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^{177}Lu-DOTATATE Peptide Receptor Radionuclide Therapy: Indigenously Developed Freeze Dried Cold Kit and Biological Response in In-Vitro and In-Vivo Models

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Abstract

Somatostatin receptors (SStR) based ^{177}Lu-DOTATATE therapy is known as one of the highly effective neuroendocrine tumors (NETs) treatment strategy. Development of DOTATATE freeze-dried kit for imaging and therapy of SStR positive NETs is a prime goal in neuroendocrine cancer research. The present work describes the development of ^{177}Lu-DOTATATE freeze dried cold kit for indigenous needs, through technology development fund (TDF) program offered by Higher Education Commission (HEC) Pakistan. The parameters for freeze dried kit production was optimized and tested the stored lyophilized cold kits for different time intervals after labeling with ^{177}Lu radioisotope. The effect of ligand to radionuclide ratio, pH and reaction time at 90°C was recorded. Five times greater molar concentration of ligand, pH 5 and 30 min reaction time were the effective reaction conditions for maximum radiochemical yield. The radiolabeling yield at 1 day, 1-week and 4-week post storing period showed ~100% radiochemical yield. The biodistribution study using rat model depicted the absence of non-targeted accumulation while glomerular filtration rate also explains the rapid renal washout. Cytotoxicity study showed quite favorable results for subjecting the radiopharmaceutical to clinical practice in Pakistan.

Keywords

^{177}Lu-DOTATATE, neuroendocrine tumors, NET, SStR, radiopharmaceuticals

Introduction

Molecular imaging technique (MIT) is well known to oncology setup due to its efficiency in imaging, staging and fixing the malignant diseases. MITs such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) are frequently practiced at oncology centers using target specific radiolabeled biological molecules for diagnosis of deep-seated infections and malignancies.[1] Gamma or positron emitter radionuclide such as technetium-99m (^{99m}Tc), indium-111 (^{111}In), gallium-68 (^{68} Ga) and fluorine-18 (^{18}F) labeled biological molecules are used for imaging process,[2-5] while beta or alpha emitter radionuclide such as leutitium-177 (^{177}Lu), yttrium-90 (^{90}Y), rhenium-188 (^{188}Re), actinium-225 (^{225}Ac) are used for therapeutic procedures.[6-9] A variety of radiolabeled regulatory peptides are in clinical trials and few are in clinical practice for imaging and therapy of NETs. NET imaging and therapy bases on peptide-receptor affinity.

NETs form a heterogeneous group of neoplasms that have ability to grow at any place of human body with the secretion of peptides and neuroamines. The gastroenteropancreatic endocrine tumors are the most common (~67.5%) among NETs including pancreas carcinoma (30%-40%), the small-intestine (15%-20%) and the rectum cancers

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(5%-15%) followed by bronchopulmonary tumors (25.3%).[10-12] An exclusive feature of these cancers/tumors is the overexpression of SStR which act as NET markers and the principle targets of radionuclide-labeled somatostatin peptide analogues for therapy.[13] Somatostatin is a naturally occurring hormone commonly known as growth hormone-inhibiting factor. It is cyclic peptide hormone bearing disulphide cyclic linkage with either 14 (SS-14) or 28 amino-acids (SS-28).[14] The former form predominant in brain while latter sequence produced by intestinal enteroendocrine cells. These receptors also express in the adrenals, and the pancreas. It mainly regulates the endocrine system via interaction with G-protein coupled SStR which mainly comprises of 5 subtypes (SStR1-5).[15] There are many neuroendocrine origin tissues that show overexpression of SStR in its malignancy state. NETs, predominantly characterized by the overexpression of any 1 of the 5 subtypes of SStR. Molecular imaging and therapy, based on targeting the SStR is justified with the coherence between the structure of the somatostatin analogue and SStR subtype.[16,17] Highly attractive somatostatin analogue that target SStR2 with its maximum efficiency ($\sim$100%) is [DOTA0, Tyr3, Thr8]-octreotide (DOTATATE).[18] SStR2 is overexpressed in a majority of NETs, including small-cell lung carcinomas (SCLCs), breast tumor tissues, and pancreatic cancer.[18-20] DOTATATE is being practiced clinically, either for imaging NETs by labeling with ^{111}In or ^{68} Ga, or for therapy by labeling with ^{177}Lu. This particular type of therpy which base on radiolabeled peptide and receptors interaction is termed as peptide receptor radionuclide therapy (PRRT). The procedure is gaining ample attention for its $\sim$100% efficiency to fix NETs, which on contrary are hard to treat either through surgery or chemotherapy. PRRT, however fix the malignancy at molecular level that is acomplished by using radionuclide labeled somatostatin peptide analogues which transfer therapeutic dose of betta or alpha radiations to cancer cells.[21]

^{177}Lu labeled DOTATATE has shown promising results in phase-3 clinical trials which sufficiently satisfy the requirements for FDA approval. Recently, Spain has approved the ^{177}Lu-DOTATATE therapy on the bases of its well tolerable efficiency in treating SStR2 positive NETs.[13] Currently Europe USA and few other developed countries offer ^{177}Lu-DOTATATE therapy at tolerable cost for thier peoples. In Pakistan the ^{177}Lu-DOTATATE therapy is also practicing in few oncology centers by importing freeze dried cold kit at very high cost-per-patient. The cost-per-patient could be reduced at lowest possible level by indigenous development of ^{177}Lu-DOTATATE freeze dried cold kit and production of $^{177}LuCl_3$ at high specific activity level. HEC-Islamabad through TDF scheme provided the opportunity to develop ^{177}Lu-DOTATATE freeze dried cold kit to fulfill the indigenous need at affordable cost per patient. This manuscript is a show-case of partial pre-clinical results to share effectiveness of indigenously developed ^{177}Lu-DOTATATE cold kit and providing the ground for further clinical tests and to initiate the process for regional approval for clinical practice in country.

Material and Methods

Materials

All the chemicals were of analytical grade and purchased from Sigma-Aldrich, Fisher Scientific and Alfa Aesar. Whatman 3 chromatographic paper was purchased from Agilent

Germany. [DOTA^0, Tyr^3, Thr^8]-octreotide were synthesized in GL-Biochem laboratory, China under sterile conditions. The ^{177}Lu radionuclide in the form of $^{177}LuCl_3$ was obtained from Pakistan Atomic Research Reactor-1 (PARR-1), PINSTECH, Islamabad with sufficiently good specific activity required for research purposes and animal study.

Formulation of Freeze Dried DOTATATE kit

In order to formulate freeze dried cold kit of DOTATATE, the radiochemical preparation was assessed at different reaction conditions and test the effect of ligand-radioactivity ratio, pH and reaction time to obtain maximum labeling yield. The optimized reaction conditions at which $\sim$100% labeling yield of ^{177}Lu-DOTATATE was obtained was used to prepare freeze dried kits. Brief description of freeze-dried DOTA-TATE kit preparation is summarized as follow; DOTATATE acetate was dissolved in ultrapure filtered water to prepare the stock solution having concentration 1μg/ μL and stored in 50 μL aliquots. A solution of gentisic acid was prepared by dissolving 40 mg of gentisic acid in 2 mL of 0.4 M acetate buffer in dark. Stock solution of ascorbic acid was prepared by dissolving 210 mg of ascorbic acid in 2 mL 0.4 M acetate buffer having pH 4.8 and stored the solution in dark. 50 μg of DOTATATE (1μg/ μL in water) was taken in to an amber vial and added 5 mg of gentisic acid along with 26 mg of ascorbic acid from stock solution. The resulting solution was mixed thoroughly and pH was adjusted to 5. The solution was passed through 0 .22 μm millipore filter and lyophilized for 24 h. The kits were stored at 4°C in refrigerator.

Production of $^{177}LuCl_3$

Production and radiochemical processing of ^{177}Lu in the form of $^{177}LuCl_3$ salt was carried out at Pakistan Atomic Research Reactor-1(PARR-1), PINSTECH, Islamabad, Pakistan. The $^{177}LuCl_3$ obtained from PARR-1 was used for preparation and optimization of cold kit parameters. Typically, 100 μg of isotopically enriched Lu_2O_3 target (52% in ^{176}Lu) was dissolved in concentrated HCl to get $LuCl_3$. The resulting solution was then dried in a quartz tube in desiccators and reconstituted with ultrapure water—dried it again, sealed the tube in aluminum sheet for irradiation. The sample was irradiated at a thermal neutron flux of 1.5×10^{14} ncm^{-2} s^{-1} for a period of 18 h. The irradiated target was allowed to cool for 12 h and dissolved in gently warm HCl solution (pH 3-4) followed by cooling to room temperature and filtering through 0.22 μm millipore filter

paper to obtain pure $^{177}LuCl_3$. Thus obtained $^{177}LuCl_3$ could be used for therapeutic dose of ^{177}Lu-DOTATATE.

Labeling of DOTATATE Kit With ^{177}Lu

The freeze dried cold kit of DOATATAE was reconstituted with sterile water and diluted to pH 5 followed by the addition of $^{177}LuCl_3$ solution. The mixture was heated to 90°C for 35 min to label the DOTATATE with ^{177}Lu. Different labeling reaction was performed by increasing DOTATATE concentration such as 1,2,3,4, or 5 times as compared to ^{177}Lu.

Quality Control Studies

Following the preparation of ^{177}Lu-DOTATATE and cooling at room temperature, the radiochemical mixture was subjected for quality control analysis using chromatography process.

Paper Chromatography Analysis

The radiochemical purity of ^{177}Lu-DOTATATE was determined by paper chromatography. The analysis was carried out by spotting an aliquot of 1 µL reaction mixture at 1.5 cm from one end of the 14 cm long chromatography paper strip (Whatman 3). The strip was developed using 50% acetonitrile aqueous solution. After drying the strip, it was scanned through Radio-TLC scanner having flat type NaI(Tl) detector. The R_f value was calculated through software B-SCAN.

Stability of ^{177}Lu-DOTATATE Freeze Dried Kits

The radiochemical yield of ^{177}Lu-DOTATATE which reconstituted using freeze dried cold kits which have been stored for 24 h, 1 week and 4 weeks, was investigated by incubating the radiopharmaceutical at room temperature for predefined time intervals through paper chromatography. The percent yield after radiolabeling reaction revealed that DOTATATE analogue remained stable in one month storing period.

Biodistribution Studies

Biodistribution pattern of ^{177}Lu labeled FDK of DOTATATE was studied in normal wistar rats each weighing 40-50 g in a group of 3-5. The ^{177}Lu-DOTATATE solution was further diluted with saline to 25 MBq activity/mL. An aliquot of 200 µL of ^{177}Lu-DOTATATE solution was then administration in animals through the tail vein of rats. For each time point, 3 animals were injected. The animals were given chloroform anesthesia prior to sacrifice at 2 h, 24 h, 48 h, and 72 h post-injection. Immediately, after animal sacrifice, blood was collected through cardiac puncture and counted the radioactivity using well-type NaI(Tl) scintillation counter. Different organs of the sacrificed animals were also excised, saline-washed, dried over filter paper, weighed and counted the radioactivity. The percentage of injected activity (%IA) accumulated in various organs was calculated. The activity excreted was indirectly determined from the difference between total injected activity (IA) and %IA accounted for all the organs.

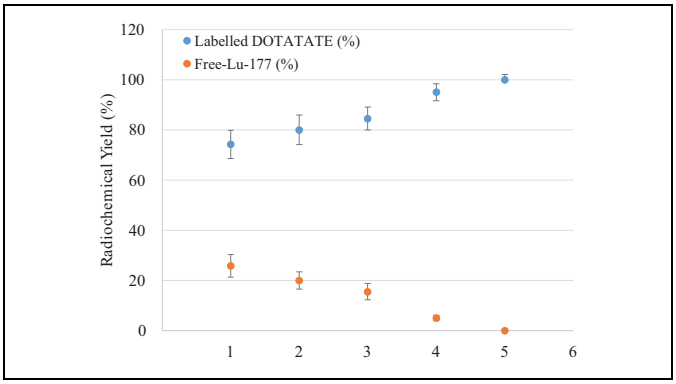

Figure 1. Effect of ligand concentration against fixed radioactivity of ^{177}LuCl.

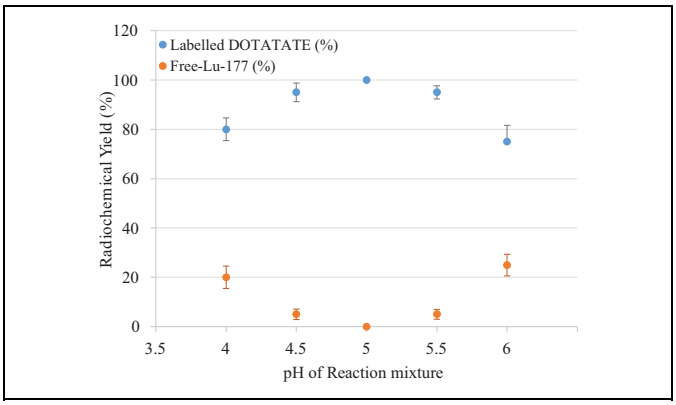

Figure 2. Effect of pH on radiochemical yield.

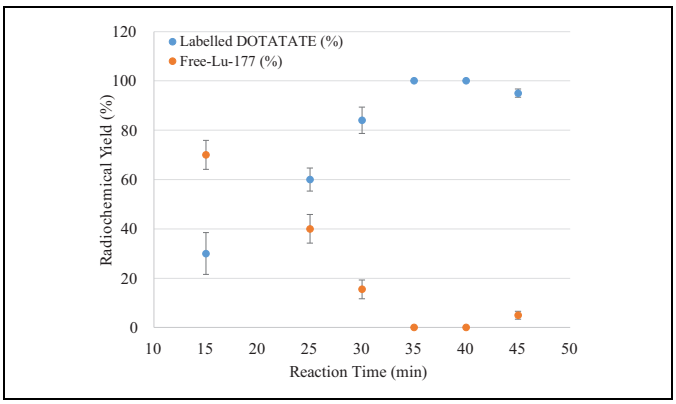

Figure 3. Effect of on reaction time on radiochemical yield.

Glomerular Filtration Rate

Glomerular filtration rate (GFR) study was performed using protocol as described previously published reports.[22] Briefly, an aliquot of 200 µL of ^{177}Lu-DOTATATE was administered into the ear vein of a group of 3 New Zealand white rabbits

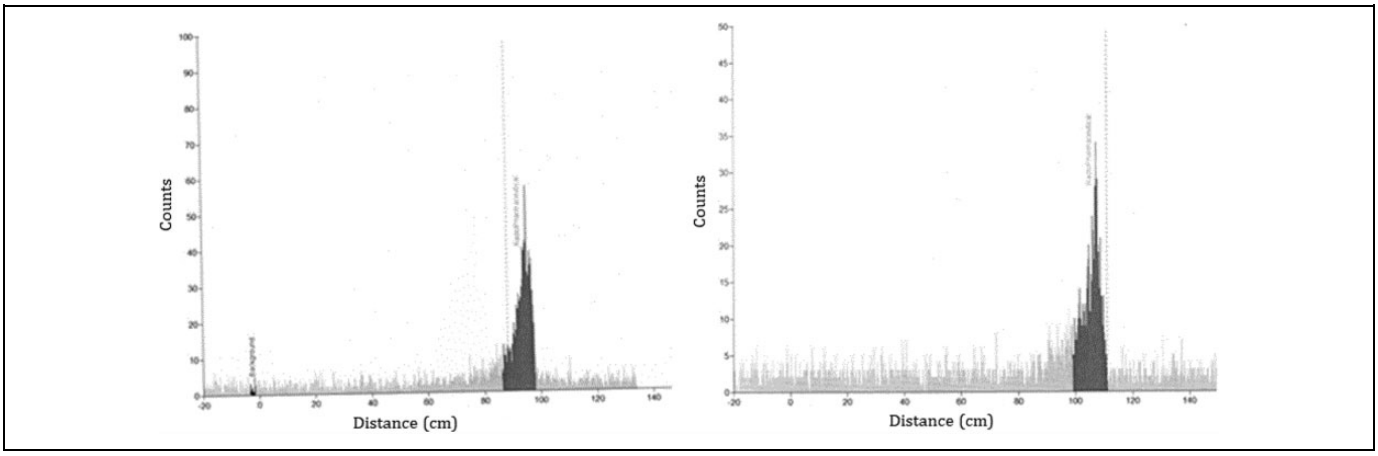

Figure 4. Radiochromatogram of ^{177}Lu-DOTATATE quality control developed in 2 different.

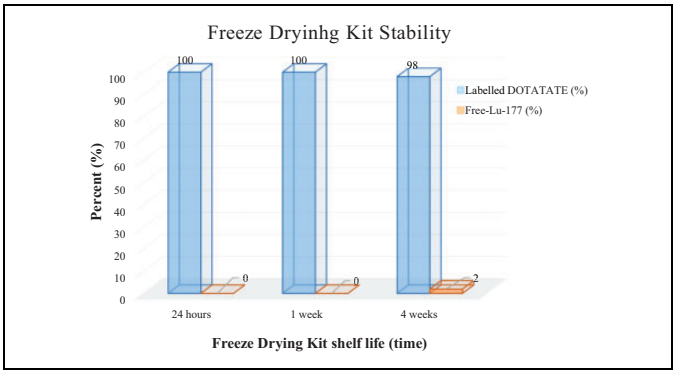

Figure 5. Freeze dried kit stability study by labeling with ^{177}Lu.

(kept under starvation conditions) early morning on the day of experiment. The GFR was analyzed with built-in software in SPECT camera software, and the urine activity was also calculated simultaneously with GFR counting.

Hematology, Cytotoxicity and Histopathology Studies

Cytotoxicity of ^{177}Lu-DOTATATE against the normal biological tissues was evaluated by injecting the labeled peptide to the New Zealand white rabbit each weighing about 2-3 Kg. The cytotoxicity in animals were studied in following 3 groups; i) using over-dose of the ^{177}Lu-DOTATATE in which 555 MBq was injected and sacrificed after 3 weeks (21 days) postinjection period, ii) using therapeutic dose of ^{177}Lu-DOTATATE according to weight of the rabbit and completed the 3 doses each after 1 month, and iii) injecting maximum dose of the cold DOTATATE. The pre-injection (Pr-I) and post-injection (Ps-I) rabbits' blood samples were collected for biochemical and hematology studies to analyze the systemic effect on blood parameters in all 3 conditions. All the animals were sacrificed and specific vital organs were excised. The tissues of heart, kidney and Lungs were taken and fixed with neutral formalin (10%), embedded in paraffin, and then manually sectioned with a microtome to obtain 4–5 mm-thick paraffin sections. Dewaxed sections were then stained with hematoxylin and eosin (H&E) method and observed under the light microscope for cytotoxicity and histopathology results.

Results

Quality Control Parameter Study and Radiochemical Yield

The radiochemical yield greatly affected by ligand to radionuclide ratio, pH and reaction time. In order to develop freeze dried DOTATATE kit and to achieve maximum radiochemical yield, and consequently maximum therapeutic advantages; the radiolabeling of ^{177}Lu with DOTATATE were studied in detail using different reaction conditions such as ligand to radionuclide ratio in which ligand concentration was increased from 1 to 5 times as compared to ^{177}Lu (Figure 1), pH in the range of 3.5 to 6.5 units (Figure 2) and 10 to 50 min reaction time (Figure 3) at 90°C reaction temperature. At each set of reaction conditions the reaction mixture was tested for radiochemical yield using paper chromatography. At optimized reaction conditions, the radiochromatogram of reaction mixture was developed in 2 mobile systems as shown in Figure 4.

Stability of DOTATATE Freeze Dried Kit

Shelf life stability of DOTATATE freeze-dried kit was assessed after 24 h, 1 week and 4 weeks of its preparation by labeling with ^{177}Lu. The results of radiochemical yield of labeled freeze dried kits are shown in Figure 5.

Biodistribution Study

Biodistribution of ^{177}Lu-DOTATATE was assessed in wister rates. Briefly, at 2, 24, 48 and 72 h post injection of 200 µL of an aliquot of ^{177}Lu-DOTATATE through tail vein, the rats were anesthetized, sacrificed, organ excised, washed and subjected for radioactivity counts. The data were analyzed and summarized in the form of graph as shown in Figure 6.

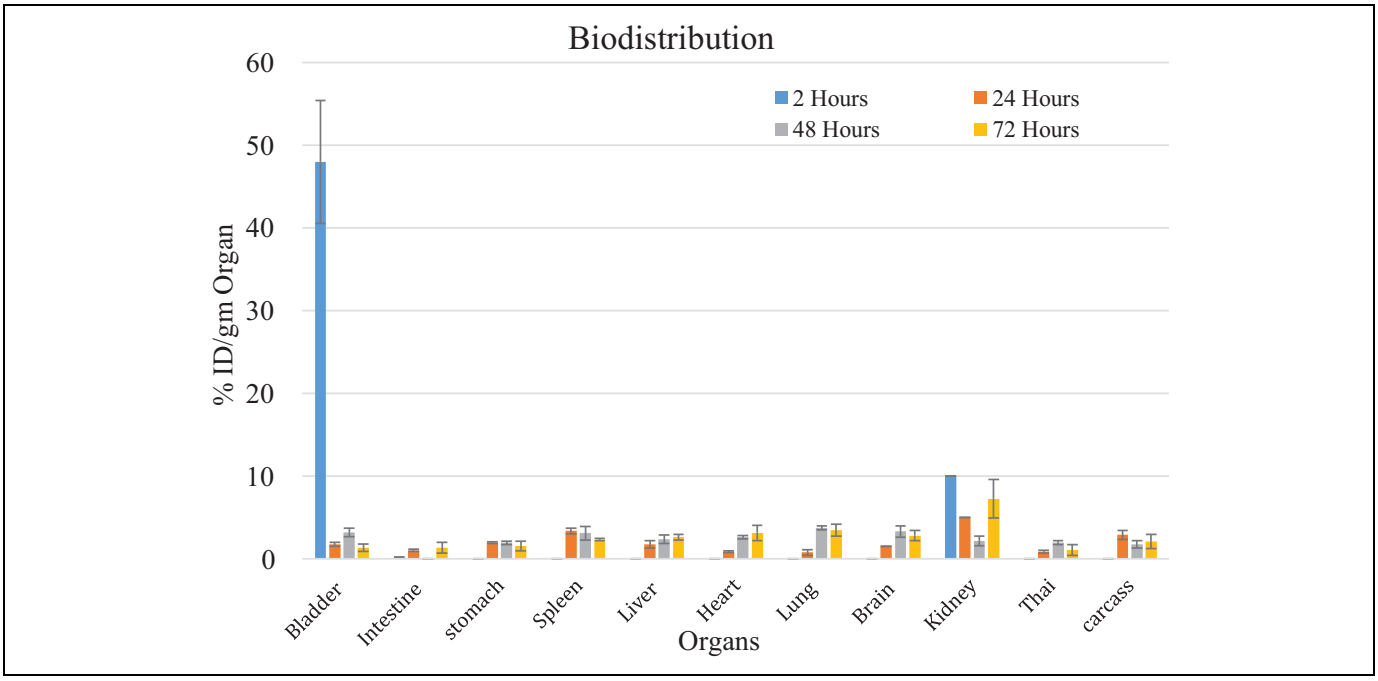

Figure 6. Biodistribution pattern of ^{177}Lu-DOTATATE in normal wister rats' models.

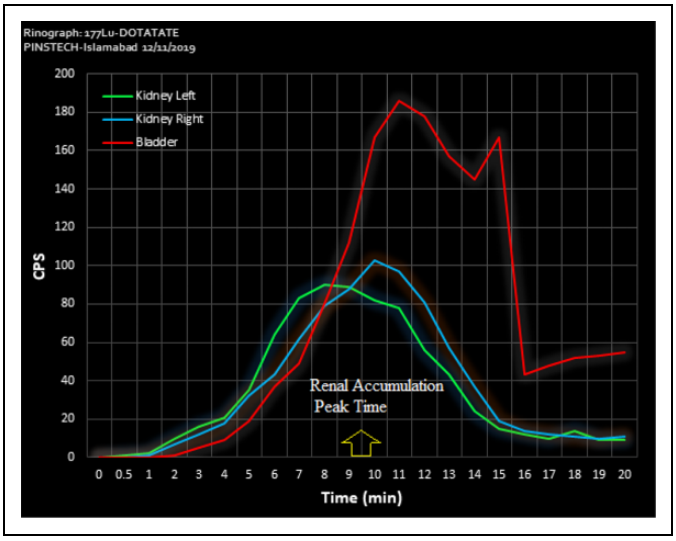

Figure 7. Glomerular filtration rate study to evaluate renal functioning and renal accumulation behavior of ^{177}Lu-DOTATATE.

GFR Study

GFR study was conducted to test the effect of indigenously developed ^{177}Lu-DOTATATE kit on renal function. Renal filtration rate was noted at regular intervals that was analyzed through built in GFR software in SPECT camera. The results of GFR study are shown in Figure 7.

Hematology, Cytotoxicity and Histopathology Study

Table 1 shows the hematology results of ^{177}Lu-DOTATATE administrated animal model. Due to the radionuclide involvement in cancer therapeutic procedures, it is more likely that the disturbance of blood parameters by ionizing radiations may take place. Further, the ionizing radiations can also cause cytotoxicity in animal body. The results of cytotoxicity study using histopathology are shown in Table 1 and Table 2; while the effect on tissues of different key organs are shown in Figure 8.

Discussion

Currently, ^{177}Lu-DOTATATE is gaining breathtaking attention in therapy of NETs followed by ^{68}Ga-DOTATATE mediated PET imaging for initial diagnosis, and selection of patients for PRRT.[23] The agent is under different clinical trials to get approval from respective authorities in different regions of world.[24-26] In Pakistan, we have also developed the freeze-dried kit of DOTATATE for imaging and therapy of NETs at affordable cost.

The quality control analysis for developing freeze-dried kit at different ligand to radionuclide ratio, pH and reaction time indicate 5 times higher ligand ratio as compared to radionuclide facilitate 100% labeling yield at pH 5 and 30 min reaction time at 90°C temperature. Less than this value increases the free radioactivity. Varying the pH and reaction time directly affect the labeling yield. At optimized reaction conditions 100% labeling yield guarantied the promising efficacy of therapeutic procedure. The freeze-dried kit was tested at 24 h, 1-week and 4-weeks of post-preparation/storing period. The results indicate consistency in radiochemical purity and stability of the kit which are the primary requirements for freeze dried kit to provide at remote areas or to store for number of week to utilize on demand for therapeutic procedures. The ^{177}Lu-labeling with freeze dried kit in intervals of weeks, analyzed using

Table 1. Blood Parameter Study to Evaluate Cytotoxicity of ^{177}Lu-DOTATATE and Free ^{177}LuCl3.

		^{177}Lu-DOTATATE (20 μg)		$^{177}LuCl_3$		
Sr. No.	Parameter	Pr-I	Ps-I	Pr-I	Ps-I	Normal range
1.	WBC /μl	4800	4700	8600	8400	4000-11000
2.	RBC m/μl	4.0	3.89	5.47	6.22	4.5-6.0
3.	Hemoglobin g/dl	11.6	11.4	12.9	12.3	
4.	Hematocrit %	30.0	32.3	35	36.4	40-50
5.	MCV fl	51	52.8	63.9	58.6	76-96
6.	MCH pg	21	19.4	23.6	19.8	27-31
7.	MCHC g/dl	32	31	36.9	32.3	32-36
8.	Platelets/μl	490000	489000	311000	545000	140000-450000
	Differentia Count					
9.	Neutrophils%	70	75	62	76	50-75
10.	Lymphocytes%	25	20	31	16	20-45
11.	Monocytes%	1	3	4	5	02-08
12.	Eosinophils%	1	2	3	3	0-6

Table 2. Liver and Kidney Function Parameters Study.

		^{177}Lu-DOTATATE		Free $^{177}LuCl_3$		
Sr. No.	Liver & kidney function parameters	Pr-I	Ps-I	Pr-I	Ps-I	Normal range
1.	Serum Bilirubin (total) (mg/dl)	0.5	0.7	0.5	0.7	~1.0
2.	Serum ALT(SGPT) (μ/L)	39	68	80	84	~46
3.	Serum AST(SGOT)(μ/L)	22	82	30	71	~35
4.	Serum ALP (μ/L)	134	111	98	114	Adults <258 children <600
5.	Serum uric acid (mg/dl)	3.2	4.2	5.1	3.3	~3.5-7
6.	Serum urea (μ/L)	42	3.7	38	32	~10-50
7.	Serum creatinine (μ/L)	1.1	0.8	1.1	0.7	~0.6-1.2

chromatography, the results show the promising ability to bind with ^{177}Lu with maximum labeling yield.

The ^{177}Lu-DOTATATE kit was subjected to biodistribution in animal model to record the accumulated activity in different body organs. All organs showed normal uptake which gradually washed-out from organ tissue. The normal uptake phenomenon of ^{177}Lu-DOTATATE is mainly due to its selectivity for target which indicated the efficacy of freeze dried kit as well. Kidneys, however showed slightly more accumulation of ^{177}Lu-DOTATATE and the blood creatinine level remain in the limit. Previously, reported data showed slight increase (11.1%) of blood creatinine level along with renal accumulation.[24] The whole biodistribution pattern, however not showed unusual accumulation which expresses that there were no overexpressed SStR cells/tissues.[27,28] The GFR results showed the peak time 8.1 and 9.8 min, percentage uptake 56.5% and 67.23% and GFR value 34.67 mL/min was calculated. The excretion rate of ^{177}Lu-DOTATATE indicated the normal renal filtration which indicate thevalidity of the kit for clinical study.[29] The GFR value of gold standard GFR agent (^{99m}Tc-DTPA) is 72 mL/min which is small organic molecule and non-proteinous in nature, but due to protein nature of DOTATATE the renal filtration was recorded slow.[7,22] The radiolabeled peptide therapy, however face the renal accumulation in general such as in case of imaging and therapy using radiolabeled minigastrin peptides[29] but in contrast to other radiolabeled peptides ^{177}Lu-DOTATATE shows rapid renal filtration which is the success point of ^{177}Lu-DOTATATE therapy.[30]

Cytotoxicity study of ^{177}Lu-DOTATATE kits studied after 21st day of its preparation, by collecting the blood of ^{177}Lu-DOTATATE administrated animal at Pr-I and Ps-I time points. The complete blood profile results indicated non-significant difference between Pr-I and Ps-I blood analysis. The minute difference in blood parameters is mainly due to ionizing potential of β-radiations, however it could be tolerating. The histopathology, study also showed the physiological changes as exist in hematology study of blood. The cytotoxicity was further analyzed by studying liver and kidney functioning parameters. The values of serum bilirubin remained within normal limit in Pr-I and Ps-I ^{177}Lu-DOTATATE and free $^{177}LuCl_3$ administration. Other parameters; serum ALP, serum uric acid, serum urea and serum creatinine remained in-range after administrating the ^{177}Lu-DOTATATE and free $^{177}LuCl_3$, while serum ALT (SGPT) and serum AST (SGOT) increased in

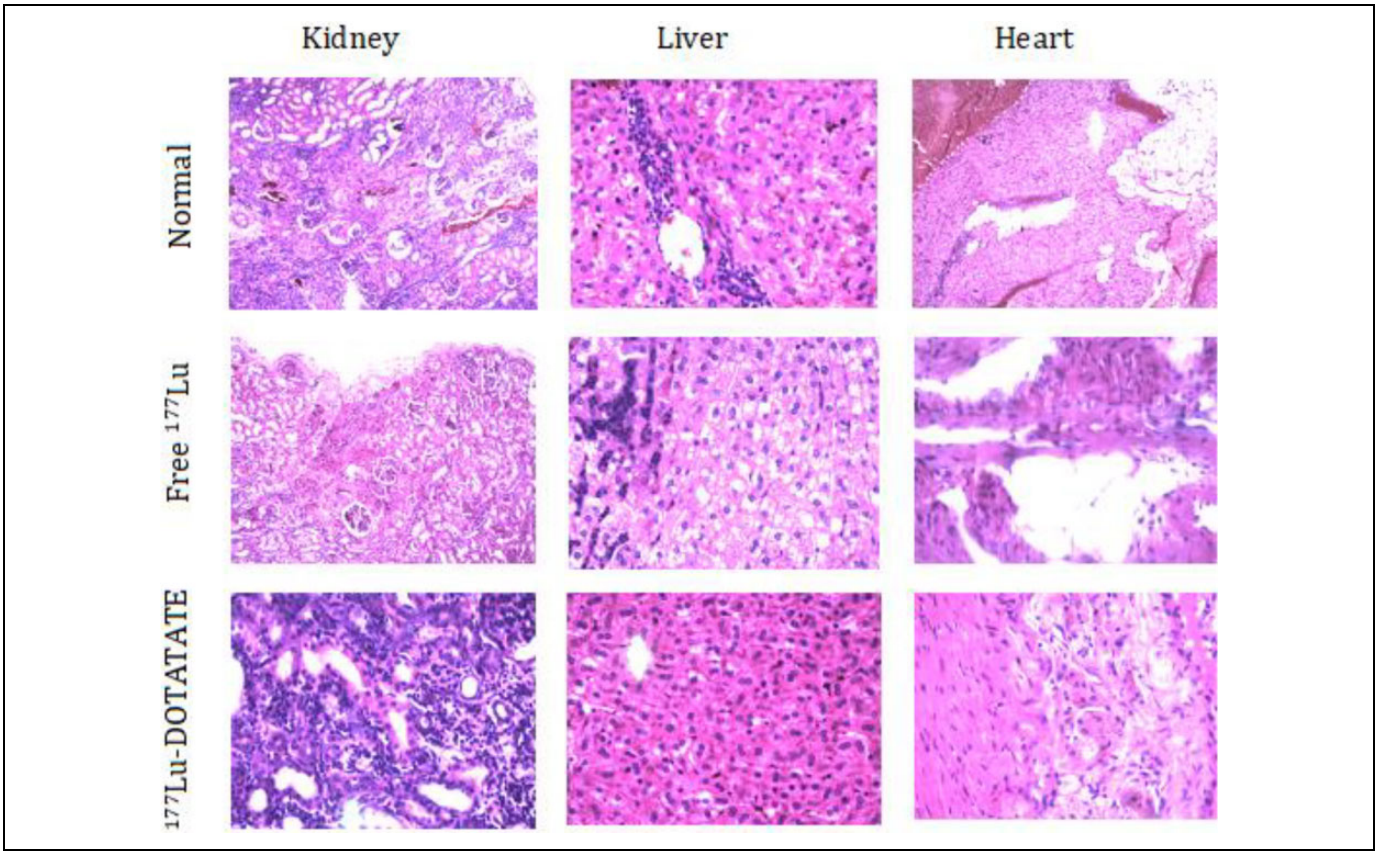

Figure 8. Histopathology images of control, free ^{177}Lu and ^{177}Lu-DOTATATE administrated kidney, liver and heart tissues.

either case of administration, ^{177}Lu-DOTATATE or free $^{177}LuCl_3$. Serum ALT(SGPT) was increased to 68 μ/L in case of ^{177}Lu-DOTATATE Ps-I and 84 μ/L in case of free $^{177}LuCl_3$ Ps-I analysis. The Serum ALT(SGPT) limit in blood is ∼46. The other liver parameter, serum AST(SGOT) also showed increase in its level i.e. 82 μ/L in case of 177Lu-DOTATATE Ps-I and 71 μ/L in case of free 177LuCl3 Ps-I as compared to its higher limit, ∼35 μ/L. The increase in both liver markers indicate minor liver damage. It can be considered that some damaging effect may possible due to β-radiations. Moreover, the liver was the most common site of NET metastasis (91.7%) which also indicate the presence of SStR which facilitate the accumulation of DOTATATE and hence the damaging.[13] The injection of free ^{177}Lu dose equivalent to the ^{177}LuDOTATATE dose, however showed comparable values. The histopathology analysis showed non-significant alteration in tissue morphology except some changes were seen in liver slides.

Conclusion

The radiochemical yield, stability study of freeze-dried kit, biodistribution, GFR values, and cytotoxicity results showed good development, however it needs further improvement to make the kit of world health standards and to submit the experimental data to "*Animal and Human Ethics Board*" of institute for further guidelines and experimentation to conduct Phase-I & II trials.

Authors' Note

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declaration of Conflicting Interests

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References

1. Ahpin C, Robin P, Samaan S, et al. Use of SPECT-CT in high-risk breast cancer patients results in a decrease in the number of downstream radiographic studies ordered as a function of increased reader confidence. *J Nucl Med*. 2020;61(supplement 1):1486.
2. Rasheed R, Usmani S, Raza Naqvi SA, Alkandari F, Marafi FA, Rasheed S. Optimization of radiation exposure in (18)F-sodium fluoride positron emission tomography-computed tomography in bone imaging: Quo Vadis. *Indian J Nucl Med*. 2019;34(2):157-159.
3. Naqvi SA, Matzow T, Finucane C, et al. Insertion of a lysosomal enzyme cleavage site into the sequence of a radiolabeled neuropeptide influences cell trafficking in vitro and in vivo. *Cancer Biother Radiopharm*. 2010;25(1):89-95.
4. Rasheed R, Usmani S, Al-Kandari F, Mansha A, Farooq Z, Naqvi SAR. Radiosynthesis and clinical evaluation of 68Ga-DOTANOC as somatostatin receptor positive benign sub-dural meningioma PET imaging agent. *Pak J Pharm Sci*. 2020;33(3(Suppl)):1277-1280.
5. Rasheed R, Raza Naqvi SA, Siraj R, et al. Development of 99mTc-SDP-choline SPECT radiopharmaceutical for imaging of cerebrovascular diseases. *Pak J Pharm Sci*. 2020;33(1(Supplementary)):241-244.
6. Rasheed R, Usmani S, Naqvi SAR, Alkandari F, Marafi F. Alpha therapy with (225)actinium labeled prostate specific membrane antigen: reporting new photopeak of 78 kilo-electron volts for better image statistics. *Indian J Nucl Med*. 2019;34(1):76-77.
7. Rasheed R, Tariq S, Naqvi SA, et al. (177) Lu-5-Fluorouracil a potential theranostic radiopharmaceutical: radiosynthesis, quality control, biodistribution, and scintigraphy. *J Labelled Comp Radiopharm*. 2016;59(10):398-403.
8. Lepareur N, Lacœuille F, Bouvry C, et al. Rhenium-188 labeled radiopharmaceuticals: current clinical applications in oncology and promising perspectives. *Front Med*. 2019;6:132.
9. Hosono M, Ikebuchi H, Kinuya S, et al. Manual on the proper use of yttrium-90-labeled anti-P-cadherin antibody injection for radionuclide therapy in clinical trials. *Ann Nucl Med*. 2019;33(11):787-805.
10. Yao J, Hassan M, Phan A, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol*. 2008;26(18):3063-3072.
11. Garcia-Carbonero R, Capdevila J, Crespo-Herrero G, et al. Incidence, patterns of care and prognostic factors for outcome of gastroenteropancreatic neuroendocrine tumors (GEP-NETs): results from the National Cancer Registry of Spain (RGETNE). *Ann Oncol*. 2010;21(9):1794-1803.
12. Johnbeck CB, Knigge U, Kjær A. PET tracers for somatostatin receptor imaging of neuroendocrine tumors: current status and review of the literature. *Future Oncol*. 2014;10(14):2259-2277.
13. Abou Jokh Casas E, Pubul Núñez V, Anido-Herranz U, et al. Evaluation of (177)Lu-Dotatate treatment in patients with metastatic neuroendocrine tumors and prognostic factors. *World J Gastroenterol*. 2020;26(13):1513-1524.
14. Mull RW, Harrington A, Sanchez LA, Tal-Gan Y. Cyclic peptides that govern signal transduction pathways: from prokaryotes to multi-cellular organisms. *Curr Top Med Chem*. 2018;18(7):625-644.
15. Cakir M, Dworakowska D, Grossman A. Somatostatin receptor biology in neuroendocrine and pituitary tumours: part 1—molecular pathways. *J Cell Mol Med*. 2010;14(11):2570-2584.
16. Qian ZR, Li T, Ter-Minassian M, et al. Association between somatostatin receptor expression and clinical outcomes in neuroendocrine tumors. *Pancreas*. 2016;45(10):1386-1393.
17. Reubi JC, Maecke HR. Peptide-based probes for cancer imaging. *J Nucl Med*. 2008;49(11):1735-1738.
18. Kharmate G, Rajput PS, Lin Y-C, Kumar U. Inhibition of tumor promoting signals by activation of SSTR2 and opioid receptors in human breast cancer cells. *Cancer Cell Int*. 2013;13(1):93.
19. Lehman JM, aMassion PP. Somatostatin receptor 2 targeting in small cell lung carcinoma: perspectives. *Oncotarget*. 2019;10(46):4727-4730.
20. Guenter R, Aweda T, Carmona Matos DM, et al. Overexpression of somatostatin receptor type 2 in neuroendocrine tumors for improved Ga68-DOTATATE imaging and treatment. *Surgery*. 2020;167(1):189-196.
21. Jahn U, Ilan E, Sandström M, Garske-Román U, Lubberink M, Sundin A. 177Lu-DOTATATE peptide receptor radionuclide therapy: dose response in small intestinal neuroendocrine tumors. *Neuroendocrinology*. 2020;110(7-8):662-670.
22. Khan NUH, Naqvi SAR, Roohi S, Sherazi TA, Khan ZA, Zahoor AF. Technetium-99 m radiolabeling and biological study of epirubicin for in vivo imaging of multi-drug-resistant *Staphylococcus aureus* infections via single photon emission computed tomography. *Chem Biol Drug Des*. 2019;93(2):154-162.
23. Ivanidze J, Roytman M, Sasson A, et al. Molecular imaging and therapy of somatostatin receptor positive tumors. *Clin Imaging*. 2019;56:146-154.
24. Demirci E, Kabasakal L, Toklu T, et al. 177Lu-DOTATATE therapy in patients with neuroendocrine tumours including high-grade (WHO G3) neuroendocrine tumours: response to treatment and long-term survival update. *Nucl Med Commun*. 2018;39(8):789-796.
25. Sitani K, Parghane RV, Talole S, Basu S. Long-term outcome of indigenous (177)Lu-DOTATATE PRRT in patients with metastatic advanced neuroendocrine tumours: a single institutional observation in a large tertiary care setting. *British J Radiol*. 2021;94(1117):20201041.

26. Jaiswal SK, Sarathi V, Memon SS, et al. 177Lu-DOTATATE therapy in metastatic/inoperable pheochromocytoma-paraganglioma. *Endocr Connect*. 2020;9(9):864-873.
27. Desai H, Borges-Neto S, Wong TZ. Molecular imaging and therapy for neuroendocrine tumors. *Curr Treat Options Oncol*. 2019;20(10):78.
28. Mittra ES. Neuroendocrine tumor therapy: (177)Lu-DOTATATE. *AJR Am J Roentgenol*. 2018;211(2):278-285.
29. Rizvi SFA, Naqvi SAR, Roohi S, Sherazi TA, Rasheed R. (177)Lu-DOTA-coupled minigastrin peptides: promising theranostic agents in neuroendocrine cancers. *Mol Biol Rep*. 2018; 45(6):1759-1767.
30. Sundlöv A, Sjögreen-Gleisner K, Svensson J, et al. Individualised (177)Lu-DOTATATE treatment of neuroendocrine tumours based on kidney dosimetry. *Eur J Nucl Med Mol Imaging*. 2017;44(9):1480-1489.