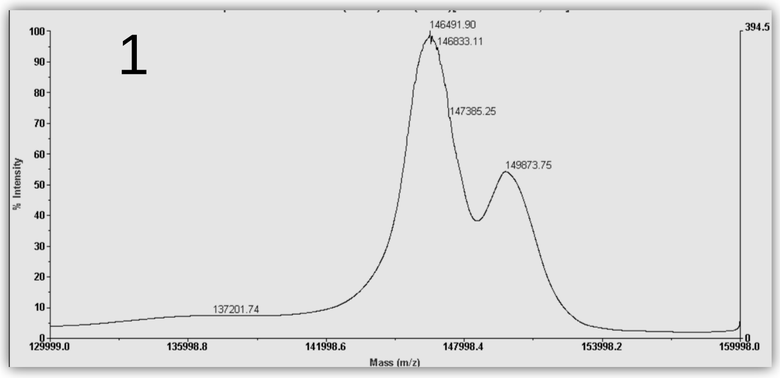


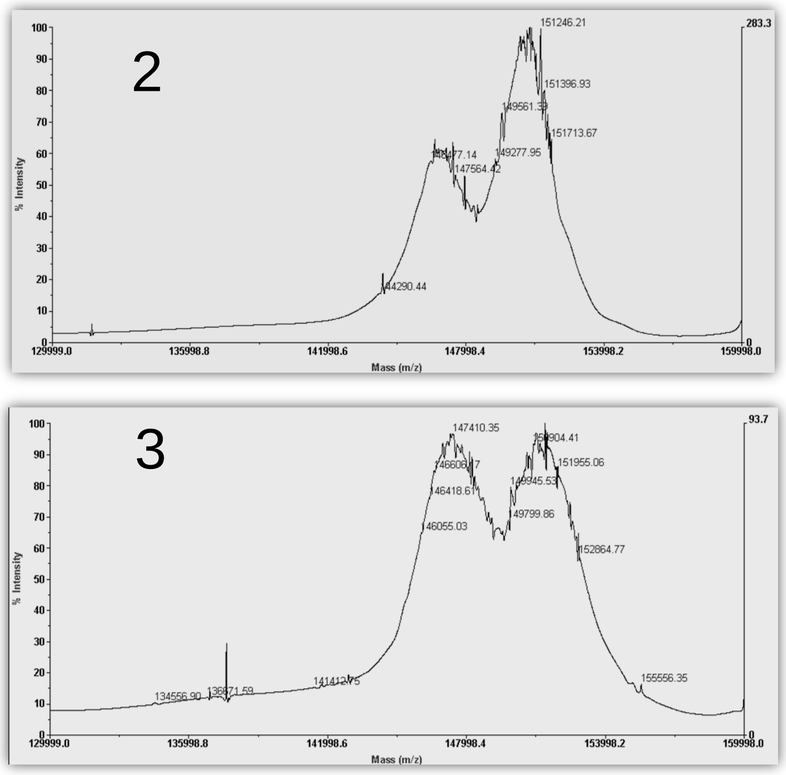
Fig. 1: Schematic process of conjugation and labeling of three BFCA-rituximab

Characterization of the conjugated BFCA-MoAb and determination of the average number of BFCA attached to each antibody molecule was performed by Matrix-Assisted Laser Desorption Ionization time-of-flight (MALDI-TOF) mass spectrometry. 10 µL of the solution of the conjugated BFCA-MoAb is diluted (1:10) with a matrix solution of 3,5-dimethoxy-4-hydroxycinnamic acid to a concentration of about 10 pmol/µL. An aliquot (1-2 µL) of the final solution is applied to the sample target prior to insertion into the high vacuum chamber of a mass spectrometer (Voyager-De MALDI-ToF, Applied Biosystems).
In order to examine relevant quality parameters, including detection, and purity assessment, SDS-PAGE experiments, under reducing conditions, were performed.

Results

MALDI-TOF experiments revealed the presence of two major peaks corresponding to a MW of conjugated and unconjugated rituximab equivalent to an average of 7.7 (p-SCN-Bn-DOTA), 11 (p-SCN-Bn-DTPA) and 9.8 (1B4M-DTPA) groups per molecule of antibody (Figure 2).
All three BFCA-rituximab conjugates (labeled and non-labeled) were resolved in two distinct Mr species which migrated in two bands (upper band at ~50 kDa and lower band ~30 kDa) confirming the migration behavior typical for IgG antibodies which are composed of two identical subunits each composed by two polypeptide chains: two heavy and two light chains, linked via disulfide bonds (Figure 3).





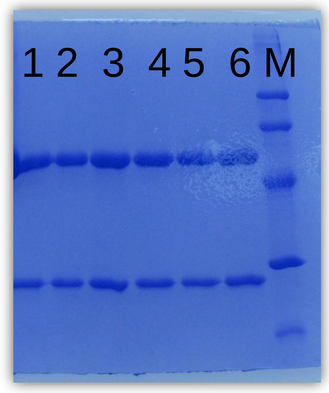


Fig. 3: SDS-PAGE paterns for three BFCA-rituximab (1: *p*-SCN-Bn-DOTA; 2: *p*-SCN-Bn-DTPA; 3: 1B4M-DTPA; 4: *p*-SCN-Bn-DOTA-Lu; 5: *p*-SCN-Bn-DTPA-Lu and 6: 1B4M-DTPA-Lu; M is molecular marker)

Fig. 2: MALDI-TOF results for three BFCA-rituximab (1: *p*-SCN-Bn-DOTA; 2: *p*-SCN-Bn-DTPA and 3: 1B4M-DTPA)

Conclusions

Results of this study showed that "cold" labeling of the lyophilized formulation (kit) of BFCA-rituximab results in immunoconjugates with good purity and stability.
Findings of this study suggest that further investigations may result in a lyophilized (kit) formulation which could be easily radiolabeled with ^{177}Lu in order to be used for radioimmunotherapy of patients with relapsed and refractory non Hodgkin's lymphoma.

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