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Biodistribution Studies of ^{177}Lu-DOTATOC in Mouse Tumor Model: Possible Utilization in Adenocarcinoma Breast Cancer Treatment

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***Abstract*—**Despite the appropriate characteristics of ^{177}Lu and DOTATOC, to our best knowledge, the therapeutic benefit of ^{177}Lu-DOTATOC complex in breast cancer has not been reported until now. In this study, biodistribution of ^{177}Lu-DOTA-TOC in mouse tumor model for evaluation of possible utilization of this complex in breast cancer treatment was investigated.^{177}Lu was prepared with the specific activity of 2.6-3 GBq.mg^{-1} and radionuclidic purity higher than 99%. The radiolabeled complex was prepared in the optimized conditions with the radiochemical purity higher than 99%. The final solution was injected to the BALB/c mice with adenocarcinoma breast cancer. The biodistribution results showed major accumulation in the kidneys as the major excretion route and the somatostatin receptor-positive tissues such as pancreas compared with the other tissues. Also, significant uptake was observed in tumor even in longer time after injection. According to the results obtained in this research study, somatostatin receptors expressed in breast cancers can be targeted with DOTATOC analogues especially with ^{177}Lu-DOTATOC as an ideal therapeutic agent.

***Keywords*—**^{177}Lu, DOTATOC, adenocarcinoma, breast cancer, BALB/c mice.

I. Introduction

NOWADAYS, radiolabeled peptides are used to target the specificity receptors overexpressed on tumors with high affinity in the procedure called peptide receptor radionuclide therapy (PRRT). In fact, in PRRT, the oligopeptides are designed to target cellular proteins, commonly cell surface receptors such as the somatostatin receptor (SSTR) [1]. SSTRs are expressed on a wide range of human tumours including neuroendocrine, breast, lung, lymphomatic tissue and nervous system; so they can be potential targets for PRRT [2], [3].

Breast cancer is one of the most spread malignancies among different cancers in women. Unfortunately, this type of cancer is increasing over the past decades and a review of National Cancer Institute data shows invasive breast cancer incidence was 24% higher in 2007 than in 1976 [4]. Monoclonal antibodies (mAbs) have increasingly been applied for Radio Immune Scintigraphy (RIS) and Radio Immune Therapy (RIT) of breast cancer [5].

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A series of octreotide analogues were synthesized and used for somatostatin expressing tumour targeting. Octreotide is an eight amino acids synthetic peptide analogue developed with higher elimination half-time compared with native somatostatin analogues [6]. Among the octreotide analogues, [DOTA-DPhe1, Tyr3] octreotide (DOTATOC), where Tyr3-octreotide is the modified octreotide and DOTA (1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid) is the bifunctional chelating molecule, as a derivatized somatostatin analogue peptide indicated advantageous properties in tumour models [7], [8]. Recently, various radiolabeled complexes of DOTATOC such as ^{68}Ga-DOTATOC, ^{90}Y-DOTATOC and ^{177}Lu-DOTATOC have been introduced as valuable options for diagnostic and therapeutic purposes [8]-[10].

^{177}Lu with a physical half-life of 162 h (6.73 days) decays by the emission of γ and β$^-$ rays while maximum and mean β-particle energies are 0.498 and 0.133 MeV, respectively, which are much lower compared with ^{90}Y. Also, this radionuclide has two main gamma emission lines with low abundance [113 keV (6%) and 208 keV (11%)] which permits scintigraphy and subsequent dosimetry during the treatment [1]. According to the desirable characteristics of ^{177}Lu, a wide range of ^{177}Lu radiopharmaceuticals have been developed [11]-[13]. However, the optimized production of ^{177}Lu-DOTATOC as well as its efficacy in the therapy of patients with neuroendocrine tumors has been reported formerly [9], [14]; more studies on the benefit of ^{177}Lu-DOTA-TOC as the adjuvant therapy in breast cancer are still needed. In this study, to investigate the possible utilization of ^{177}Lu-DOTA-TOC in breast cancer treatment, the radiolabeled complex was prepared in the optimized conditions and its biodistribution in the mice with adenocarcinoma breast cancer was studied.

II. Materials and Methods

A. Production and Quality Control of ^{177}Lu

Lutetium-177 was produced via ^{176}Lu(n,γ)^{177}Lu reaction at TRR and according to the previously reported literature [15]. The radionuclide purity of the solution was checked by means of an HPGe spectrometer for the detection of various interfering gamma emitting radionuclides. The radiochemical purity of the solution was evaluated by instant thin layer chromatography (ITLC) method using two different solvent systems [A: 10 mmol.L-1 diethylene triamine pentaacetic acid (DTPA) at pH.5 and B: 10% ammonium acetate:methanol (1:1)].

B. Production and Quality Control of ^{177}Lu-DOTATOC

Briefly, a stock solution of DOTATOC in the diluted water with the concentration of 1 µg/µL was prepared. 150 µL of the stock solution was added to the vial containing 7 mCi of $^{177}LuCl_3$ solution and the pH was adjusted to 4. The reaction vial was then heated to 95 °C for 30 min and then the solution was passed through a C18 Sep-Pak column.

Radiochemical purity of the radiolabeled complex was checked using both ITLC and HPLC method. Paper chromatography was performed utilizing Whatman No. 3 paper and 0.9% NaCl as the mobile phase.

C. Stability Studies of ^{177}Lu-DOTATOC

The stability of the complex in room temperature and in human serum was studied up to 48 h by ITLC method using 0.9% NaCl as the mobile phase and Whatman No. 3 paper as the stationary phase.

D. Mouse Model with Breast Tumor

The tumor was established by subcutaneous implantation of spontaneous breast carcinoma tumor fragments (2-3 mm^3) in the right side of the abdominal region (Flank) of inbred female BALB/c mice (15-26 g, 6-8 weeks old, Pasteur Institute, Tehran, Iran). The studies were performed when the tumor volume reached 70-80 mm^3.

E. Biodistribution of the Radiolabeled Complex in Tumoral Mice

The final ^{177}Lu-DOTATOC solution was passed through 0.22 µm biological filter for sterilization and pH was adjusted to 7 by means of 0.9% normal saline. Then, 100 µL of the final solution was injected intravenously into mice through their tail vein. The animals were sacrificed at the exact time intervals (2, 4, 24 and 48 h) after injection (three mice for each interval time). The tissues were weighed and their activities were measured with a calibrated HPGe detector. %ID/g for each tissue was calculated by dividing the activity of each tissue to the decay corrected injected activity and the mass of each tissue.

III. Results and Discussions

A. Production and Quality Control of ^{177}Lu

^{177}Lu was prepared with the specific activity of 2.6-3 $GBq.mg^{-1}$ and radio nuclidic purity of more than 99% (Fig. 1). The result of radiochemical purity of the $^{177}LuCl_3$ solution showed the radiochemical purity of higher than 98% (Fig. 2).

B. Production and Quality Control of ^{177}Lu-DOTATOC

Radiochemical purity of the radiolabeled complex was checked using both HPLC and ITLC methods. In this case, ITLC showed radiochemical purity of higher than 99% (Fig. 3). HPLC analysis showed that the fast eluting compound was hydrophilic $^{177}LuCl_3$ cation (0.85 min), while ^{177}Lu-DOTATOC with high molecular weight was eluted after 4.4 min (Fig. 4).

C. Stability Studies of ^{177}Lu-DOTATOC

The radiochemical purity of the complex remained more than 98% after 48 h of preparation. Also the stability of the complex in human serum at 37 °C showed no decrease in the radiochemical purity even after for 48 h (Fig. 5).

D. Biodistribution of the Radiolabeled Complex in Tumoral Mice

The percentage of injected dose per gram (%ID/g) of ^{177}Lu-DOTATOC for different organs up to 168 h after injection was calculated (Fig. 6). The results demonstrated significant uptake in SSTR-positive tissues. Approximately no activity was found after 2 h in the blood samples due to the rapid clearance from blood circulation.

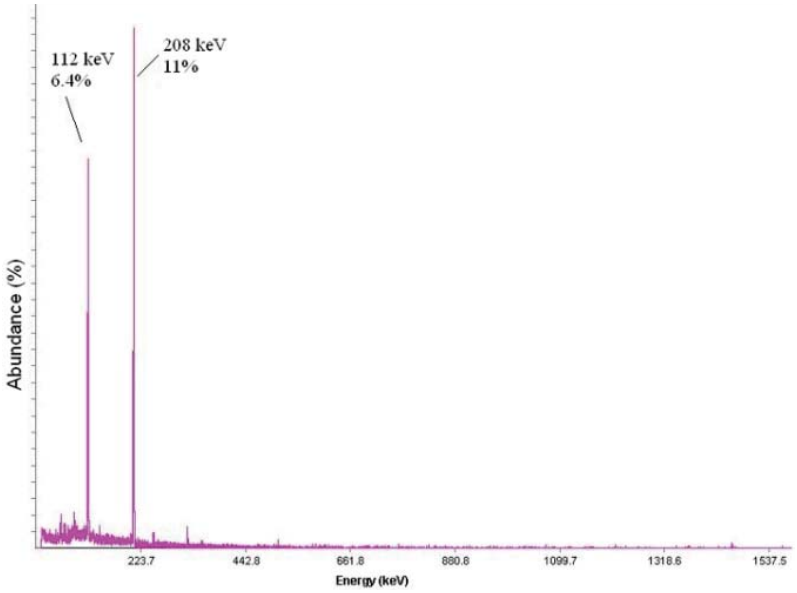
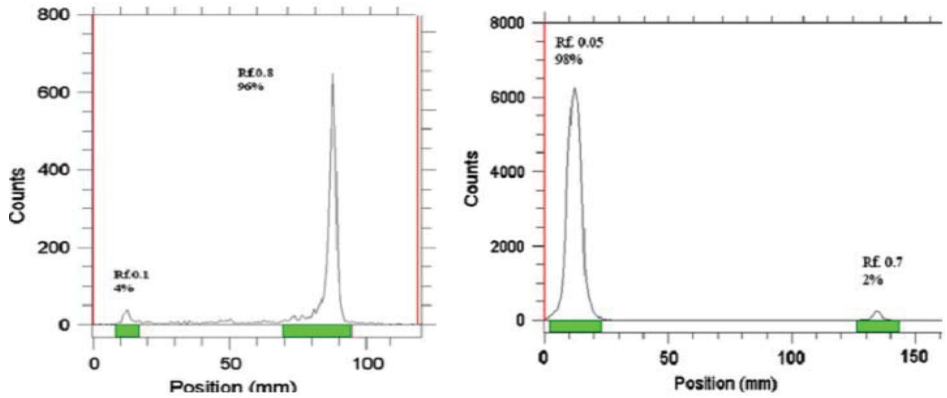
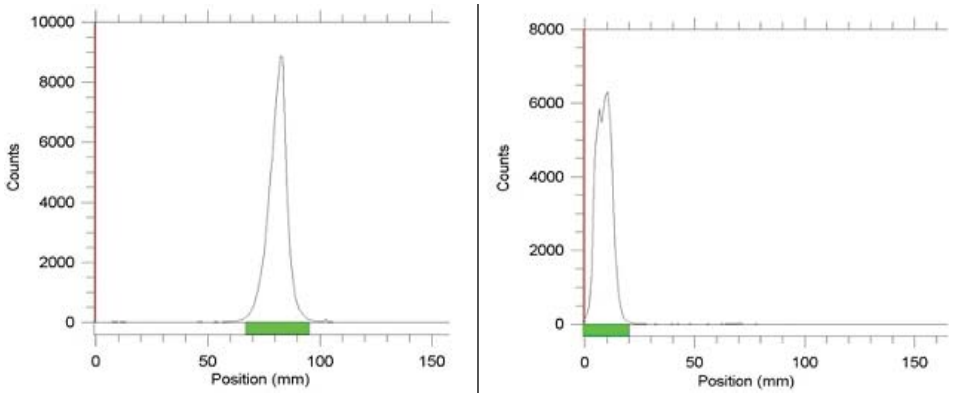


Fig. 1 HPGe spectrum of $^{177}LuCl_3$



(a) (b)

Fig. 2 ITLC chromatograms of $^{177}LuCl_3$ in 10 mM DTPA (a) and 10% ammonium acetate:methanol (1:1)solution (b) on Whatman No. 2 paper



(a) (b)

Fig. 3 ITLC chromatograms of $^{177}LuCl_3$ (a) and ^{177}Lu-DOTATOC solution (b) on Whatman No. 3 paper using 0.9% NaCl as the mobile phase

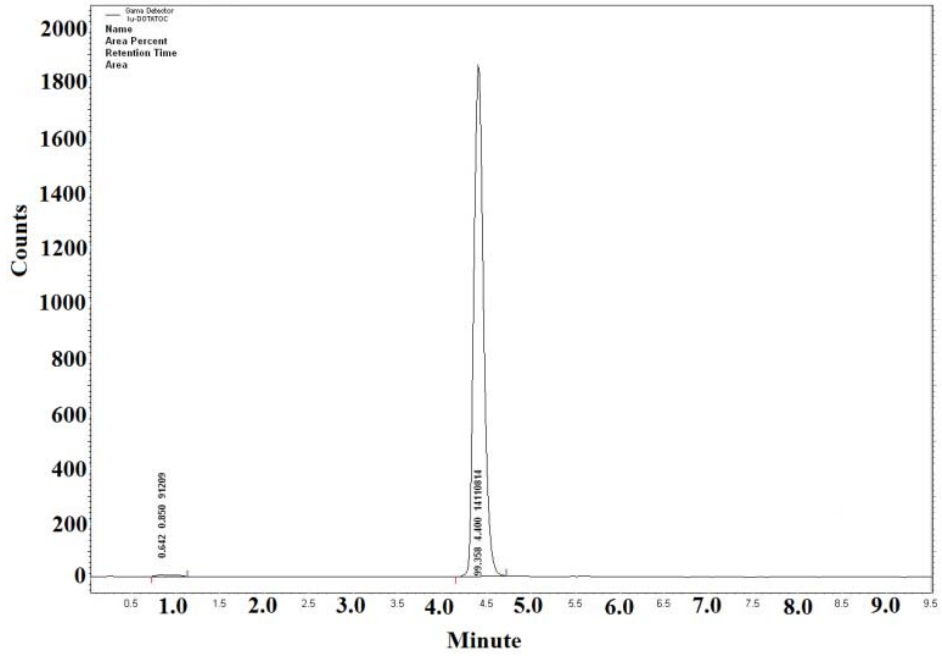


Fig. 4 HPLC chromatogram of ^{177}Lu-DOTATOC

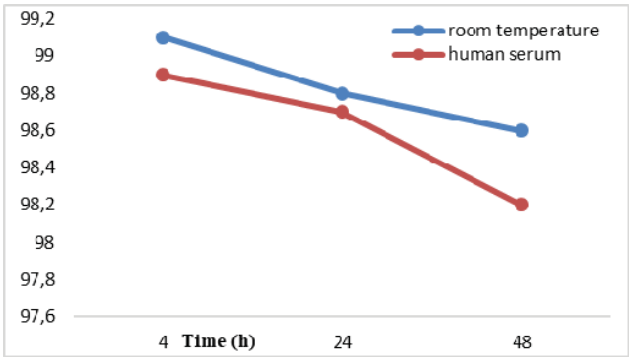


Fig. 5 Radiochemical purity of ^{177}Lu-DOTATOC in room temperature and in human serum at 37 °C

Considerable accumulation was observed in tumor at all intervals. On the other hand, the kidneys indicated considerable uptake as the major excretion route which is consistent with ^{90}Y-DOTATOC where the kidney has been introduced as the dose limiting organ [16]. Also, for better comparison, the tumor/kidney, tumor/liver, tumor/spleen and tumor/lung uptake ratio were calculated (Table I). As it can be seen in Table I, tumor to critical organs uptake ratio increases with time which shows the clearance of the complex from these organs.

Tumor to liver, lung and spleen uptake ratio is considerable, however, the kidney uptake is higher than tumor until 72 h. Considering these results, the kidney is the dose limiting organ and the amount of injected activity should be determined with regards to the absorbed dose delivered to kidney.

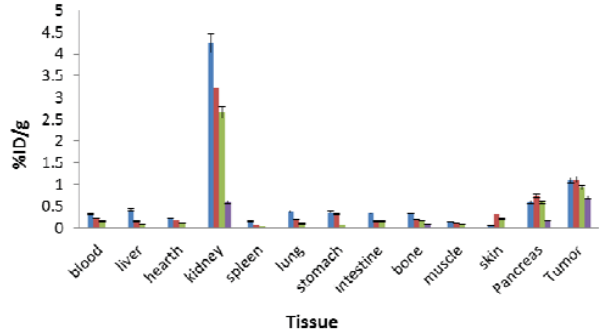


Fig. 6 Percentage of injected dose per gram (%ID/g) at 4, 24, 72 and 168 h after intravenously injection of ^{177}Lu-DOTATOC into tumoral mice

TABLE I
TUMOR TO CRITICAL ORGANS UPTAKE RATIO

	4 h	24 h	72 h	168 h
Tumor/kidney	0.40	0.54	0.54	1.80
Tumor/liver	2.66	7.15	10.05	45.19
Tumor/lung	2.89	5.66	9.05	108.42
Tumor/Spleen	7.22	14.13	18.97	81.86

According to the significant uptake of tumor as well as its durability in tumor site, the radiolabeled complex can be utilized in breast tumor treatment, however, further studies on its usefulness in the management of adenocarcinoma breast cancer is needed.

IV. CONCLUSION

In this research, ^{177}Lu-DOTATOC radiolabeled complex was prepared successfully with radiochemical purity of higher than 99% in the optimized conditions. The final complex was injected to the BALB/c mice with adenocarcinoma breast cancer. The kidneys indicated considerable uptake because of the high water solubility of the complex. Also, SSTR-positive tissues such as pancreas would have more uptake compared with the other tissues. The significant uptake of tumor as well as its durability in tumor site in longer time approved the possible usage of the complex in breast cancer treatment.

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