Freeze-dried kits of DOTA-TATE sourced from in-house synthesized peptide

A formulation of ^{177}Lu-DOTA-TATE dose for administration in cancer patients

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Abstract

^{177}Lu-DOTA-TATE is the first radiopharmaceutical that has been approved in Europe, USA and Canada for peptide receptor radionuclide therapy (PRRT) of somatostatin receptor-positive neuroendocrine tumors (NETs). Broader utilization of ^{177}Lu-DOTA-TATE in nuclear medicine centres burdened with huge volume of patients requires a rapid and robust dose formulation protocol. One of the simpler methodologies for radiopharmaceutical preparation is the use of freeze-dried kits, which are pre-assembled with sterile ingredients (peptide, buffer) and hence, reduce the number of steps and manipulations during radiolabeling. Therefore, the task of freeze-dried kit preparation for formulation of ^{177}Lu-DOTA-TATE was undertaken by Radiopharmaceuticals Division. Towards this, the peptide DOTA-TATE was indigenously synthesized manually by solid phase peptide synthesis. The peptide content was then optimized for formulation of DOTA-TATE kits. Quality checks for testing sterility, pyrogen absence and stability of the kit were performed. Several batches of kits were tested to ensure high radiochemical yield of ^{177}Lu-DOTA-TATE. The freeze-dried kits were then preliminary evaluated in patients presented with neuroendocrine tumors.

Keywords: Freeze-dried kits, ^{177}Lu, DOTA-TATE, peptide synthesis, neuroendocrine tumor, quality control

Introduction

The peptide DOTA-TATE radiolabeled with diagnostic radionuclide ^{68}Ga and therapeutic radionuclide ^{177}Lu is an established standard for imaging and treatment respectively of neuroendocrine tumors (NETs) [1-4]. Though, ^{177}Lu-DOTA-TATE (Lutathera®) has been approved by Food and Drug Administration (FDA) for treatment of NETs [5] a simpler protocol for its preparation is needed to expand the utilization across different nuclear medicine centers. Three general methods adopted at hospitals for the preparation and use of radiopharmaceuticals are: (i) *Manual (wet) radiolabeling* - preparation of radiopharmaceuticals from raw materials and radionuclide following the cumbersome radiolabeling procedure, (ii) *Freeze-dried kits*-addition of appropriate amount of radioactivity to the freeze-dried kit pre-dispensed with ligand, buffer and radical scavenger (gentisic acid, ascorbic acid), (iii) *Finished radiopharmaceutical* in readymade form.

Generally, ^{177}Lu-DOTA-TATE is prepared in hospitals by manual labeling procedure which involves preparation of buffer solution and peptide solution, followed by addition of appropriate volume of buffer for pH maintenance (pH 5) and lastly, addition of required radioactivity of $^{177}LuCl_3$ and heating for patient dose formulation. This multi-step preparation is a time consuming process and also increases scope for errors. Moreover, therapeutic radiopharmaceuticals are preparations containing high radioactivity (~100-200 mCi) and also have stringent requirement of very high radiochemical yield (> 98%). Hence, a rapid and efficient radiolabeling process with adequate safety measures, needs to be in place for ^{177}Lu-DOTA-TATE preparation [6,7]. Freeze-dried kits containing pre-dispensed ligand, buffer and other excipients in powder form are attractive, viable option for simplified radiolabeling. Kit formulation requires simple addition of appropriate amount of radioactivity followed by heating. Being an easy procedure, use of kits omits the

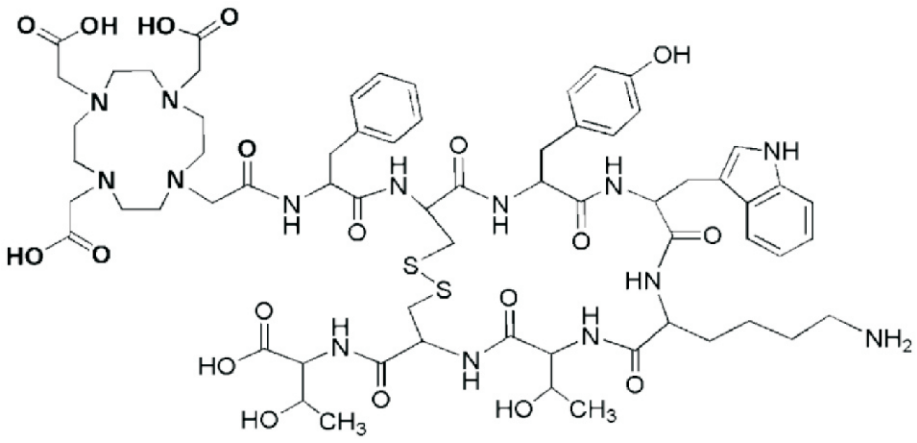

Fig.1: Chemical structure of DOTA-TATE.

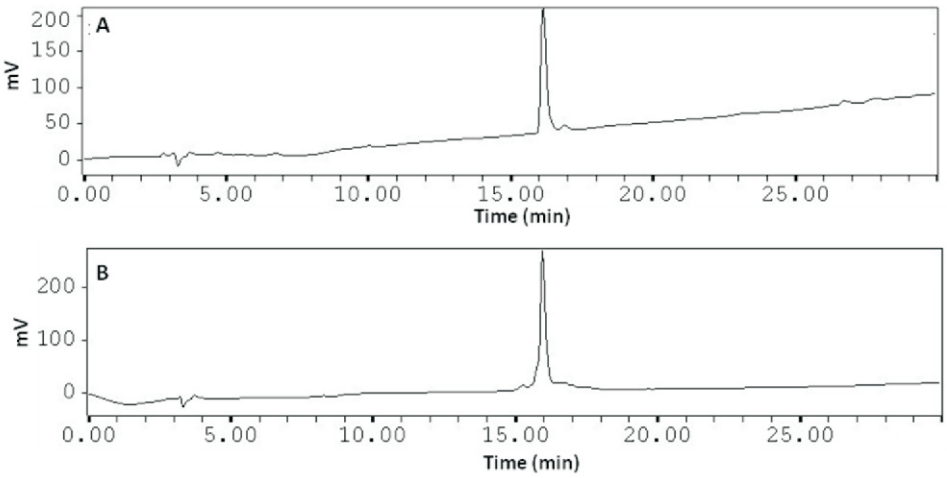

Fig.2: UV-HPLC chromatogram of (A) commercial DOTA-TATE peptide and of (B) in-house synthesized DOTA-TATE.

requirement of highly skilled personnel and the rapidity of the methodology leads to reduced radiation exposure. Despite these advantages, there are no commercial suppliers of ready-to-use freeze-dried DOTA-TATE kits for ^{177}Lu-DOTA-TATE formulation. Availability of kits will encourage more number of nuclear medicine centers to adopt the PRRT mode of treatment of NET patients.

^{177}Lu-DOTA-TATE, as a ready-to-use radiolabeled product, has been approved by Radiopharmaceutical Committee (RPC) for supply to hospitals through Board of Radiation and Isotope Technology (BRIT)/ Radiation Medicine Center (RMC). Inconsistent transportation logistics of radioactive materials is a major impediment to the use of ready radiopharmaceuticals. Geographically remote locations face troubles and transportation delays. Sometimes patients reach the nuclear medicine center for treatment after a long and difficult journey, any delay in the arrival of the radiopharmaceutical increases the waiting period and anxiety of the patient.

Preparation of ^{177}Lu-DOTA-TATE using freeze-dried kits can overcome the limitations posed by the other two methods. Hence, DOTA-TATE kits were synthesized using the in-house prepared peptide. Experiments towards optimization of kit contents were performed so as to achieve high radiochemical yield. Prepared freeze-dried kits were also supplied to a nuclear medicine hospital for therapy of patients suffering from neuroendocrine tumors.

Synthesis of DOTA-TATE

The peptide DOTA-TATE was synthesized by standard Fmoc solid phase peptide synthesis method using 2-chlorotrityl chloride resin. The fully protected peptide chain D-Phe-Cys(Acm)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Acm)-Thr(tBu)-OH was prepared by successive coupling of protected amino acids using Fmoc strategy, where 3-fold excess of amino acid as well as O-(7-azabenzotriazol-l-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) was used along with 6-fold excess of (N,N)-di-isopropylethylamine (DIPEA) in dimethylformamide (DMF) for 120 min. After assembling the fully protected peptide, coupling of the chelator DOTA-tris-(tBu)-ester at the N-terminus of the

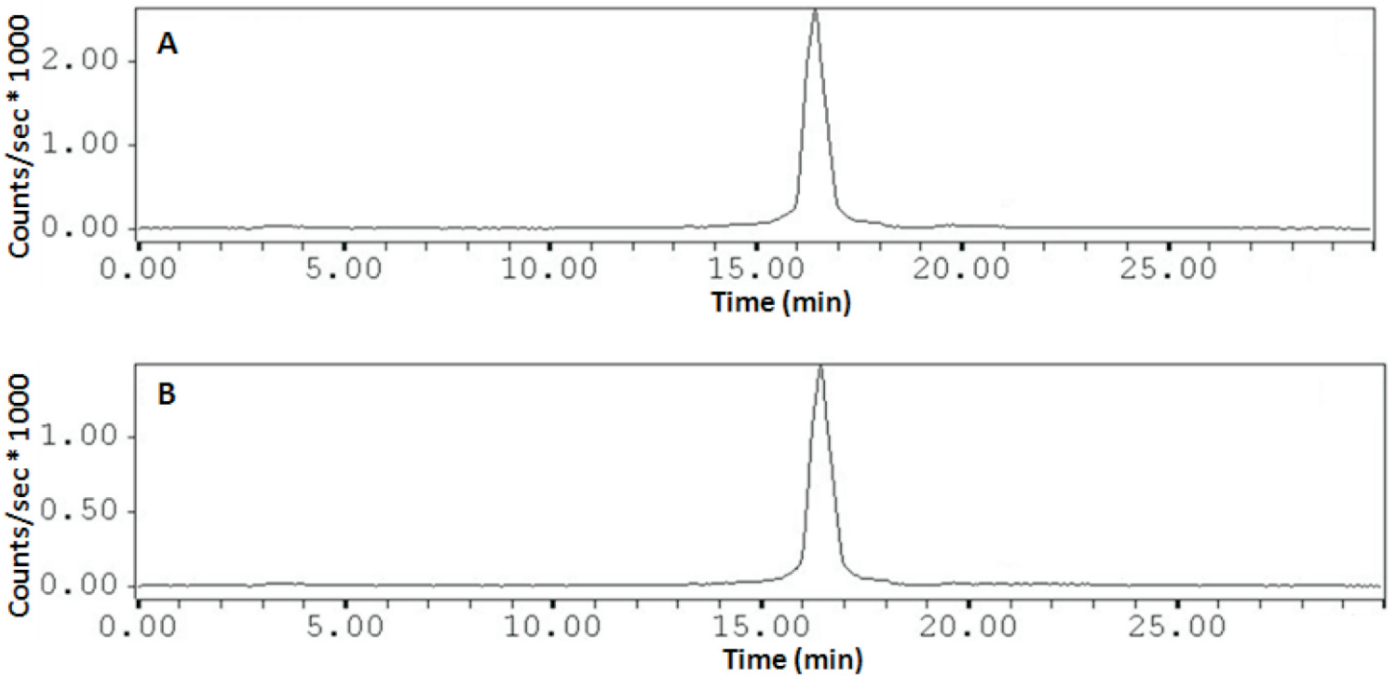

Fig.3: Radio-HPLC elution profiles of (A) ^{177}Lu-DOTA-TATE prepared using DOTA-TATE kit (B) ^{177}Lu-DOTA-TATE prepared using 1-year-old DOTA-TATE kit stored at 0°C.

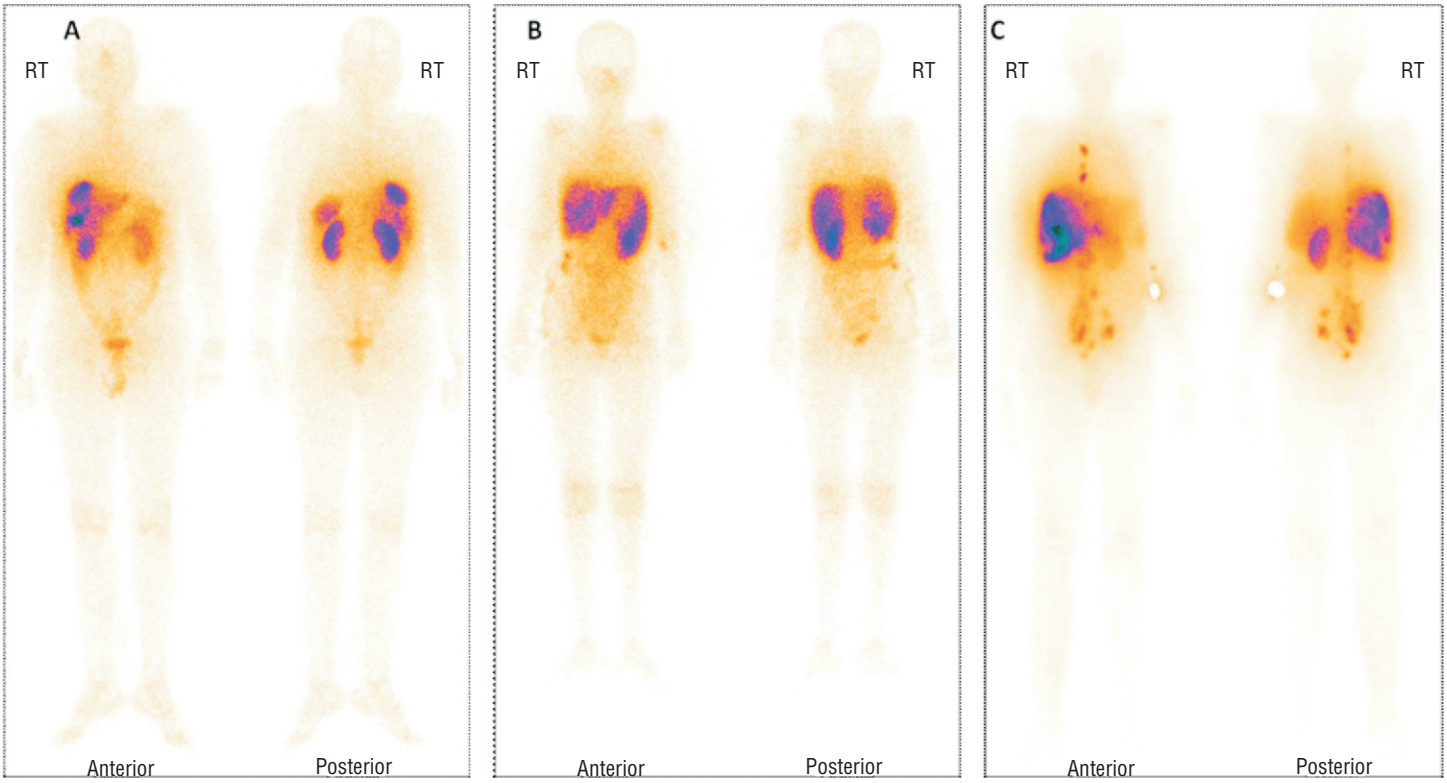

Fig.4: Whole-body scintigraphic images of kit formulated ^{177}Lu-DOTA-TATE (200 mCi) in three different patients suffering from NETs.

peptide was performed followed by on-resin cyclization with 1.2 eq. of thallium(III) trifluoroacetate in DMF. The cleavage of the peptide from the solid phase and simultaneous deprotection of side chain groups was carried out using the cocktail mixture (1 mL) of TFA/H_2O/EDT/TIPS (94:1:2.5:2.5 v/v/v/v). The crude peptide was purified by semi-preparative HPLC and characterized by mass spectroscopy. MS (ESI^+): m/z observed = 718.9 $[M+2H]^{2+}$ (calculated for $C_{65}H_{90}N_{14}O_{19}S_2$: 1434.6). The chemical structure of DOTA-TATE is presented in Fig. 1. The UV-HPLC chromatogram of in-house synthesized peptide was compared with the DOTA-TATE procured from ABX advanced biochemical compounds, GmbH (Fig. 2A & 2B).

Freeze-dried kits of DOTA-TATE

The initial radiochemical studies, carried out in order to optimize various parameters, indicated that 300 µg of DOTA-TATE is required for the formulation of patient dose of ^{177}Lu-DOTA-TATE (200 mCi) with >98% radiochemical yield using $^{177}LuCl_3$ having a minimum specific activity of 17.5 mCi/µg. Thus, each freeze-dried DOTA-TATE kit was formulated using 300 µg of DOTA-TATE peptide. Freeze-dried DOTA-TATE kits (ten numbers in each batch) were prepared according to the protocol: aqueous solution of DOTA-TATE was prepared by dissolving 3 mg of peptide in 3 mL of HPLC grade water. A solution of gentisic acid (300 mg) was prepared by dissolving it in sodium acetate buffer (0.5 M, 3 mL) by gentle warming and final pH was adjusted to ~5 by using 2 M NaOH. The peptide solution was then added to the solution of gentisic acid and the resultant solution was thoroughly mixed. The final solution was sterilized by passing through 0.22 µm Millipore filter and aliquoted into ten sterile glass vials, each vial containing 0.7 mL of the solution.

All the above procedures were carried out under aseptic conditions. Finally, the vials were frozen in dry ice at - 70˚C for 30 min and lyophilized under vacuum in a lyophilizer for 6-8 h, whereby the freeze-dried kits were obtained. Each kit vial consisted of 300 µg of DOTA-TATE, 30 mg of gentisic acid and 12.05 mg of sodium acetate. The kit vials were stored at 0˚C after lyophilization.

Kit formulation and evaluation of ^{177}Lu-DOTA-TATE

Therapeutic dose of ^{177}Lu-DOTA-TATE was prepared by addition of $^{177}LuCl_3$ (200 mCi) to the kit vial followed by addition of saline to make up the final volume of 1 mL. Subsequently, the kit was heated at 100˚C for 30 min. Radiochemical yield of ^{177}Lu-DOTA-TATE, as determined by reversed-phase high performance liquid chromatography (RP-HPLC) and paper chromatography, was >98% (Fig 3A). The radiolabeled product, ^{177}Lu-DOTA-TATE, when stored at room temperature, was observed to be stable till 3 days post reconstitution as there was no significant change in the HPLC profile.

The long term storage stability of kit vials, stored at 0˚C, was assessed by performing radiolabeling with $^{177}LuCl_3$ at periodic time intervals. The kits stored at 0˚C were found to be stable till 1 year post preparation (Fig. 3B).

The pharmacokinetics of ^{177}Lu-DOTA-TATE formulated using DOTA-TATE freeze-dried kits was studied by carrying out biodistribution studies in normal Swiss

mice. ^{177}Lu-DOTA-TATE was intravenously injected into the tail vein of each mice (n = 3). Animals were sacrificed at different time intervals (3 h, 1 d, 2 d and 7 d p.i) and activity associated with each organ/tissue was counted in flat type NaI(Tl) detector. The biodistribution study revealed rapid clearance of activity from blood and the other major organs except kidneys. More than 80% of injected activity got excreted out within 3 h p.i. and preferential route of excretion was observed to be renal pathway.

DOTA-TATE kits were clinically evaluated on formulation with $^{177}LuCl_3$ at Sri Venkateswara Institute of Medical Sciences (SVIMS, Tirupati). Therapeutic dose of ^{177}Lu-DOTA-TATE was prepared by addition of 200 mCi of $^{177}LuCl_3$ for radiotherapy of patients suffering from neuroendocrine cancers. Three doses using three kits were prepared and injected in patients presented with well differentiated NET's and associated metastasis. Single photon emission computed tomography (SPECT)/ computed tomography (CT) images in patients indicated high uptake of kit-formulated ^{177}Lu-DOTA-TATE in primary as well as metastatic sites (Fig. 4).

Conclusion

Single vial freeze-dried kits of DOTA-TATE, containing in-house synthesized DOTA-TATE peptide, were developed and successful formulation of therapeutic dose of ^{177}Lu-DOTA-TATE (200 mCi) was demonstrated. ^{177}Lu-DOTA-TATE could be prepared with >98% radiochemical purity and high stability using these kits. Preliminary clinical studies in patients suffering from NETs indicated high uptake of kit-formulated ^{177}Lu-DOTA-TATE in primary as well as metastatic sites. DOTA-TATE freeze-dried kits prepared using in-house synthesized peptide provides a convenient and cost-effective alternative for facile preparation of ^{177}Lu-DOTA-TATE in clinical setting.

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Acknowledgements

The authors are thankful to Dr. Anupam Mathur of BRIT for performing mass spectroscopy. The help provided by Dr. H. D.Sarma in performing bio distribution studies is being thankfully acknowledged. The authors are also thankful to Dr. T. C. Kalawat, SVIMS, Tirupati for carrying out the clinical evaluation studies.

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