

Scientific Session-8 Radioimmunotherapy

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Radioimmunotherapy of Lymphoma

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Low Grade B cell non-Hodgkin's lymphoma [NHL] presents a worthwhile target for radiolabeled antibody therapy. Although most patients respond to initial course of chemotherapy, the chemotherapy is unpleasant and relapse is frequent. NHL regularly expresses an epitope CD-20 which is not expressed on precursor stem cells or "descendant" plasma cells.

Two versions of anti-CD20 antibody [Bexxar® and Zevalin®] have been demonstrated to be effective and are approved for clinical use in the United States and Canada. Zevalin® is a combination of the non-radiolabeled antibody rituximab and ⁹⁰Y-labeled ibritumomab. It is now available also in Western Europe and other trans-Atlantic locations whereas Bexxar®, a combination of unlabeled and ¹³¹I-labeled tositumomab, is not yet available beyond North America.

At the present time, there are no publications directly comparing the efficacy of the two antibodies. Both antibodies were initially approved for use in NHL patients with low grade follicular lymphoma who were resistant to rituximab or had relapsed following rituximab. In separate, non-randomized trials following relapse after chemotherapy and/or rituximab, Overall Response Rates of 65-80% were observed. The duration of response for Bexxar® appears to be longer than following Zevalin®. The earliest trials involved patients who failed chemotherapy at least twice. Subsequently, patients have been treated with these radiolabeled antibodies earlier in the course of their disease with even better results. There are small numbers of patients who received this therapy as part of their initial treatment. Recently, Zevalin® has been shown to prolong the time to progression when used as "consolidation" therapy following the frequently used chemotherapy regimen CHOP. Several clinical trials are underway assessing the efficacy of one or the other agent in patients with other than follicular NHL and with various combinations of chemotherapeutic agents.

Both therapeutic regimen include pre-treatment one week earlier with a full dose of the respective "cold" antibody. Bexxar® use also requires a 5-6 mCi dose of ¹³¹I-labeled tositumomab in order to determine the Residence Time of the labeled antibody. Residence time determination is necessary because the treatment dose is calculated based on the whole body Radiation Absorbed Dose [75 cGy] for

patients with platelets greater than 150,000. The treatment dose for Zevalin® is based on weight [0.4 mCi/kg] for patient with platelets greater than 150,000. In both instances, patients with platelet counts below 150,000 but greater than 100,000 receive an attenuated dose [65 cGy and 0.3 mCi/Kg respectively]. Patients are required to have less than 25% bone marrow involvement based on a recent [within 6-8 weeks] bone marrow aspirate. In the United States, Zevalin® use currently requires administration of 5-6 mCi of ¹¹¹In-labeled ibritumomab followed by whole body imaging at 24-48 hours to confirm normal biodistribution.

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Radioimmunoscinigraphy and Radioimmunotherapy: Summary of 20 years of experience and the state of the art"

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Abstract not available

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RADIOIMMUNOTHERAPY OF PROSTATE CARCINOMA

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While several antibodies have been assessed for the radioimmunotherapy of prostate carcinoma, our group has had considerable experience with a "humanized" version of J591. J591 is a monoclonal antiPSMA antibody that recognizes the external epitope of PSMA and is subsequently internalized. Accordingly, pre-clinical and clinical trials of the radiometals ¹⁷⁷Lu and ⁹⁰Y were performed to assess therapeutic efficacy for the treatment of Castrate Resistant prostate carcinoma. Based upon the physical properties such as short range to allow higher doses to tumor and less to marrow, ¹⁷⁷Lu was selected for further evaluation even though it may be suboptimal for bulky tumors. A phase II trial (15 pts @ 65 mCi/m²; 17 pts @ 70 mCi/m²) showed significant efficacy. Analysis reveals a dose-response effect and imaging data may predict response. Based upon these favorable results, 4 additional trials have been initiated.

Methods:

1. An expansion trial at 70 mCi/m² is underway to further investigate variables such as the degree of ¹¹¹In-DOTA-J591 localization prior to administration of the therapeutic dose.

2. Since dose fractionation may decrease toxicity while maintaining or increasing efficacy, a phase I dose study is underway with cohorts of 3-6 pts receiving 2 escalating doses of ¹⁷⁷Lu-J591 2 weeks apart with the primary endpoint of maximum tolerated dose (MTD) determination and secondary endpoints of efficacy.

3. Based upon improved tolerability of fractionated radioimmunotherapy (RIT) and the known radiosensitizing effect of taxanes, a phase I study utilizing docetaxel plus fractionated dose ¹⁷⁷Lu-J591 has begun enrollment to determine MTD and explore efficacy for the combination.

“Targeted salvage radiation” may lead to significant improvements in the setting of biochemical relapse.

4. A randomized phase II study utilizing ketoconazole/hydrocortisone + ¹⁷⁷Lu-J591 vs ¹¹¹In-J591 (i.e. placebo) is underway in men with biochemically progressive CRPC without evidence of metastatic disease with the primary endpoint of delay in time to appearance of metastases. Since this is an adjuvant trial, it will require large numbers of patients and considerable duration before efficacy can be determined.

Results:

1. Four pts have been received pre-treatment ¹¹¹In-J591 infusion/imaging followed by treatment with ¹⁷⁷Lu-J591 in the expansion phase II single-dose study at the phase I MTD. Analysis of efficacy, toxicity, and the predictive ability of scans and PSMA expression in circulating tumor cells is ongoing.

2. 22 pts have been treated with fractionated dose ¹⁷⁷Lu-J591, with completion of cohort 5 at 40 mCi/m² x2; cumulative dose > single dose MTD. Myelosuppression appears lower compared to a single-dose of ¹⁷⁷Lu-J591.

3. Enrollment has begun in the phase I study of docetaxel/prednisone + fractionated dose ¹⁷⁷Lu-J591. No DLT's have been observed in the initial 2 pts.

4. The initial 2 pts have been treated in the randomized trial of radiolabeled J591 plus ketoconazole. Additional study sites across the U.S. are in the approval process.

Conclusions:

Radiolabeled J591 is tolerable and efficacious. Current trials are focusing on improvements in patient selection, dose fractionation to improve tolerance and efficacy, combination with chemotherapy, and “salvage radioimmunotherapy” to delay the onset of metastases in men with progressive biochemical evidence of disease.

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Development of ¹⁷⁷Lu- Dendrimer-Anti-CD20 : preliminary studies

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Introduction: Monoclonal antibodies against several antigens have been used as agents to carry drugs, toxins and radionuclides. These abilities can be increased significantly combining them with a nanoreservoir such as polymer Dendrimers. This would enable drug release specifically in tumor cells as well as a high specific activity in the case of therapeutic radionuclides. Anti-CD20 monoclonal antibody (Rituximab®) generated specifically against the surface antigen CD20 of human B lymphocytes is used as an immunotherapeutic agent for the treatment of non-Hodgkin's lymphoma, alone or associated with chemotherapy. Labeling of anti-CD20 antibodies with β-emitters would increase the therapeutic effectiveness due to radiological and cytotoxic effects of ionizing radiation. Dendrimers PAMAM G4 (polyamidoamine) are polymeric nanosystems with special properties like their nanometric size, low dispersion and defined superficial structure. Their amine groups at their surface allow radionuclides to bind directly or through other ligands. Lutetium 177 (¹⁷⁷Lu, T_{1/2} 6.7 d), is a β- (497 keV) and γ emitter (150 keV) and has a range of tissue penetration of 2 mm; these properties makes it possible to obtain scintigraphy images that would enable biodistribution studies, perform dosimetry calculations and produce the desired therapeutic effect specific to its β-emission. Our objective was to optimize conjugation and labeling of ¹⁷⁷Lu-(DOTA)G4 - Antibody anti-CD20. Methodology The conjugation of anti CD20 and dendrimers was carried out in 4 steps. Phosphate buffer 0.1M pH 7.5 and PD-10 short columns (Pharmacia) for purification were used in all steps. Step 1 Preparation of DOTA-Ossu was performed dissolving 128μmol DOTA and 128μmol NHS in 960 μl MilliQ-water, then 16.3 μl of EDC 50 mg/ml (12.8μmol) was added to the solution. The mixture was incubated for 45 min. at 4°C. Methanol was removed from the dendrimer solution (100μl, 100mg/ml) by nitrogen and recovered with 150 μl of phosphate buffer 0.1 M at pH 7.5, then 150 μl of DOTA-Ossu preparation was added. The mixture was incubated overnight at 4°C, with subsequent purification. Step 2 1 mL of purified dendrimer-DOTA-Ossu and 200 μl de SPDP (N-Succinimidyl 3-(2-pyridyldithio)-propionate) 20mM were incubated during 30 minutes at room temperature and then purified. Step 3 2 mg of anti CD20 was purified and conjugated with 0.2 mg de SMCC (succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate), incubated for 30 minutes at room temperature, and purified. Step 4.1 Dendrimer-DOTA-SPDP (step 2) and Anti CD20-SMCC (step 3) were incubated in a molar ratio of 1:2 over night at room temperature and then lyophilized. Step 4.2 Dendrimer-DOTA-SPDP (step 2) and Anti CD20-SMCC (step 3) were lyophilized each other. The conjugation was carried out dissolving both in 0.5 mL of phosphate buffer 0.1M pH 7.5 and then incubated as in step 4.1. The radio labeling was carried out immediately after this reaction. Radiolabeling The labeling of these conjugates were performed through the addition of 1.5 - 7 mCi of ¹⁷⁷LuCl₃ and 100 μl of

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gentisic acid (10mg/ml) to 10 mg of DOTA-dendrimer-AntiCD-20 (step 4), incubating 30 minutes at 37°C and purifying. Labelling yield, radiochemical purity and stability was evaluated by chromatography by using 2 chromatographic systems: ITLC-SG and saturated ITLC-SG strips (BSA 5%) as carrier, and sodium acetate 14% and EtOH-NH₄OH-H₂O (2:1:5) as solvents, respectively and gel filtration (PD-10 column G-25, eluent: phosphate buffer 0.1 M pH 7.5). Results: This method allows us to obtain a dendrimer conjugated anti CD20 antibody using a 4-step process. Labelling yield for conjugated obtained from step 4.1 was 90% and it was stable at least 24 hours post labelling. The labelling yield for conjugated obtained from step 4.2 was 50%. The controls systems can discriminate good chromatographic species involved so as to obtain a reliable system of radiochemical purity. ITLC-SG with Sodium acetate 14% as solvent allowed free ¹⁷⁷Lu to migrate with the solvent, ¹⁷⁷Lu Dendrimer and ¹⁷⁷Lu Dendrimer-antiCD-20 remained at the origin using saturated ITLC-SG strips (BSA 5%) as carrier, and using EtOH-NH₄OH-H₂O (2:1:5) as solvent free ¹⁷⁷Lu and ¹⁷⁷Lu Dendrimer remained at the origin. ¹⁷⁷Lu Dendrimer-antiCD-20 migrated with solvent, gel filtration in which ¹⁷⁷Lu and ¹⁷⁷Lu dendrimer anti CD20 dendrimer was found present in fraction 3 to 4 mL and free ¹⁷⁷Lu was present in fractions 7 to 8 mL. The conjugation (step 4) before lyophilisate gives more desirable results than the conjugation carried out from the precursors Dendrimer-DOTA-SPDP (step 2) and Anti-CD20 SMCC (step 3) lyophilized Conclusions We conclude that the best strategy for the conservation of the conjugation is to make the incubation of Dendrimer-DOTA-SPDP (step 2) and Anti-CD20 SMCC (step 3) and then freeze-dry. These promising results warrant further studies and open a new strategy for tumor specific treatment.

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Development of ¹⁵³Sm-DTPA-Rituximab for radioimmunotherapy

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Combining beta-particle effect with therapeutic properties of anti-CD20 monoclonal antibody in lymphomas, Mabthera™ (rituximab) was targeted in this study. The antibody was labeled with ¹⁵³Sm-samarium chloride (185 MBq) after conjugation with freshly prepared ccDTPA. Conjugated-Rituximab was obtained by the addition of 1 ml of a Rituximab pharmaceutical solution (5 mg/ml, in phosphate buffer, pH=7.8) to a glass tube pre-coated with freshly prepared ccDTPA (0.01-0.1 mg) at 25°C. Sm-153 chloride was obtained by thermal neutron flux (5×10^{13} n.cm⁻².s⁻¹) of an enriched ¹⁵²Sm₂O₃ sample, dissolved in acidic media. Radiolabeling was performed in one hour by the addition of DTPA-rituximab conjugate at room temperature. Radiochemical purity of 96% (ITLC) and 98% (HPLC) were obtained for final radioimmunoconjugate (Specific activity = 560 TBq/mM). The final isotonic ¹⁵³Sm-rituximab complex was checked by gel electrophoresis for protein integrity retention. Biodistribution studies in normal rats performed to determine radioimmunoconjugate distribution up to 24h. SPECT images were also obtained using 103 keV photons up to 48 hours.

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¹⁷⁷-Lu-Anti-CD20 monoclonal antibody: A potential radiopharmaceutical for treatment of non-Hodgkin's lymphoma.

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Rationale: Anti-CD20 monoclonal antibody generated specifically against the surface antigen CD20 (transmembrane) of human B-lymphocytes is used for the treatment of non-Hodgkin's lymphoma as immunotherapeutic agent, alone or associated with chemotherapy. The labelling of this anti-CD20 antibody with β-emitters increases the therapeutic effectiveness due to radiological and cytotoxic effects of ionizing radiation. Lutetium-177 (¹⁷⁷Lu, T_{1/2} 6.7 d), is a β- (497 keV) and γ emitter (150 keV) and has a range of tissue penetration up to 2 mm; these properties give the possibility to obtain images of the biodistribution, to do dosimetric calculations and produce the desired therapeutic effect specific to this β-emission. Our objective was the optimization of the labelling, the evaluation of the biological behavior and

dosimetric studies of ^{177}Lu -anti-CD20 as radiopharmaceutical for treatment of non-Hodgkin's lymphoma. Methodology: Before labelling, mab-anti-CD20 (Mabthera®) was purified by gel filtration and conjugated with 235 μl (3 μmol) of DOTA-Ossu, at pH 7.5 and incubated 18 hours at 4°C, with subsequent purification by gel-permeation with Sephadex G-25 (PD-10 columns from Pharmacia). Fractions of conjugated antibody were stored at 4 and -20°C for further stability evaluation. The labelling of this conjugate was performed through the addition of 55,5 – 259 MBq of $^{177}\text{LuCl}_3$ and 100 μl of gentisic acid (10 mg/mL) to 100 μL (500 μg) of anti CD20-DOTA-OSSu, incubating 30 minutes at 37°C and purifying by PD-10 column. Stability of ^{177}Lu -anti-CD20 in human serum and in saline was analyzed by using 2 chromatographic systems: ITLC-SG and saturated ITLC-SG strips (BSA 5%) as carrier, and Sodium acetate 14% and EtOH-NH₄OH-H₂O (2:1:5) as solvents, respectively. Biodistribution studies were carried out in CD-1 normal mice in triplicate at 4, 16 and 24 hours post injection of 22,6 - 40,7 MBq of anti-CD20-DOTA- ^{177}Lu . In order to study the effect of previous administration of unlabelled anti-CD20 in the uptake by critical organs, biodistribution studies were also done at the same conditions using 150 mg/m² and 250 mg/m² of unlabelled anti-CD20. Dosimetric studies were accomplished using Monte Carlo Simulation method, Subroutine Penelope, considering ^{177}Lu -anti-CD20 uniformly distributed in a spheroid as a model of tumor mass. Results The Mab labelling with ^{177}Lu -Lu gave yields ranging from 60% to 75% and a radiochemical purity higher than 97% after purification for up to 24 hours elapsed time, both in human serum and in saline. Also, there was no significant difference in the performance and stability studies, between antibody stored at 4°C and at -20°C. The biological distributions without previous administration of unlabelled anti-CD20 showed significant uptake by liver up to 40% and urinary elimination up to 30% at 24 hours. Biodistributions studies with blocking by previous administration of unlabelled anti-CD20 showed a significant dose-dependent decrease, reaching half the initial values in the uptake of ^{177}Lu -anti-CD20 by liver tissue, while in blood there is not significant change. Dosimetric studies using Monte Carlo Simulation showed that 83% of the total dose was deposited at the tumor mass, while only the remaining 17% affect the surrounding non- pathological tissue. Conclusions: Optimization of the labelling of ^{177}Lu -anti-CD20 was achieved giving reliable results with a radiochemical purity consistent with its potential clinical application as a therapeutic radiopharmaceutical for treatment of non-Hodgkin's lymphoma. Biodistributions studies with blocking by previous administration of unlabelled anti-CD20 showed the importance of this previous administration in order to decrease the uptake by critical organs to achieve a protective effect, optimizing the therapeutic desired effect in terms of the administered dose.

Dosimetric studies using the simulation method determined that ^{177}Lu is a radionuclide suitable for treatment of tumor masses of small and medium size. Acknowledgments: OIEA. PEDECIBA Química, Roche.

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Sterically stabilized ^{177}Lu de DTPA-Liposomes and ^{177}Lu DTPA-Liposomes: development of labelling procedure and biologic evaluation

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Introduction: Liposomes are hollow microspheres that spontaneously form when water is added to a dried lipid mixture. Their membrane is constituted by one or several curved lipid bilayers, which entrap part of the aqueous medium in which they are suspended. In general, two different approaches to label preformed liposomes can be distinguished: firstly, the radionuclide can be transported through the lipid bilayer and trapped in the internal aqueous phase of the liposome. Secondly, liposomes can be labeled by coupling the radiolabel to the lipid bilayer, either directly to the surface or via chelator, as the case of this work through DTPA. Liposomes that have the correct size act as carrying nanosystems capable to accumulate in tumors by diffusion. ^{177}Lu is a relatively low energy β -particle emitter that has successfully been used for therapy. The objective of this work was to label liposomes with ^{177}Lu and to evaluate their biologic properties in normal mice. Materials and Methods: DTPA-liposomes are composed of: phosphatidylcholine: cholesterol: 1,2; dimiristoil-sn-glicero-3 phosphoethanolamine-N- DTPA (9:4:1 p/p). Sterically stabilized liposomes are composed of phosphatidylcholine: cholesterol: 1, 2; dimiristoil-sn-glicero-3 phosphoethanolamine-N-DTPA and 1.2 dimiristoil-sn-glicero-3 phosphoethanolamine-N-[Methoxy (polietilenglicol-5000)]. Both kinds of liposomes were prepared using the hand shaken method. ^{177}Lu radiolabeling was performed as follow: 2 mL ^{177}Lu (2.7mCi) and 0,8 ml liposomal dispersion, were incubated for 20 minutes at room temperature. Radiolabeling yield and radiochemical purity was evaluated by ITLC using sodium acetate 14% solution and piridine: acetic acid: water (3:5:1.5 v/v) as mobile phase. Chromatography controls were performed after 5 hours post -labeling to check stability using the systems mentioned earlier. We also performed molecular exclusion chromatography using a PD 10 column eluting with 0.9% NaCl. Biodistribution studies were performed in two groups of normal CD1 mice. Group 1 had a liver blockage with 0.3 ml (20mg/ml) of cold liposomes, weight 23.65 \pm 2.3 g (n=6). Group 2 had a liver blockage with 0.2ml (20 mg/ml) of cold liposomes, weighing 20.88 \pm 2,0 g (n = 6). Mice were sacrificed at 4 and 72 hours post intravenous injection. Results: Labeling

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yield was higher than 95% for both types of liposomes, maintaining a radiochemical purity over 90% during the course of the study. Studies of biodistribution notes that for both kinds of liposomes, the main route of elimination is the hepatic as expected for the elimination of these nanosystems. Blood clearance studies of these sterically stabilized liposomes show a slower clearance than the conventional liposomes. Conclusions: These results encourage further investigation of these liposomes as potential agents for therapy. Acknowledgements: PDT, OIEA

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177Lu –Bevacizumab: a novel therapeutic radiopharmaceutical

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Introduction: Bevacizumab (Avastin®) is a humanized anti-human vascular endothelial growth factor-A (VEGF-A) monoclonal antibody. It targets and inhibits the function of VEGF that stimulates new blood vessel formation. Bevacizumab inhibits angiogenesis and has been approved for treatment of lung, colon and breast cancer. 177Lu is a relatively low energy β^2 -particle emitter that has successfully been used for therapy. 177Lu labeled Bevacizumab could be used for tumor treatment. The objective of this paper was to describe the labeling of Bevacizumab with 177LuCl₃ and to perform its chemical and biological evaluation. Materials and Methods: Bevacizumab was purified by size exclusion chromatography using PD-10 column equilibrated and eluted with NaHCO₃ 0.1 M, pH=8.5 and elution profile was determined by UV spectrophotometry at 280 nm, fractions containing the antibody were pooled and the final monoclonal concentration was determined. DOTA (Sulfo NHS, EDC) was activated for 1 hour at 4Â°C, and incubated overnight with purified Bevacizumab at 4Â°C with continuous gentle stirring. Bevacizumab-DOTA was purified by size exclusion chromatography using PD-10 column equilibrated and eluted with Ammonium Acetate 0.25M, pH=7 and detected by UV spectrophotometry at 280 nm and the final monoclonal-DOTA concentration determined. Immediately it was labeled with 177LuCl₃ with an activity of 111-185 MBq/mg. Radiochemical purity of 177Lu-DOTA-Bevacizumab was evaluated by ITLC-SG using MEK and Saline as solvents and by saturated ITLC-SG strips (BSA 5%) using EtOH-NH₄OH-H₂O (2:1:5). It was also evaluated by HPLC using an SW300 protein pack column and eluting with phosphate buffer 0.01M pH 7.4 at 1.0 mL/min. 177Lu-DOTA-Bevacizumab was purified by size exclusion chromatography using PD-10 column equilibrated and eluted with Ammonium Acetate 0.25 M,

pH=7. In vitro stability was verified in a Ammonium Acetate solution 0.25M, pH=7 for 72 hours post labeling. Biodistribution studies of 177Lu-DOTA-Bevacizumab were done in healthy C57 black mice and C57 black mice previously inoculated with B16F1 cells. Scintigraphy images were acquired at 24 and 48 hours post-labeling. Results: Radiochemical purity of 177Lu-DOTA-Bevacizumab was higher than 90%. After purification in vitro stability remains higher of 90% during 3 days. Biodistribution and scintigraphy studies in healthy C57 mice show that 177Lu-DOTA- Bevacizumab has significant liver uptake with negligible uptake in other organs. In C57 melanoma black mice, it was observed liver take and a high tumoral uptake that persisted at 24 and 48-hrs images. Conclusion: 177Lu-DOTA-Bevacizumab was easily and rapidly labeled and showed a high tumor uptake due to angiogenesis which is one of the first steps of tumor development. This new radiopharmaceutical has potential application for the treatment of metastasis and micrometastasis. Further research is warranted in order to evaluate the dosimetry in normal tissues and in tumors. Acknowledgements: OIEA, Laboratorios Roche, Dr Gustavo Arroyo.

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In vivo characterization of a 177Lu-bombesin-based radiopharmaceutical for GRP-positive tumors diagnosis and treatment

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In designing of radiometal-based radiopeptides for cancer diagnosis and treatment, important factors to consider are half-life, mode of decay, cost and availability of the radionuclide and the chemical and biological properties of the labeled molecule. In the field of radiolabeled peptides, bombesin (BBN) appears as focus of interest. BBN is a 14-amino acid analog of human gastrin releasing peptide (GRP). BBN was originally isolated from the skin of the frog *Bombina bombina* in 1970. Bombesin receptors – in particular the gastrin-releasing peptide (GRP) receptor – have been shown to be massively over expressed in several human tumor cells, including prostate and breast cancer, and could be an alternative as target for their treatment by radionuclide therapy (RNT). A large number of BBN analogs had already been synthesized for this purpose and have shown to reduce tumor growth in mice. Nevertheless, most of the studied analogs exhibit high abdominal accumulation, especially in pancreas and intestine. This abdominal accumulation may represent a problem in clinical use of radiolabeled BBN analogs probably due to serious side effects to patients. The goal of the present work was to radiolabel a novel peptide based on bombesin structure with lutetium-177, a beta emitter with optimal

physical characteristics for RNT of small tumors and metastases, and to evaluate its behavior in Balb-c and Nude mice bearing prostate tumor (PC-3) xenografts. Bombesin derivative was radiolabeled with 97.5 MBq of ^{177}Lu at 90°C for 30 minutes and pH 4.5. Biodistribution, pharmacokinetics, whole body and scintigraphic studies were performed in both healthy Balb-c and xenografted Nude mice, in order to characterize the biological properties of labeled peptide. In addition, the specificity of labeled bombesin derivative targeting to PC-3 tumor cells was analyzed by in vivo competition assays. Bombesin derivative was successfully labeled with high yield. Investigations in Balb-c mice showed fast blood clearance, rapid excretion, performed mainly by renal pathway, and low abdominal accumulation and retention. The studies in Nude mice bearing human prostate tumor showed that the radiopeptide can target tumor cells. Higher tumor uptake was observed at 1 hour post- injection and showed to be specific in competition assays in which the pre-injection of unlabeled peptide reduced 88% of labeled peptide target to tumor. Moreover, tumor uptake allowed tumor detection by scintigraphy imaging, especially 1 hour post injection. The results of this work showed that this novel radiopharmaceutical based on bombesin structure is promising for applications in GRP positive tumor detection and treatment.

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^{177}Lu -DOTA-TATE and Sandostatin LAR as a promising therapy of Esthesioneuroblastoma – case report

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Introduction: Esthesioneuroblastoma (ENB) is a rare tumour arising from the olfactory epithelium of the nasal vault which frequently invades the cranial base and orbit. For the first time, ENB was described by Berger and Luc in 1924. About 900 cases of ENB have been reported since then. ENB represents 1-5% of intranasal cancers and has bimodal age distribution between 11-20 years and 51-60 years. Case. A 49 year old man with esthesioneuroblastoma confirmed in histopathological examination, was referred to our department in 2009. He had seven years history of headache, nasal obstruction, anosmia, and rhinorrhoea. At the beginning, this patient was treated with diagnosis of recurrent sinusitis. Histopathological diagnosis was done in 2004 after surgical treatment. Tumour fully filled the left nasal cavity, left maxillary sinus, infiltrated nasal septum and ethmoid sinus at this time. From 2004 to 2008 patient passed: surgical craniofacial resection, radiotherapy (60 Gy in 30 fractions - 2 Gy per day) and 6 courses of chemotherapy (cisplatin and etoposide) without good

effect. In the end of 2008, the tumor filled left orbit, left lacrimal duct, base of skull and dura (stage C in Kadish's system). Patient was referred to our department in January 2009 and underwent somatostatin receptor scintigraphy with $^{99\text{m}}\text{Tc}$ -HYNIC-TATE. After confirmation of presence of somatostatin receptors in tumour, we decided to treat the patient with the "hot" somatostatin analog labelled with lutetium 177 - ^{177}Lu -DOTA-TATE and with the "cold" somatostatin analog - Sandostatin LAR. The patient received three courses of combined treatment with 7,4GBq ^{177}Lu -DOTA-TATE in 6-8 weeks intervals and three injections of 20mg Sandostatin LAR two weeks after each radiopharmaceutical treatment. After the last (third) course of such a treatment, the patient underwent PET-CT with ^{68}Ga -DOTA-TATE. PET-CT showed less than 25% reduction of the neoplasm which means stabilization of disease. The next three courses of treatment with "hot"- ^{177}Lu -DOTA-TATE and "cold" – somatostatin analog Sandostatin LAR are planned.. Conclusion: ^{177}Lu -DOTA-TATE and Sandostatin LAR are promising therapies for Esthesioneuroblastoma

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^{177}Lu - Dendrimer PAMAM G4: Evaluation in a murine melanoma model.

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Introduction: Dendrimers PAMAM G4 (polyamidoamine) are polymeric nanosystems with special properties like their nanometric size, low dispersion and defined superficial structure. Their amine groups at their surface allow radionuclides to bind directly or through other ligands. Dendrimers can easily pass through vessels at sites of inflammation, thus labeled dendrimers can be used as potential agents for tumor treatment. Lutetium -177 (^{177}Lu , $T_{1/2}$ 6.7 d), is a β^- - (149 and 497 keV) and β^+ emitter (113 and 208 keV) and has a range of tissue penetration of 0.5 to 2 mm; these properties allow to obtain scintigraphy images of the biodistribution, to perform dosimetric calculations and to produce the desired therapeutic effect specific to its β^- -emission in small sized tumors or metastasis. Our objective was to optimize the conjugation of dendrimers to DOTA and the labeling of previously conjugated dendrimers to DOTA, and to evaluate its biological behavior. Methodology: The preparation of DOTA-Ossu was performed dissolving 20 μg DOTA and 128 μmol NHS in 320 μl MilliQ-water, and then 16,3 μl of EDC 50 mg/ml (12,8 μmol) was added to the solution. The mixture was incubated for 45 min. at 4°C . Methanol was removed from the dendrimer solution 100 μl (100 mg/ml) by nitrogen and recovered with 150 μl of phosphate buffer 0.1 M at pH 7.5, then 150

ABSTRACTS

1/4l of DOTA-Ossu preparation was added. The mixture was incubated overnight at 4°C, with subsequent purification by gel-permeation with sephadex G-25 (PD-10 short columns from Pharmacia). The labelling of this conjugate was performed through the addition of 1.5-7 mCi of $^{177}\text{LuCl}_3$ to 1 mg of dendrimer-DOTA-Ossu, incubating 30 minutes at 37°C and purifying by PD-10 column. Labelling yield, radiochemical purity and stability were evaluated by using 2 chromatographic systems: ITLC-SG and saturated ITLC-SG strips (BSA 5%) as carrier, and Sodium acetate 14% and EtOH-NH₄OH-H₂O (2:1:5) as solvents. Gel filtration (PD-10 column G-25, eluent: phosphate buffer 0.1 M pH 7.5) as well Biodistribution studies were done in C57 Black mice with induced murine melanoma (B16-F1 cell line) as well as in normal mice, age 2 months, weight 30±1 g (n=3), at different distribution times. Results: Labelling yield was 99%, remaining 95% of the radionuclide bound to dendrimers for at least 24 hours post labelling. Elution volume in PD-10 columns was 6-8 mL for free ^{177}Lu and 3-4 mL for radiolabelled dendrimers. Biodistribution in normal mice show a fast blood clearance and a high liver and spleen uptake. In mice with induced melanoma a high uptake was evidenced even at one hour after IV administration and confirmed by scintigraphic studies. Conclusions: PAMAM G4 dendrimers (polyamidoamine) was labelled with ^{177}Lu reaching a high yield and radiochemical purity. Preliminary biodistribution studies in the animal model envisage good potential as a radiopharmaceutical for melanoma tumour treatment; and further research is encouraged. Acknowledgments: OIEA, PEDECIBA, CHLCC.