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Tracer level radiochemistry to clinical dose preparation of ^{177}Lu-labeled cyclic RGD peptide dimer☆

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ABSTRACT

Aim: Integrin $\alpha_v\beta_3$ plays a significant role in angiogenesis during tumor growth and metastasis, and is a receptor for the extracellular matrix proteins with the exposed arginine(R)-glycine(G)-aspartic acid(D) tripeptide sequence. The over-expression of integrin $\alpha_v\beta_3$ during tumor growth and metastasis presents an interesting molecular target for both early detection and treatment of rapidly growing solid tumors. Considering the advantages of ^{177}Lu for targeted radiotherapy and enhanced tumor targeting capability of cyclic RGD peptide dimer, an attempt has been made to optimize the protocol for the preparation of clinical dose of ^{177}Lu labeled DOTA-E[c(RGDfK)]$_2$ (E = Glutamic acid, f = phenyl alanine, K = lysine) as a potential agent for targeted tumor therapy.
Methods: ^{177}Lu was produced by thermal neutron bombardment on enriched Lu_2O_3 (82% in ^{176}Lu) target at a flux of 1×10^{14} n/cm^2.s for 21 d. Therapeutic dose of ^{177}Lu-DOTA-E[c(RGDfK)]$_2$ (7.4 GBq) was prepared by adding the aqueous solution of the ligand and $^{177}LuCl_3$ to 0.1 M NH_4OAC buffer containing gentisic acid and incubating the reaction mixture at 90 °C for 30 min. The yield and radiochemical purity of the complex was determined by HPLC technique. Parameters, such as, ligand-to-metal ratio, pH of the reaction mixture, incubation time and temperature were varied using tracer quantity of ^{177}Lu (37 MBq) in order to arrive at the optimized protocol for the preparation of clinical dose. Biological behavior of the radiotracer prepared was studied in C57/BL6 mice bearing melanoma tumors.
Results: ^{177}Lu was produced with a specific activity of 950 ± 50 GBq/mg (25.7 ± 1.4 Ci/mg) and radionuclidic purity of 99.98%. A careful optimization of several parameters showed that ^{177}Lu-DOTA-E[c(RGDfK)]$_2$ could be prepared with adequately high radiochemical purity using a ligand-to-metal ratio ~2. Based on these studies therapeutic dose of the agent with 7.4 GBq of ^{177}Lu was formulated in ~63 GBq/μM specific activity with high yield (98.2 ± 0.7%), radiochemical purity and in vitro stability. Biodistribution studies carried out in C57/BL6 mice bearing melanoma tumors revealed specific accumulation of the radiolabeled conjugate in tumor (3.80 ± 0.55% ID/g at 30 min p.i.) with high tumor to blood and tumor to muscle ratios. However, the uptake of the radiotracer in the tumor was found to be reduced to 1.51 ± 0.32 %ID/g at 72 h p.i.
Conclusions: The present work successfully demonstrates the formulation of an optimized protocol for the preparation of ^{177}Lu labeled DOTA-E[c(RGDfK)]$_2$ for PRRT applications using ^{177}Lu produced by direct neutron activation in a medium flux research reactor.

1. Introduction

Over the last two decades, a large number of radiolabeled peptide derivatives containing Arg-Gly-ASP (RGD) tripeptide sequence have been evaluated as potential radiotracers for non-invasive monitoring of tumor growth, metastases and therapeutic response by SPECT and PET [1–13]. This strategy utilizes the high affinity and specificity of RGD peptides for integrin $\alpha_v\beta_3$, which plays a pivotal role in tumor malignancies and are over-expressed in the activated endothelial cells of the neovasculature as well as in different variety of growing tumor cells [4,5,14–19]. Moreover, RGD peptide derivatives have also been used as integrin $\alpha_v\beta_3$ antagonists for tumor therapy by inhibiting tumor angiogenesis or enhance the efficacy of other tumor therapeutics [20,21]. In contrast to these, the utilization RGD peptides in receptor targeted radionuclide therapy are typically rare till date. Only in the last couple of years a few reports describe the use of tumor targeting properties of radiolabeled RGD peptides beyond diagnostic applications and therapy monitoring to targeted tumor therapy using suitable therapeutic radionuclides viz. ^{90}Y and ^{177}Lu [10,21–24].

The success of peptide receptor radionuclide therapy (PRRT) relies on the maximized accumulation and retention of the radiolabeled peptides in tumor targets along with minimum exposure of healthy tissues. The tumor targeting capability and clearance properties of the radiolabeled conjugate as well as the emission properties of the

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radionuclide are the contributing factors. Among the radiolabeled RGD peptide derivatives proposed for use in in vivo diagnostic or therapeutic applications, the preferred targeting biomolecules are cyclic penta-peptide derivatives containing the Arg-Gly-Asp sequence and an aromatic D-amino acid [2–5,25,26]. Conformational restriction by ring closure of the peptides and further chemical modification, such as the use of D-amino acids, like in the c(RGDfK) (with f = phenylalanine, K = lysine), not only increased their bioavailability but also improved integrin $\alpha_v\beta_3$ binding affinity [21,27]. Further, it is already well documented that with increase in peptide multiplicity there is an increase in radiotracer tumor uptake and retention of radiolabeled RGD multimers. This trend is observed from monomer to tetramer. However, a steady increase of uptake in other non-target organs e.g. liver, intestine and kidneys is also reported. A comparison of biological behavior of radiolabeled RGD monomer, dimer and tetramer revealed that dimers exhibit considerable tumor uptake with best target to non-target ratio among the three and is a suitable candidate for tumor imaging and therapy [13,28–31]. The cyclic RGD peptide dimer E[c(RGDfK)]$_2$ (E = glutamic acid) coupled with DOTA (1,4,7,10-tetra-azacyclododecane-1,4,710-tetraacetic acid) has been widely used as the targeting biomolecule to develop the integrin $\alpha_v\beta_3$-targeted radiotracers using metallic radionuclides for diagnostic (^{64}Cu, ^{68}Ga and ^{111}In) and therapeutic (^{90}Y and ^{177}Lu) applications [10,12,13,22–24].

^{177}Lu has excellent nuclear decay properties for in vivo targeted therapy of small and metastatic tumors. Although, the radionuclide has potential drawback for the treatment of large tumors and tumors with large heterogeneity of receptor expression, where ^{90}Y is especially effective. ^{177}Lu decays with a half-life of 6.65 d by emission of β^- particles with maximum energies of 497 keV (78.6%), 384 keV (9.1%) and 176 keV (12.2%) to stable ^{177}Hf. The emission of suitable energy gamma photons of 113 keV (6.4%) and 208 keV (11%) with relatively low abundances provides the opportunity to carry out simultaneous scintigraphic studies as well as to perform dosimetric evaluations. An important aspect of consideration for the countries with limited reactor facility is the comparatively longer half-life of ^{177}Lu which provides logistic advantage towards facilitating supply to locations far away from the reactors. Besides this, the high thermal neutron capture cross-section (σ = 2090 b) of [^{176}Lu(n,γ)^{177}Lu] reaction ensures that ^{177}Lu can be produced with sufficiently high specific activity for preparing receptor specific agents using the moderate flux reactors [32,33]. These favorable nuclear parameters also ensure that there will be no constraints with respect to large scale production of the radionuclide [32]. The excellent potential of ^{177}Lu in PRRT has been amply demonstrated especially using somatostatin analog peptides [34–36]. Taking these factors into consideration, ^{177}Lu labeled DOTA-E[c(RGDfK)]$_2$ [DOTA-(RGD)$_2$] could be envisaged as a promising agent for targeted tumor therapy.

Shi et al. and Luna-Gutierrez et al. have recently reported the preparation and biological evaluation of ^{177}Lu labeled DOTA-E[c(RGDfK)]$_2$ in BALB/c mice bearing U87MG human glioma xenografts [23,24]. The radiotracer showed specific accumulation in tumor. Considering the future scope of the agent towards extensive clinical investigation, developing an optimized protocol for its preparation in clinically relevant dose (~7.4 GBq of ^{177}Lu, single patient dose of ^{177}Lu used in therapy of neuroendocrine originated tumors) using ^{177}Lu produced by direct neutron activation route was felt pertinent. Herein, we report the optimization of the protocol for the preparation of ^{177}Lu-DOTA-E[c(RGDfK)]$_2$ in clinically relevant dose of ~7.4 GBq using (n,γ) produced ^{177}Lu of moderate specific activity (~925 GBq/mg), investigation of its in vitro stability and preliminary evaluation in animal model.

2. Experimental

2.1. Materials and methods

Lutetium oxide (82% enriched in ^{176}Lu, spectroscopic grade, >99.99% pure) for the production of ^{177}Lu was procured from Trace Science International, Canada. The RGD peptide conjugate viz. DOTA-E[c(RGDfK)]$_2$ [DOTA-(RGD)$_2$] [Fig. 1] was custom synthesized by Ms. ABX Advanced Biochemical Compounds, Radeber, Germany. Gentisic acid (2,5-dihydroxybenzoic acid) was obtained from Aldrich Chemical Company, St. Louis, MO, USA. Supra-pure hydrochoric acid was purchased from Merck, Germany. MilliQ water (resistivity 18.2 MΩ.cm) was used for all the studies. All other chemicals used in the experiments were of AR grade and supplied by reputed chemical manufacturers.

^{177}Lu was produced by thermal neutron irradiation of isotopically enriched lutetium target (82% in ^{176}Lu). A stock solution of was prepared by dissolving enriched Lu_2O_3 (82% in ^{176}Lu) powder in 0.1 M supra-pure HCl (typically, 1 mg.mL^{-1} concentration). A known aliquot of this solution was taken in a quartz ampule and carefully evaporated to dryness. The ampule was subsequently flame sealed and irradiated in the nuclear reactor at a thermal neutron flux of 1×10^{14} n/cm^2.s for 21 d after placing inside an aluminum can. The irradiated target was radiochemically converted to $^{177}LuCl_3$ solution after dissolving in 0.01 M supra-pure HCl [32,33]. Assay of ^{177}Lu activity produced was carried out by measuring the ionization current obtained when an aliquot of the batch was placed inside a pre-calibrated well-type ion-chamber (the calibration factor for the ion chamber for ^{177}Lu was arrived at 15.65 A.MBq^{-1}). Radionuclidic purity of ^{177}Lu was ascertained by high resolution gamma ray spectrometry using an HPGe detector (Canberra Eurisys, France) coupled to a 4 K multichannel analyzer (MCA) system after radiochemical processing. ^{152}Eu reference source used for energy as well as efficiency calibration of the detector was obtained from Amersham Inc., USA. All other radioactivity measurements were carried out by using a well type NaI(Tl) scintillation counter (Mucha, Raytest, Germany) utilizing the 208 keV gamma photon of ^{177}Lu. A high performance liquid chromatography (HPLC) system equipped with a PU 1575 UV/VIS detector was obtained from JASCO (PU 1580), Japan. A well-type NaI(Tl) scintillation detector (Mucha, Raytest, Germany) was coupled

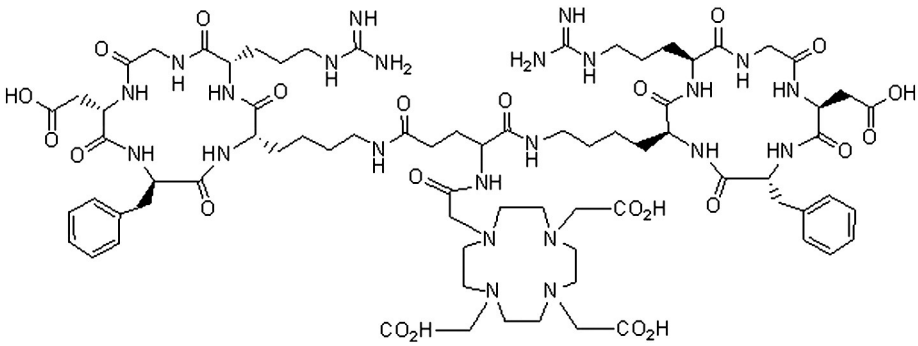

Fig. 1. Structure of DOTA-E[c(RGDfK)]$_2$ [DOTA-(RGD)$_2$].