Development of Rituximab Radioimmunoconjugates as PET-Radiopharmaceuticals

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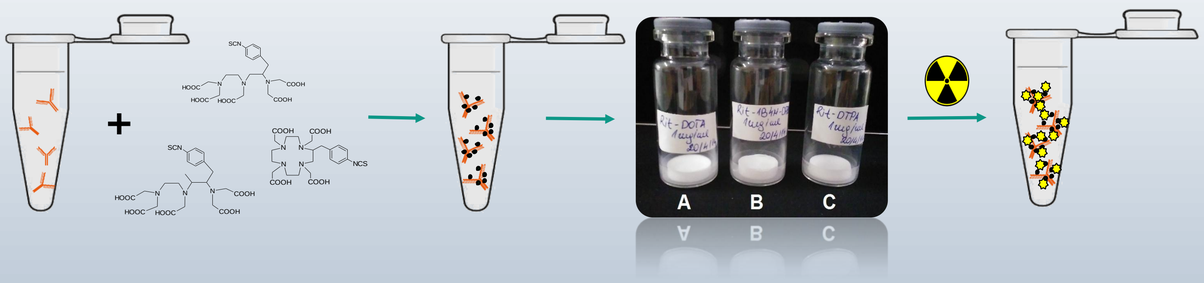
Introduction

Positron emission tomography (PET) has a growing use in medical decision making and management of patients. To further apply the unique properties of PET, more clinically validated PET-radiopharmaceuticals are required and subsequently new and better radiochemical preparation methods are under development stimulates the use of different radiometals as ^{68}Ga, ^{89}Zr and ^{64}Cu for clinical use.

Depending on the biological target of interest and the properties of the PET-radiopharmaceutical the proper radionuclide should be selected.

For the treatment and diagnosis of malignancies, various radiolabeled monoclonal antibodies have also been developed. Rituximab selectively binds with high affinity to the CD20 antigen (human B-lymphocyte restricted differentiation antigen, Bp35), which is expressed on B-lymphocytes and on >90 % of B cell non-Hodgkin lymphomas (NHL). These properties make the CD20 receptor a suitable target for radiotherapy/diagnostic purposes.

Methods



Schematic process of conjugation and labeling of three BFCA*-rituximab conjugates.
**Bifunctional Chelating Agents*

Rituximab, conjugated with three different BFCA's,

✓ ***p*-SCN-Bn-DOTA,**
(2-(4-izothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)

✓ ***p*-SCN-Bn-DTPA**
(2-(4-izothiocyanatobenzyl)-diethylenetriaminepentaacetic acid)

and

✓ **1B4M-DTPA**
(2-(4-izothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid)

in a form of freeze-dried preparation, was subjected to characterization and determination of secondary structure, quality parameters (purity, integrity, fragmentation and aggregation of the antibody), and average number of BFCA's attached with employment of different analytical techniques such as:

✓ Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS),

✓ Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE),

✓ Fourier Transform Infrared (FT-IR) and

✓ Raman Spectroscopy.

Results

After lyophilisation, diluted rituximab immunoconjugates remain stable. No modification of its physico-chemical characteristics, no aggregation, and preservation of antibody secondary structure were observed.

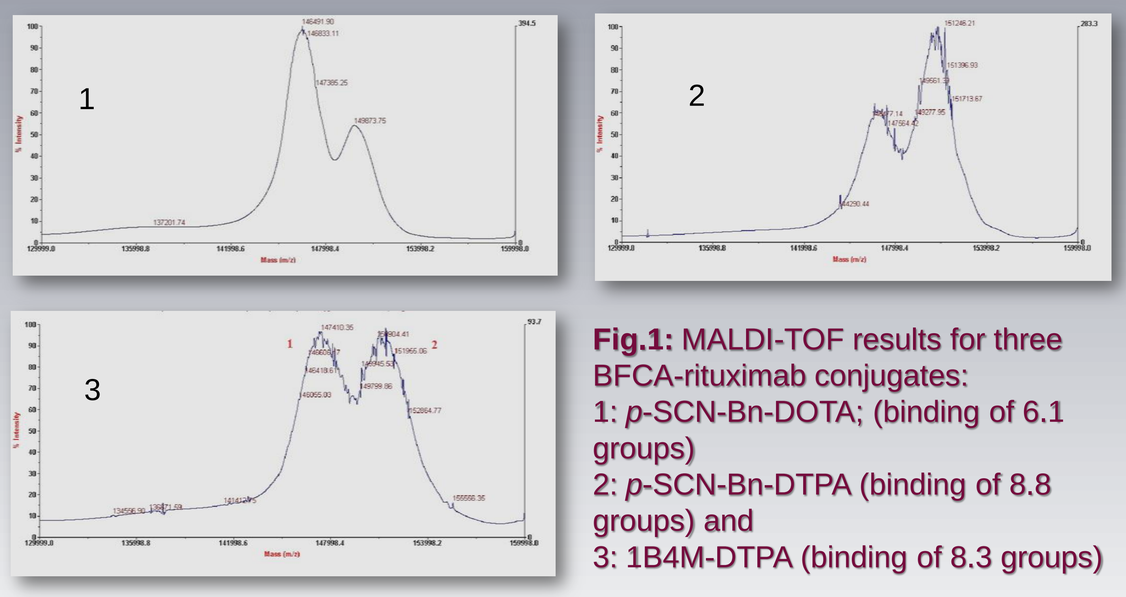


Fig.1: MALDI-TOF results for three BFCA-rituximab conjugates:
1: *p*-SCN-Bn-DOTA; (binding of 6.1 groups)
2: *p*-SCN-Bn-DTPA (binding of 8.8 groups) and
3: 1B4M-DTPA (binding of 8.3 groups)

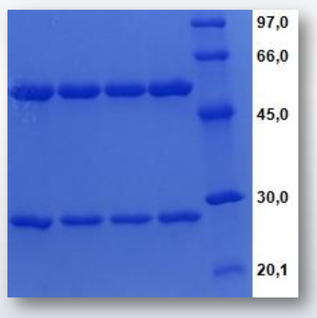


Fig. 2: Reducing SDS-PAGE lane patterns of rituximab (1), *p*-SCN-Bn-DOTA-rituximab (2), *p*-SCN-Bn-DTPA-rituximab (3), 1B4M-DTPA-rituximab (4).

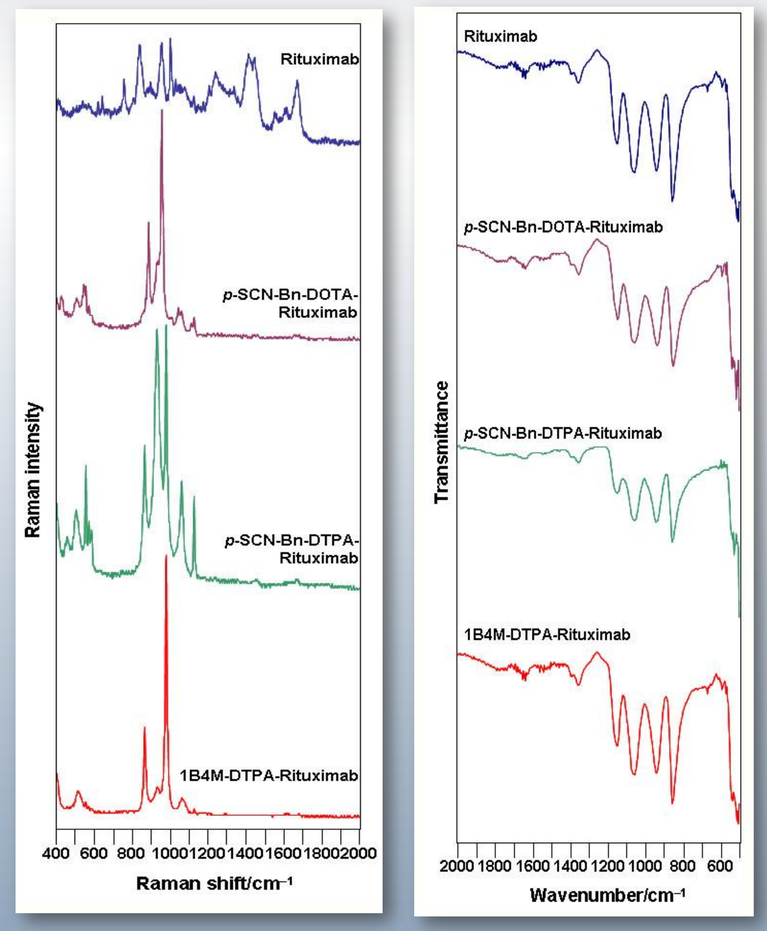


Fig. 3: Raman and ATR-IR spectra of rituximab and three types of experimental conjugates.

Conclusions

➢ The time frame for the practical use of rituximab immunoconjugates can be safely extended using lyophilisation, enabling, for example, safe and longer storage and supporting the possibility of developing a "ready-to-label" rituximab immunoconjugates for imaging/therapy.

➢ Further experiments are still needed in order to demonstrate biological and pharmacological properties.

➢ Development of ^{68}Ga-labeled rituximab immunoconjugates (in a form of lyophilised formulation) may provide PET screening of the therapeutic outcomes following ^{177}Lu therapy.

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