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It is our great pleasure to present this Supplement Issue on “*Macedonian Pharmaceutical Bulletin*” to the scientific and professional community. This supplement includes the short communications from the *Sixth Congress of Pharmacy in Macedonia with International participation*, as the largest gathering for the pharmacy profession held in the Republic of Macedonia. The main theme of the Congress was “Modern pharmacist - bridging science with practice”.

A broad spectrum of topics within the pharmaceutical sciences and practice carefully selected for this special occasion in order to build up a highly interesting and comprehensive program were covered. The contributions submitted to the Congress included 6 plenary lectures, 84 section lectures, and more than 240 posters. This Congress, followed the excellent international tradition, was attended by close to 1000 domestic and foreign participants. We received 326 short paper submissions from more than 25 countries. These numbers show that our Congress is aiming for the highest scientific standards, and that it can be considered a well-established venue for researchers in the broad fields of Pharmaceutical sciences and practice.

We would like to thank all internationally prominent researchers for their contribution to reinforcing the overall quality of the Congress. They give the state of the art of the recent advances in the field of pharmacy research.

Sincere thanks to the hosts of the Sixth Congress of Pharmacy in Macedonia with International participation, Macedonian Pharmaceutical Association and Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje for their vision and commitments.

We acknowledge the sponsoring companies: the platinum sponsor AD ALKALOID, Skopje, the golden sponsor PLIVA, the silver sponsor EUROFARM and the bronze sponsor SEPTIMA, for the permanent support to our efforts during the organization.

We would also like to thank our members of the Scientific Committee for their volunteer time and dedication to the critical peer review process and in the organization of the program. We also wish to thank all the members of the Organizing Committee, whose work and commitment was invaluable.

On behalf of the Advisory and Scientific Committees, we would like to especially thank the authors, whose work was the essential part of the congress and contributed to a very successful event. Besides the many academic staff and professionals who contributed to the success of the Congress, we are grateful to the students who participated with oral presentations and posters.

The pharmaceutical sciences continue to grow as dynamic scientific interdisciplinary fields. We believe that published short communications will be an excellent source of scientific material in the fast evolving fields in Pharmaceutical sciences and practice.

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Your hosts
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The present issue of *Macedonian Pharmaceutical Bulletin* is a special issue of the 6th Congress of Pharmacy in Macedonia with international participation.

This issue of *Macedonian Pharmaceutical Bulletin* contains short papers accepted by the scientific committee for the presentation at the Congress.

The authors are fully responsible for the contents of their short papers.

All reviewers that were involved in the short papers revision process are sincerely acknowledged.

Development and standardization of Rituximab-conjugates for labeling with Lutetium-177 and Yttrium-90

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Introduction

Our work was focused on the investigation for a ready to use prepared freeze dried rituximab immunoconjugates as potential radiopharmaceuticals for labeling with Lu-177 and Y-90 in order to increase the stability and higher efficiency and lower toxicity. We tested three bifunctional chelating agents (BFCA's), *p*-SCN-Bn-DOTA, *p*-SCN-Bn-DTPA and 1B4M-DTPA conjugated to the same antibody using previously established protocol for conjugation (Gjorgieva Ackova, 2014, 2015; Smilkov, 2014).

The main goal was to investigate chemical characterization of the immunoconjugates, labeled with "cold" non-radioactive isotopes of Lutetium and Yttrium in the same conditions as with radioactive Lutetium 177 and to show the chemical behavior and toxicological properties.

Material and method

The conjugation of antibody with three different bifunctional cleaving agents was performed using using previously established protocol for conjugation. The concentrations

were adjusted to 1 mg/mL and the solutions were then lyophilized.

The purified immunoconjugates were formulated in absence of any cryoprotectant at the concentration of 10 mg/mL, and subsequently lyophilized according to selected protocols.

The process of freeze drying was completed using Labconco Free Zone Stoppering Tray Dryer, (USA), using protocol described by Park in 2013, modified to our experience.

Concentration of the antibody/immunoconjugate was determinate before and after freeze drying and reconstitution using UV spectrophotometer (Jenway UV/VIS spectrophotometer 6715), and semi-micro UV polypropylene tubes with 0.1M PBS pH=8.0, at 280 nm in triplicate.

After freeze drying both characterization of the conjugates and determination of the average number of BFCA attached to each antibody molecule is performed by MALDI-TOF mass spectrometry and integrity of the antibody was evaluated using SDS-PAGE electrophoresis, on 12% bis-tris acrylamide gel.

The spectroscopic characterization of all three freeze dried immunoconjugates, in terms of monitoring the secondary protein structure (and its preservation), was achieved

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by FT-IR and Raman spectroscopy.

The freeze drying immunoconjugates after reconstitution were labeling with Lu-177 (555 MBq/mg in 0,5 M NH_4OAc) and radiochemical purity was determined by instant thin-layer chromatography on silica plates with a mobile phase of ammonium acetate : methanol (1 : 1) using Cyclone Plus Phosphore Imager (Perkin Elmer).

The obtained radioimmunoconjugates were characterized by SE-HPLC, using a Zorbax Bio Series GF-250 column and the elution process was monitored on UV detector at 280 nm and radiodetector (Wojdowska, 2014).

Toxicological studies were performed in Wistar rats after injection of rituximab labeled with cold Lutetium and Yttrium. Biodistribution studies were performed in 5-6 week old nude mice grafted with Raji cells (2×10^6 cells in 0.5 mL medium solution) after injection of radioactive Lu-177-Rituximab.

Results and discussion

The 3 day protocol of freeze drying without the presence of mannitol, showed the greatest similarity in the elution profiles of the immunoconjugate prior lyophilization (Gholipour, 2014).

After freeze drying, the pellets obtained corresponded to the composition and the time until complete reconstitution after addition of saline showed no significant difference in the time of complete dissolution of the lyophilisates, i.e. all tested samples were completely reconstituted in 2 min.

The average number of BFCA attached to each antibody molecule performed by MALDI-TOF mass spectrometry shows presence of two main peaks corresponding to MW of 146491 Da (unconjugated antibody) and 149873 Da (conjugated antibody) which corresponds to an average of 6.1 groups of *p*-SCN-Bn-DOTA ($M = 551.61 \text{ g}\cdot\text{mol}^{-1}$), two peaks also, corresponding to MW of 146477 Da (unconjugated antibody) and 151246 Da (conjugated antibody) corresponds to an average of 8.8 groups of *p*-SCN-Bn-DTPA ($M = 540.54 \text{ g}\cdot\text{mol}^{-1}$) attached to a molecule of rituximab and two peaks corresponding to MW of 146848 Da (unconjugated antibody) and 151506 Da (conjugated antibody) corresponding to average of 8.3 groups 1B4M-DTPA ($M = 555.58 \text{ g}\cdot\text{mol}^{-1}$) attached to a molecule of rituximab.

All immunoconjugates (both before and after lyophilization) were separated in two distinct Mw species which migrated in two bands (upper at $\sim 50 \text{ kDa}$ and lower at $\sim 25 \text{ kDa}$) 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 4 disulfide bonds. The obtained fragments correspond to molecular masses of rituximab heavy and light chain given at the literature (Bil, 2007;)

In the experimental IR (in the region $2000\text{-}500 \text{ cm}^{-1}$) and Raman spectra ($2000\text{-}400 \text{ cm}^{-1}$ region) we observed retaining of native structure of the antibody and no obvious aggregation.

The radiochemical purity and determination of

radioimmunoconjugates by SE-HPLC, obtained after radiolabeling the with Lu-177 was higher than 5%. These conjugates were stable for 48h in 0.9% NaCl, however, progressive aggregation was observed.

Animal studies showed no toxicity and SPECT images in mice showed good localization of the tumor, as confirmed by ex-vivo organ counting.

Conclusion

After evaluation of all the obtained results obtained we can conclude:

- Three immunoconjugates were synthesized, using *p*-SCN-Bn-DOTA, *p*-SCN-Bn-DTPA and 1B4M-DTPA using the a selected ratio, 1:20
- Protocol for lyophilization was established, yielding lyophilisates with favorable physicochemical properties.
- The non-radioactive labeling with Y and Lu showed preserved secondary structure in all three types of immunoconjugate, confirming their stability in conditions of freeze-drying and labeling
- During labeling with Lu-177 all three types of radioimmunoconjugates showed high radiochemical purity, over 95%, which was confirmed both in ITLC and SE-HPLC.

The selection of the most appropriate immunoconjugate kit suitable for labeling with Lu-177 or with Y-90 can be made after stability study of the formulation and completion of cell culture studies.

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