Impact of DOTA-Chelators on the Antitumor Activity of ^{177}Lu -DOTA-Rituximab Preparations in Lymphoma Tumor-Bearing Mice

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Abstract

Background: This work aimed to evaluate the influence of two chelators: DOTA(SCN) and DOTA(NHS) on radioimmunotherapy using ^{177}Lu -DOTA-Rituximab preparations in murine lymphoma xenograft models. Subsequently, based on animal data, the organ radiation-absorbed doses were extrapolated to humans (adult male).

Materials and Methods: Therapeutic efficacy of ^{177}Lu -DOTA-Rituximab was evaluated in male nude mice bearing either Raji (B lymphocyte, CD20⁺) and Jurkat (T lymphocyte, CD20) xenografts, utilizing an anti-CD20 antibody—Rituximab conjugate with either DOTA(SCN) or DOTA(NHS). The DOTA-Rituximab conjugates were prepared in the form of freeze-dried kits.

Results: All radioimmunoconjugates were obtained with high radiolabeling yield (radiochemical purity, RCP > 95%) and specific activity of ca. 0.5 GBq/mg. Therapeutic effects of ^{177}Lu -DOTA-Rituximab were observed in animals regardless whether DOTA(SCN) or DOTA(NHS) were used for conjugation. Importantly, therapy involving ^{177}Lu -DOTA-Rituximab was more effective than use of Rituximab alone.

Conclusions: The degree of antitumor efficacy was dependent on the type of applied bifunctional chelators conjugated to mAb. However, this difference was not statistically significant. Dosimetry calculations showed that the absorbed radiation doses extrapolated to humans were very low for osteogenic cells regardless of the conjugates. Organs like the liver and spleen, treated with ^{177}Lu -DOTA(SCN)-Rituximab, showed similar radiation absorbed doses when compared with ^{177}Lu -DOTA(NHS)-Rituximab.

Keywords: anti-CD20, ^{177}Lu , radiolabeling, Rituximab, DOTA chelator, animal study

Introduction

Although antibodies with a very high specificity for their target antigen overexpressed in tumors can display a direct therapeutic effect, their effectiveness significantly increases when combined with therapeutic radionuclides. One of the first mAb implemented in oncology, and still the most widely used, is the CD20-targeting mAb rituximab (the chimeric mouse/human monoclonal antibody). Contrary to other drugs and toxins, immunoconjugates of radiolabeled mAbs

can kill cells that are at a distance from the targeting site, depending on the choice of radionuclide, without the mAb conjugate being internalized. Preclinical and clinical applications of ^{131}I -, $^{99\text{m}}\text{Tc}$ -, ^{177}Lu -labeled,³ or ^{90}Y -^{4–6} antiCD20 have been previously reported.^{7–12} Other radionuclides used for radiolabeling of antibodies for tumor imaging or therapy were ^{64}Cu , ^{111}In , ^{89}Zr , ^{153}Sm , ^{166}Ho , and ^{188}Re .

Radiolabeling antibodies with radiometals typically involves the use of a bifunctional chelating (BFC) agent, which is able to stably coordinate the isotope and also

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allows for covalent attachment to protein functional groups. Such radioimmunoconjugates bind to the CD20 antigen, a transmembrane phosphorylated protein located on pre-B and matured B-lymphocytes, and destroy tumor cells, by combining radiolytic and biological mechanisms of action.^{13–15}

Several reports have described the radiolabeling of anti-CD20 with ¹⁷⁷Lu and ⁹⁰Y after conjugation with DOTA or DTPA because they form very stable complexes.^{16,17} However, chemical modification of antibodies may alter their properties, either chemically or biologically, for example, immunoreactivity. Based on the recent experiments,¹⁸ this article focuses on the comparisons of the influence of two DOTA chelators (p-SCN-Bn-DOTA and DOTA-NHS-ester) conjugated to Rituximab, on antitumor activity of the ¹⁷⁷Lu-radiolabeled preparations. Radiotherapeutic efficacy of ¹⁷⁷Lu-DOTA-Rituximab preparations were assessed in lymphoma tumor-bearing mice.

Additionally, to evaluate potential for therapeutic application, the absorbed radiation dosage was extrapolated to humans using the OLINDA software—Organ Level Internal Dose Assessment Code.

Materials and Methods

Antibody and reagents

Rituximab (MabThera[®]) was purchased from Roche Pharma AG (Germany). The BFC: p-SCN-Bn-DOTA (2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and DOTA-NHS-ester (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono (*N*-hydroxysuccinimide ester) were purchased from Macrocyclics. Lutetium-177 (LutaPol) of specific activity (SA) higher than 555 MBq/mg Lu was produced at the Radioisotope Centre POLATOM (Poland). Chemicals and solvents were of p.a. grade.

Immunoconjugates preparation and radiolabeling

¹⁷⁷Lu-DOTA(SCN)-Rituximab and ¹⁷⁷Lu-DOTA(NHS)-Rituximab immunoconjugates were prepared as previously described.¹⁸ The specific activities of the radioimmunoconjugates were ca. 0.5 GBq/mg. The final radiochemical purity was more than 97% measured by ITLC SG (stationary phase: silica gel; mobile phase: methanol/0.4 M ammonium acetate [1:1, v/v]) and HPLC (BioSep-SEC-S3000 PEEK column; 0.1 M phosphate buffer pH 5.8 as an eluent; 1 mL/min flow rate). Due to a high radiochemical purity (RCP) of the obtained radioimmunoconjugates, no additional purification steps were necessary.

Cell lines

Two human leukemia cell lines: Raji (B lymphocyte, CD20⁺) and Jurkat (T lymphocyte, CD20⁻), were purchased from American Type Culture Collection (ATCC). The cells were maintained in RPMI 1640 culture medium supplemented with 10% heat inactivated fetal bovine serum (ThermoScientific), 1% L-glutamine (Gibco), and 1% antibiotics (penicillin and streptomycin, ATCC) in 5% CO₂ humidified incubator at 37°C. Cell viability and cell count were determined by trypan blue assay using the automated cell counter Countess[®].

Animal studies

Animal studies were approved by the Hungarian Institutional Ethics Committee (IKEB) (No.: PEI/001/2073-6/) and were performed at the National Research Directorate on Radiobiology and Radiohygiene in Budapest. In accordance with the nomenclature on the Charles River pages Nude BALB/c mice after implementation into the right and left flank of 1×10^6 of Raji cells and the same number of Jurkat cells as the control, respectively. Twenty-one days postimplantation of cells when the average tumor size achieved $>40 \text{ mm}^3$ mice were randomly divided into five groups ($n = 5$ for each group).

Antitumor activities were investigated in the following groups:

- Group (1) received a single administration of ¹⁷⁷Lu-DOTA (SCN)-Rituximab (15 MBq/kg)
- Group (2) received a single administration of ¹⁷⁷Lu-DOTA (SCN)-Rituximab (30 MBq/kg)
- Group (3) received a single administration of ¹⁷⁷Lu-DOTA (NHS)-Rituximab (15 MBq/kg)
- Group (4) received a single administration of ¹⁷⁷Lu-DOTA (NHS)-Rituximab (30 MBq/kg)
- Group (5) received a single administration of cold Rituximab (control group).

In each case the amount of injected antibody was ca. 0.01 mg. To monitor the tumor response, animals were weighed twice per week, hematological parameters and tumor volumes were measured weekly for 8 weeks. Tumor volumes were measured using calipers, and approximated using the ellipsoid formula: $V (\text{mm}^3) = \pi/6 \times \text{length} \times \text{width} \times \text{height}$.¹⁹ The antitumor activity was evaluated by growth delay factor (GDF), defined as the mean number of tumor-doubling time (TD) gained by the treatment, and by calculating the T/C values defined as the ratio of the relative tumor volumes of treated mice (RTV_{tr}) to that of control mice (RTV_{con}).

Extrapolation of absorbed dose to human

The dose extrapolation to humans involved scaling of the biodistributions and the subsequent calculation of the absorbed radiation dose from the observed data. The biodistribution scaling was based on a method considered a relative mass scaling where the SA within a certain human organ is equal to the SA within the same mouse organ multiplied by the ratio of the body mass of human to mouse.^{20,21}

The biodistribution data of ¹⁷⁷Lu-DOTA(SCN)-Rituximab and ¹⁷⁷Lu-DOTA(NHS)-Rituximab in tumor-bearing (grafted with Raji cells) male Rj: NMRI-Foxn1nu/Foxn1nu mice (Janvier Lab., France) were used for dosimetry calculations.³ Radiation doses for a selected group of organs were calculated using the OLINDA/EXM[®] software for internal dose assessment presented as an accurate and standardized method for the calculation of radiation (version 1.1, copyright Vanderbilt University, 2007).

Statistical analyses

Statistical analysis included the calculation of averages and standard deviation of the results compared using the

FIG. 1. Changes of tumor growths after ^{177}Lu -DOTA-Rituximab treatment in mice grafted Raji cells.

Student's *t*-test. The calculations were performed using the GraphPad Prism 8 software.

Results and Discussion

Conjugation of chelating agents to anti-CD20 and radiolabeling

The DOTA derivatives were conjugated to Rituximab at different molar ratios: 10:1 for DOTA(SCN) and 100:1 for DOTA(NHS), which resulted in different numbers of conjugated chelator molecules per mAb. The average number of DOTA molecules attached to the antibody molecule was determined by radiolabeling assay using ^{64}Cu .¹⁸ Literature data indicate that a small initial quantity of DOTA(NHS) used for the reaction resulted in a low radiochemical yield due to the insufficient number of DOTA attached to mAb molecule.²² Based on this observation, two immunoconjugates were selected for further investigation: (1) DOTA(SCN)-Rituximab (with 5 mole of DOTA coupled to 1 mole of mAb) and (2) DOTA(NHS)-Rituximab (with 18 mole of DOTA coupled to 1

mole of mAb). The DOTA-Rituximab conjugates were radiolabeled with ^{177}Lu to SA of ca. 0.5 GBq/mg and with radiochemical yields ranging from 97% to 100%.

Radioimmunotherapy studies in double xenografted nude mice

After the tumors developed at 3–4 weeks, mice with a mean tumor volume of $42 \pm 6 \text{ mm}^3$ (with Raji cell) and $49 \pm 8 \text{ mm}^3$ (with Jurkat cells) were treated in five groups as described earlier. The effect of treatment with ^{177}Lu -DOTA(SCN)-Rituximab and ^{177}Lu -DOTA(NHS)-Rituximab on tumor growth in athymic nude BALB/c mice with Raji and Jurkat xenografts are shown in Figures 1 and 2. The tumor size (for Raji and Jurkat) significantly increased in the group treated with the native antibody (control) in comparison to the groups treated with ^{177}Lu -Rituximab. In the group with the tumor-bearing Raji mice, both, ^{177}Lu -DOTA(SCN)-Rituximab and ^{177}Lu -DOTA(NHS)-Rituximab preparations revealed inhibiting activity on the tumor growth and size, in

FIG. 2. Changes of tumor growths after ^{177}Lu -DOTA-Rituximab treatment in mice grafted Jurkat cells.

TABLE 1. SUMMARY OF THE ANTITUMOR ACTIVITY OF THE TESTED DOTA-RITUXIMAB COMPLEXES OBSERVED IN MICE XENOGRAFTED WITH RAJI CELL

Treatment group (Raji)	TD (95% CI of TD)	GDF	T/C	Weeks of significant T/C observed
Rituximab (control)	9.8 d (9.2–10.5)	—	—	
¹⁷⁷ Lu-DOTA(SCN)-Rituximab 15 MBq	11.4 d (10.1–12.8)	0.16	0.80–0.62 ($p < 0.01$)	6–8
¹⁷⁷ Lu-DOTA(NHS)-Rituximab 15 MBq	11.6 d (10.4–13.1)	0.19	0.78–0.59 ($p < 0.01$)	6–8
¹⁷⁷ Lu-DOTA(SCN)-Rituximab 30 MBq	11.5 d (10.8–12.2)	0.17	0.81–0.57 ($p < 0.001$)	6–8
¹⁷⁷ Lu-DOTA(NHS)-Rituximab 30 MBq	11.9 d (10.9–13.1)	0.22	0.79–0.53 ($p < 0.001$)	6–8

CI, confidence interval; GDF, growth delay factor; T/C, ratio of the relative tumor volumes of treated mice (RTV_{tr}) to that of control mice (RTV_{con}); TD, tumor-doubling time.

TABLE 2. SUMMARY OF THE ANTITUMOR ACTIVITY OF THE TESTED DOTA-RITUXIMAB COMPLEXES OBSERVED IN MICE XENOGRAFTED WITH JURKAT CELL

Treatment group (Jurkat)	TD (95% CI of TD)	GDF	T/C	Weeks of significant T/C observed
Rituximab (control)	9.2 d (8.7–9.7)	—	—	
¹⁷⁷ Lu-DOTA(SCN)-Rituximab 15 MBq	10.3 d (9.3–11.5)	0.13	0.74 ($p = 0.006$)	8
¹⁷⁷ Lu-DOTA(NHS)-Rituximab 15 MBq	10.3 d (9.3–11.5)	0.13	0.74 ($p = 0.006$)	8
¹⁷⁷ Lu-DOTA(SCN)-Rituximab 30 MBq	10.7 d (9.8–11.6)	0.17	0.73 ($p = 0.044$)	8
¹⁷⁷ Lu-DOTA(NHS)-Rituximab 30 MBq	10.6 d (9.8–11.5)	0.16	0.73 ($p = 0.0038$)	8

CI, confidence interval; GDF, growth delay factor; T/C, ratio of the relative tumor volumes of treated mice (RTV_{tr}) to that of control mice (RTV_{con}); TD, tumor-doubling time.

comparison to the control group treated with the native antibody (Table 1).

However, the rate of tumor growths in the group treated with ¹⁷⁷Lu-DOTA(NHS)-Rituximab was slightly lower in comparison to ¹⁷⁷Lu-DOTA(SCN)-Rituximab, but the difference was not statistically significant. The most intensive antitumor activity was observed for the ¹⁷⁷Lu-DOTA(NHS)-Rituximab, showing an activity of 30 MBq/kg. The tumor GDF was 0.22, whereas for the group treated with ¹⁷⁷Lu-DOTA(SCN)-Rituximab it was 0.16.

In the group with the Jurkat tumor-bearing mice, no statistically significant inhibition of tumor growth was observed in the treated groups in comparison with the control group. Negative control (Jurkat cells) showed a higher T/C value 0.73–0.74 (at week 8 of the experiment) and a lower TD, which means faster tumor growth comparing to tumors growth induced by Raji cells in the animals treated with the same preparation (Table 2). Minor differences may be due to the presence of the radioisotope (Fig. 2). It is also possible that the observed low decrease of tumor growth was caused by appearing uncontrolled focal of fragile tumor blood vessels, leading to passive transport of ¹⁷⁷Lu-DOTA-Rituximab.

Consistent with our previous results,¹⁸ we found that the DOTA chelators coupled to mAb molecule influenced the stability of radiolabeled complexes. It has been shown that DOTA-NHS-ester demonstrated slightly improved *in vivo* stability and caused a lower aggregation of mAb compared to p-SCN-Bn-DOTA. In the present study we observed a significant difference in the increase of the GDF for the DOTA(NHS) in comparison to DOTA(SCN) Rituximab conjugates [the difference between the overall GDFs is ca. 20% higher for DOTA(NHS)].

The hematological and biochemical parameters remained within the normal range in all animals. Only small, non-significant, alterations were observed in WBC, RBC, and platelet counts after radioimmunoconjugate administration.

TABLE 3. EXTRAPOLATED ABSORBED DOSE IN ADULT MAN (MGY/MBQ)

Target organ	¹⁷⁷ Lu-DOTA(SCN)-Rituximab	¹⁷⁷ Lu-DOTA(NHS)-Rituximab
Adrenals	0.029	0.030
Brain	0.018	0.020
Breasts	0.017	0.018
Gallbladder wall	0.030	0.030
Lower large intestine wall	0.238	0.320
Small intestine	0.021	0.022
Stomach wall	0.118	0.125
Upper large intestine wall	0.021	0.022
Heart wall	0.647	0.541
Kidneys	0.321	0.316
Liver	0.636	0.567
Lungs	0.361	0.378
Muscle	0.068	0.068
Ovaries	0.020	0.022
Pancreas	0.029	0.030
Red marrow	0.013	0.023
Osteogenic cells	0.206	0.282
Skin	0.016	0.017
Spleen	1.220	1.400
Testes	0.017	0.017
Thymus	0.021	0.021
Thyroid	0.019	0.020
Urinary bladder wall	0.018	0.019
Uterus	0.019	0.020
Total body	0.119	0.135
Effective dose equivalent (mSv/MBq)	2.43E-01	2.54E-01

Bold values indicate statistical significance.

Dosimetry

Radiation absorbed dose to critical organs or tissue usually limits the amount of radioactivity that can be safely administered in radiotherapy. Most often the organs critically affected by radiolabeled antibodies are the liver, spleen, bone, and/or red marrow.

In this study, unsurprisingly it was noted (Table 3) that the organs/tissues receiving the highest absorbed dose from ^{177}Lu -DOTA-Rituximab were the spleen [1.22 mGy/MBq for ^{177}Lu -DOTA(SCN)-Rituximab and 1.40 mGy/MBq for ^{177}Lu -DOTA(NHS)-Rituximab] and the liver [0.64 mGy/MBq for ^{177}Lu -DOTA(SCN)-Rituximab and 0.57 mGy/MBq for ^{177}Lu -DOTA(NHS)-Rituximab]. We observed very low absorbed radiation doses in osteogenic cells [0.21 mGy/MBq for ^{177}Lu -DOTA(SCN)-Rituximab and 0.28 mGy/MBq for ^{177}Lu -DOTA(NHS)-Rituximab] and in red marrow [0.01 mGy/MBq for ^{177}Lu -DOTA(SCN)-Rituximab and 0.02 mGy/MBq for ^{177}Lu -DOTA(NHS)-Rituximab].

Conclusions

The evaluation of the therapeutic efficacy of ^{177}Lu -DOTA-Rituximab in murine lymphoma xenograft models confirmed that both immunoconjugates ^{177}Lu -DOTA(NHS)-Rituximab and ^{177}Lu -DOTA(SCN)-Rituximab demonstrate *in vivo* anticancer activity inhibiting tumor growth. This antitumor efficacy was dependent on the type of conjugated bifunctional chelators. The study pointed out that ^{177}Lu -DOTA(NHS)-Rituximab was slightly more effective in suppressing the growth of tumor in athymic nude BALB/c mice.

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References

- Behr TM, Wormann B, Gramatzki M, et al. Low versus high-dose radioimmunotherapy with humanized anti-CD20 or chimeric anti-CD20 antibodies in a broad spectrum of B cell-associated malignancies. *Clin Cancer Res* 1999;5:3304.
- Malvija G, Anzola KL, Podesta E, et al. 99mTc-labeled Rituximab for imaging B lymphocyte infiltration in inflammatory autoimmune disease patients. *Mol Imaging Biol* 2011;14:637.
- Karczmarczyk U, Wojdowska W, Mikołajczak R, et al. Influence of DOTA chelators on radiochemical purity and biodistribution of ^{177}Lu - and ^{90}Y -Rituximab in Xenografted Mice. *Iran J Pharm Res* 2018;17:1201.
- Knox SJ, Goris ML, Trisler K, et al. Yttrium-90 labeled anti-CD20 monoclonal antibody therapy of recurrent B-cell lymphoma. *Clin Cancer Res* 1996;2:457.

- Gholipour N, Jalilian AR, Khalaj A, et al. Preparation and radiolabeling of a lyophilized (kit) formulation of DOTA-Rituximab with ^{90}Y and ^{111}In for domestic radioimmunotherapy and radioscinigraphy of Non-Hodgkin's Lymphoma. *DARU J Pharm Sci* 2014;22:1.
- Forrer F, Oechsle-Oberholzer C, Campana B, et al. Radioimmunotherapy with ^{177}Lu -DOTA-Rituximab: Final results of the phase I/II study in 31 patients with relapsing follicular, mantle cell, and other indolent B-cell lymphomas. *J Nucl Med* 2013;54:1045.
- Boguradzki P, The use of monoclonal antibody in treatment of indolent non-Hodgkin's lymphoma. *Contemp Oncol* 2001;5:156.
- Kitson SL, Cuccurullo V, Moody TS, et al. Radionuclide antibody-conjugates, a targeted therapy towards cancer. *Curr Radiopharm* 2013;6:57.
- Ligiero ThB, de Souza Albernaz M, de Carvalho SM, et al. Monoclonal antibodies: Application in radiopharmacy. *Curr Radiopharm* 2013;6:231.
- DeNardo GL. Treatment of non-Hodgkin's lymphoma (NHL) with radiolabeled antibodies (mAbs). *Semin Nucl Med* 2005;35:202.
- Witzig TE, Gordon LI, Cabanillas F, et al. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *J Clin Oncol* 2002;20:2453.
- Davis TA, Kaminski MS, Leonard JP, et al. The radioisotope contributes significantly to the activity of radioimmunotherapy. *Clin Cancer Res* 2004;10:7792.
- Gause A, Berek C. The role of B cells in the pathogenesis of rheumatoid arthritis. Potential implication for treatment. *BioDrugs* 2001;15:73.
- Wache A, Gil L, Komarnicki M. Rituximab in haematology and oncology in 10 years of experience. *Contemp Oncol* 2008;12:173.
- Bil J, Winiarska M. Molecular mechanisms of the therapeutic activity of Rituximab. The monoclonal antibody against CD20 antigen. *Adv Cell Biol* 2007;34:335.
- Brouwers AH, van Eerd JEM, Frielink C, et al. Optimization of radioimmunotherapy of renal cell carcinoma: Labeling of monoclonal antibody cG250 with ^{131}I , ^{90}Y , ^{177}Lu or ^{186}Re . *J Nucl Med* 2004;45:327.
- Perico ME, Chinol M, Nacca A, et al. The humoral immune response to macrocyclic chelating agent DOTA depends on the carrier molecule. *J Nucl Med* 2001;42:1697.
- Wojdowska W, Karczmarczyk U, Maurin M, et al. Standardization of procedures for the preparation of ^{177}Lu and ^{90}Y -labeled DOTA-Rituximab based on the freeze-dried kit formulation. *Curr Radiopharm* 2015;8:62.
- Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. *Cancer Chemother Pharmacol* 1989;24:148.
- Radiation dose assessment resource (RADAR)—Available phantoms. Online document at www.doseinfo-radar.com/RADARphan.html Accessed November 7, 2019.
- Vakili A, Jalilian AR, Ghazi-Zahedi M, et al. Evaluation and comparison of human absorbed dose of ^{90}Y -DOTA-Cetuximab in various age groups based on distribution data in rats. *J Med Phys* 2012;37:226.
- Massicano AVF, Pujatti PB, Alcarde LF, et al. Development and biological studies of ^{177}Lu -DOTA-rituximab for the treatment of non-Hodgkin's lymphoma. *Curr Radiopharm* 2016;9:54.