Chemical analysis of the rituximab radioimmunoconjugates in lyophilized formulations intended for oncological applications

Darinka Gjorgieva Ackova[1], Katarina Smilkov[1], Petre Makreski[2], Trajče Stafilov[2], Emilija Janevik-Ivanovska[1]

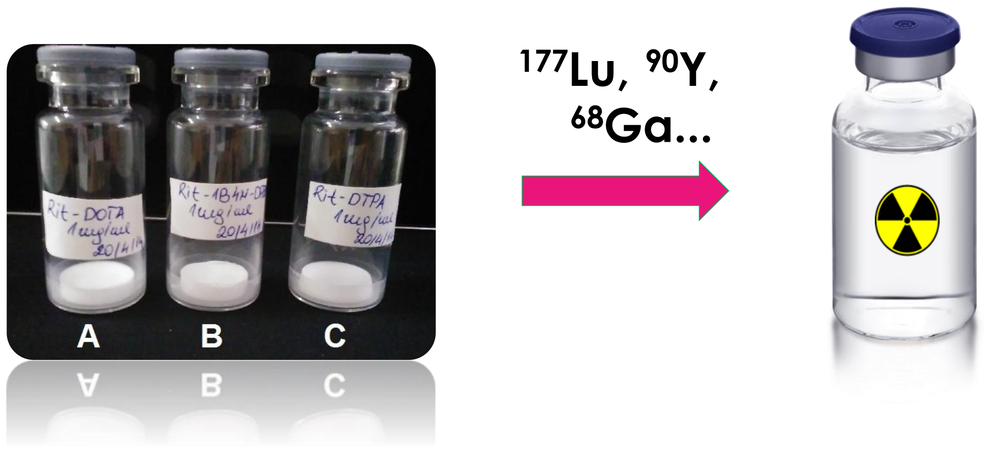
[1]Department of Pharmacy, Faculty of Medical Sciences, University Goce Delčev – Štip, R. Macedonia
[2]Department of Chemistry, Faculty of Natural Sciences and Mathematics, University "Ss. Cyril and Methodius"- Skopje, R. Macedonia

INTRODUCTION

For a protein based drugs, including antibody based radiopharmaceuticals, a structural characterization is mandatory before any possible start of a clinical trial. Vibrational spectroscopic techniques, as Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy are one of the biophysical methods for structural characterization of proteins because of their sensitivity to the composition and architecture of molecules.
Here we used vibrational spectroscopy to characterize three immunoconjugates of rituximab, intended for labeling with radioisotope of choice (^{177}Lu, ^{90}Y and/or ^{68}Ga).

MATERIALS AND METHODS

Rituximab, conjugated with three different bifunctional chelating agents (BFCAs), *p*-SCN-Bn-DOTA, *p*-SCN-Bn-DTPA and 1B4M-DTPA in a form of lyophilized preparations non-radioactive labeled with above mentioned radioisotope analogues, was subjected to characterization and determination of secondary structure and quality parameters (purity, integrity, fragmentation and aggregation of the antibody).



RESULTS

Based on the frequencies assigned for amide I, II and III bands, the studied formulations contain highest percentage of β-sheet conformation (antiparallel and parallel) in the structure, followed by a-helices. Significant changes upon processes of conjugation and lyophilization were not observed in comparison with spectra of native antibody.Vibrational spectroscopic data allow detection of alterations in investigated protein models as well as rapid assessment of conformational changes resulting from ligand binding, aggregation or macromolecular interactions. According to the obtained spectra, it is important that we observed retaining of native structure of the antibody and no obvious aggregation (the lowest band frequency detected was 1620 cm^{-1} with weak intensity) in all samples of lyophilizated conjugates.

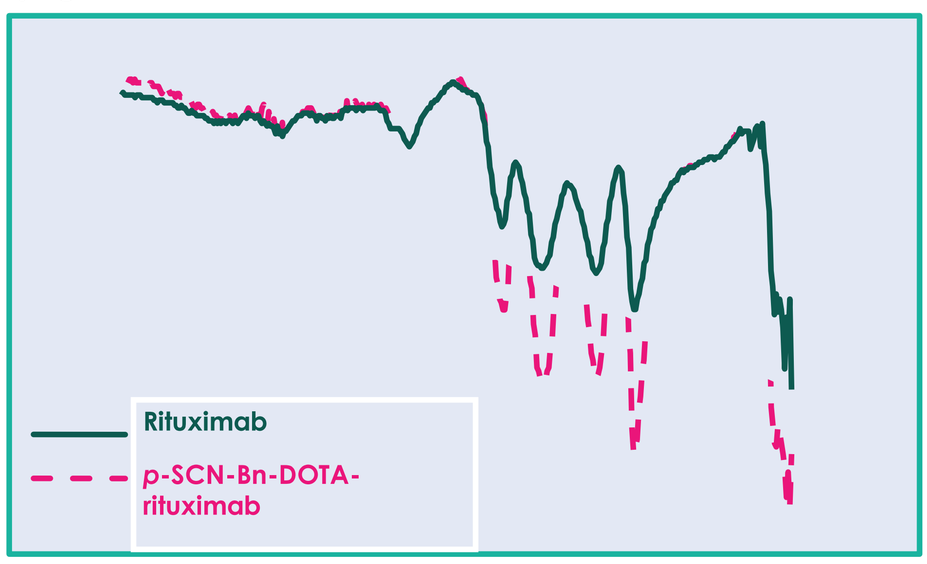


Fig.1. Comparison of ATR-IR spectra of rituximab (native antibody) and *p*-SCN-Bn-DOTA-rituximab in a form of lyophilized preparations.

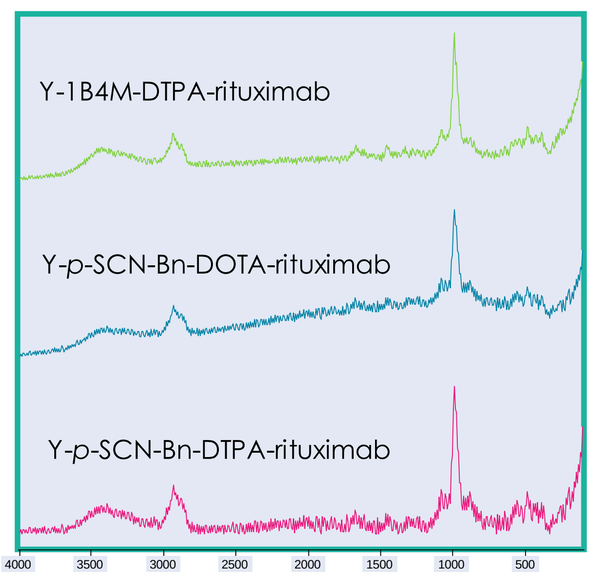


Fig.2. Raman spectra of three types of BFCA-rituximab conjugates labeled with non-radioactive Y.

CONCLUSION

We investigated the application of vibrational spectroscopy in assessment of conformational changes during stress conditions, as lyophilization and non-radioactive labeling are, using different rituximab-conjugates. The results are a good foundation for further radiolabeling studies of the lyophilized formulations for possible therapeutic application.

Acknowledgement

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