FREEZE-DRIED KIT FORMULATIONS FOR PREPARATION OF Lu-177 CONJUGATED RITUXIMAB FOR TREATMENT OF NON-HODGKIN'S LYMPHOMA

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INTRODUCTION AND AIM

Two radiolabelled monoclonal antibodies are approved for the treatment of non-Hodgkin's lymphoma, Yttrium-90-ibritumomab tiuxetan, (Zevalin®) and Iodine-131-tositumomab (Bexxar®). In the current clinical practice, rituximab, a chimeric monoclonal antibody is approved for the treatment of low-grade or follicular, CD-20 positive non-Hodgkin's lymphoma, as a single agent or in combination with chemotherapy. The radioisotope Lu-177, has a potential to be used in a radiopharmaceutical preparation as a therapy radioisotope for labelling rituximab, mainly for its advantageous characteristics, t½= 6.71 days and the 'soft' β emmision E_β of 497, 384 and 172 keV with 78.6, 9.1, and 12.2% abundance, respectively) associated with γ emission with E_γ of 113 and 208 keV and 6.4 and 11% abundance, respectively), thus enabling targeted therapy and visualization studies at the same time. The aim of this work was to present the properties of the developed freeze-dried, ready-to-label kit formulations containing rituximab, conjugated with three different bifunctional chelating agents, p-SCN-Bn-DOTA, p-SCN-Bn-DTPA and 1B4M-DTPA.

MATERIALS AND METHODS

Immunoconjugation of purified Rituximab with the bifunctional chelating agents, p-SCN-Bn-DTPA, 1B4M-DTPA and p-SCN-Bn-DOTA was performed at 4°C for 18h. The immunoconjgates were purified using Amicon® ultrafiltration. Freeze-drying was performed in three-day protocol, using Labconco Free Zone Stoppering Tray Dryer, USA, depicted in Fig. 1. The radiolabelling was performed by dissolving the freeze-dried preparations with sterile saline, and subsequent labelling with 555GBq of Lutetium-177 in total volume of 1mL at pH 7.0, and incubation for 30 min at room temperature (p-SCN-Bn-DTPA-rituximab and 1B4M-DTPA-rituximab) and 60 min at 40°C (p-SCN-Bn-DOTA-rituximab). The analysis of radiochemical purity (RCP) was performed by SE-HPLC using isocratic method and immunoreactive fraction assay (IRF) using serial dilution of CD20-positive RAJI cells (0.5-8 million/ml).

Fig.1: Temperature variations during lyophilization of the immunoconjugates

RESULTS AND DISCUSSION

The chromatograms obtained after SE-HPLC separation with radio detection, revealed high radiochemical purity of the radioimmunoconjugates in all three examined freeze-dried kits. (Fig.2). All the RCP values extend above the criteria of radiochemical purity, over 95%.

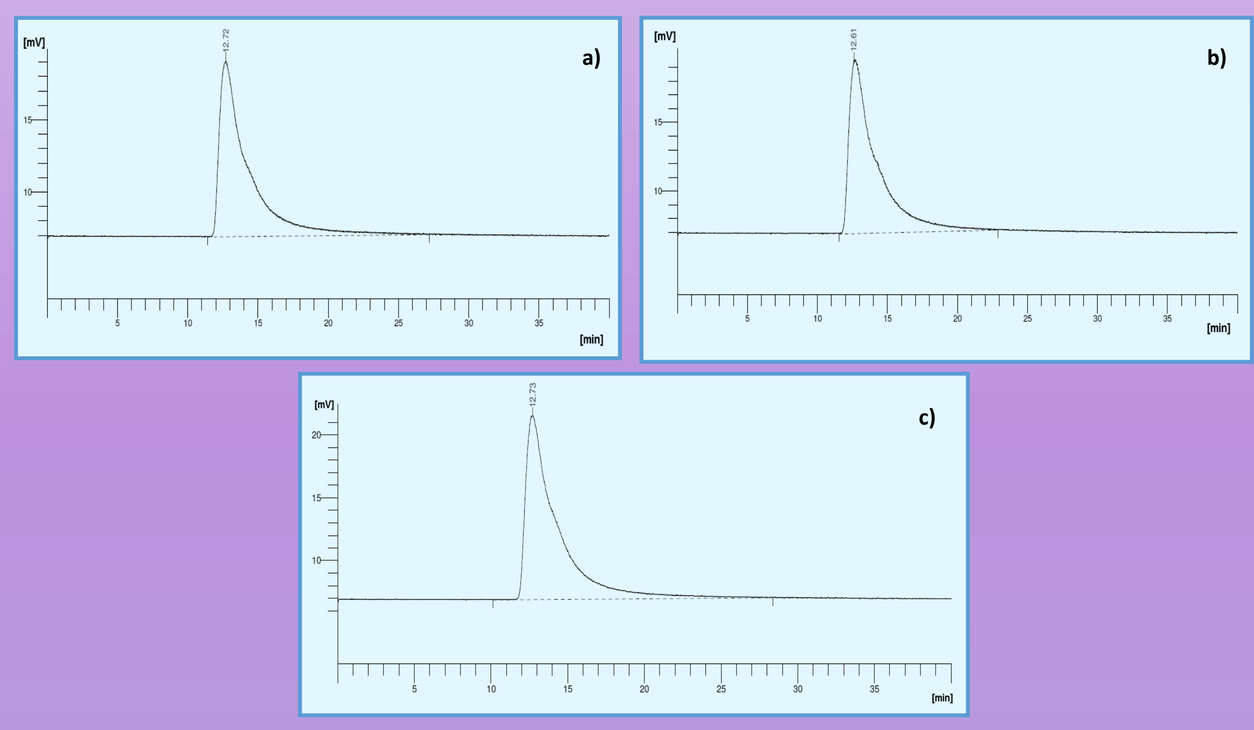

Fig.2: Representative SE-HPLC chromatograms of the radioimmunoconjugates, a)^{177}Lu-p-SCN-Bn-DTPA-rituximab; b) ^{177}Lu-1B4M-DTPA-rituximab and c) ^{177}Lu-p-SCN-Bn-DOTA-rituximab.

The immunoreactivity tested revealed 45% of immunoreactive antibody for ^{177}Lu-DTPA-rituximab and 67% for ^{177}Lu-DOTA-rituximab (Fig.3). The ^{177}Lu-1B4M-DTPA-rituximab derivative showed non-specific binding.

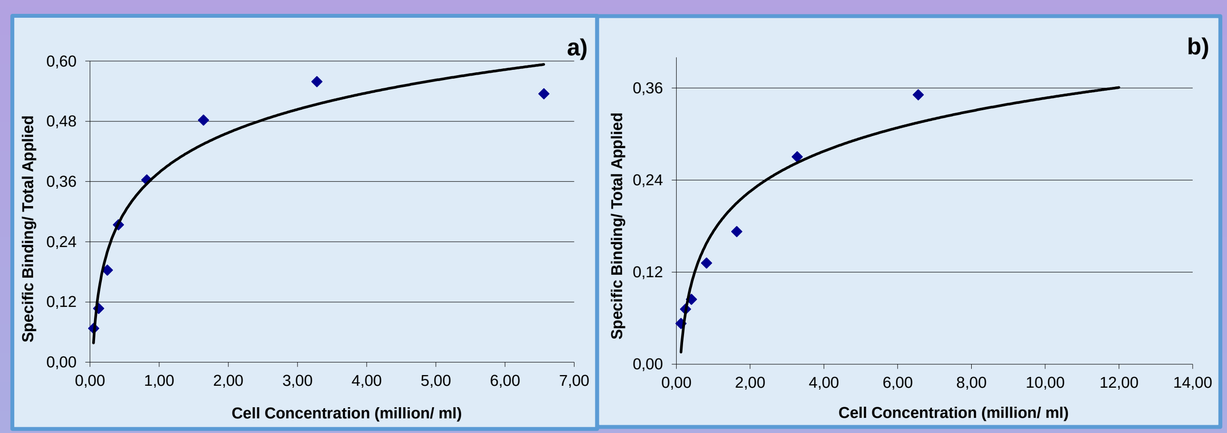

Fig.3: Specific binding over total applied radioactivity as a function of cell concentration in: a) ^{177}Lu-DOTA-rituximab and b) ^{177}Lu-DTPA-rituximab.

CONCLUSION

The obtained high RCP and maintained immunoreactivity of the tested radioimmunoconjugates confirm that the conjugation reaction and subsequent lyophilization and radiolabeling did not affect the immunoreactivity of the antibody, thus assuring binding to CD20 antigen. Further experiments are needed to reveal the non-specific binding of ^{177}Lu-1B4M-DTPA-rituximab. These results form a basis for further evaluation of these preparations in order to select a promising new radiopharmaceutical to treat non-Hodgkin's lymphoma.

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