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Absorbed Dose Estimation of ^{177}Lu-DOTATOC in Adenocarcinoma Breast Cancer Bearing Mice

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***Abstract*—**In this study, the absorbed dose of human organs after injection of ^{177}Lu-DOTATOC was studied based on the biodistribution of the complex in adenocarcinoma breast cancer bearing mice. For this purpose, the biodistribution of the radiolabelled complex was studied and compartmental modeling was applied to calculate the absorbed dose with high precision. As expected, ^{177}Lu-DOTATOC illustrated a notable specific uptake in tumor and pancreas, organs with high level of somatostatin receptor on their surface and the effectiveness of the radio-conjugate for targeting of the breast adenocarcinoma tumors was indicated. The elicited results of modeling were the exponential equations, and those are utilized for obtaining the cumulated activity data by taking their integral. The results also exemplified that non-target absorbed-doses such as the liver, spleen and pancreas were approximately 0.008, 0.004, and 0.039, respectively. While these values were so much lower than target (tumor) absorbed-dose, it seems due to this low toxicity, this complex is a good agent for therapy.

***Keywords*—**Breast cancer, compartmental modeling, ^{177}Lu, dosimetry.

I. Introduction

UNSUCCESSFUL treatment and insufficient control of metastatic diseases with conventional cancer therapy methods, led to development of new therapeutic modalities, for instance targeted radionuclide therapy (TRT).

Nowadays, abundant availability of radiolabeled somatostatin analogs opened a new way to treat some types of tumors with high expression of somatostatin receptor (sstr) and treatment by this method has a profound usage in oncology [1]. However, up to now, much attention focused on tumors expressing sstr2, such as neuroendocrine tumors (NETs), but it can be also be used for the therapy of the other neuroendocrine origin tumors with sstr as well as breast cancer [2].

As a matter of fact, in the researches of the America Cancer Society, about 12 percent of women during their lifetime will face with breast cancer which is the second leading cause of cancer death in women. Most breast cancers are often an adenocarcinoma, a type of carcinoma that initiates in glandular tissue [3].

Clinical studies about patients treated with sstr-targeted [DOTA0-Tyr3]octreotide (DOTATOC) labeled with low linear energy transfer (LET) β-emitters have illustrated the overall response rates in the range of 15–33% [4].

According to the many reports in last two decades, ^{68}Ga, ^{111}In, ^{90}Y or ^{177}Lu radiolabeled peptides are useful tools for generation of radiopharmaceuticals [5]. The number of researches was upon the use of [^{68}Ga]DOTA-TOC in patients with metastatic neuroendocrine tumors and comparison it with [^{111}In]DOTA-TOC and [^{18}F]FDG [6], [7]. In addition, the results have been demonstrated that ^{177}Lu-DOTATATE, ^{177}Lu-DOTATOC and ^{90}Y-DOTATOC played an increasingly prominent role in the treatment of NETs [8], [9].

The physical properties of ^{177}Lu offer intermediate advantages between ^{90}Y and ^{111}In. ^{177}Lu (E_β max = 0.497 MeV, half-life = 6.73 day and tissue penetration range max = 2 mm) as a shorter beta emitter is a suitable candidate radionuclide for targeting small tumors (~2 cm) and metastases, allows to perform therapy as well as imaging and dosimetry processes [10].

Generally, calculating dose of target organs is carried out using MIRD method. OLINDA/EXM software is utilized for determining accumulated activity ($\tilde{A}$) in organs of interest [11]. An alternative approach to the determination of cumulated activity and next dosimetry is the mathematical model. Compartmental modeling is the prevalent method for describing the uptake and clearance of radiopharmaceuticals in tissues. Another purpose of this model is to define the relationship between the measurable data and the physiological parameters that affect the uptake and metabolism of the radiopharmaceutical [12]-[14].

Accordance to the proper characteristics of DOTATOC and ^{177}Lu, in the present study, after preparing ^{177}Lu-DOTATOC at the optimal conditions, biodistribution of the compound was assayed for treatment of adenocarcinoma breast cancer in bearing BALB/c mice. The results have demonstrated that ^{177}Lu-DOTATOC is as a profitable selection for therapy of the tumors. Due to the vital role of internal dosimetry before and during therapy, it seems required the effort to improve the accuracy and rapidity of dosimetric calculation. For this reason, a method was accomplished to calculate the absorbed dose through mixing between compartmental model, animal dosimetry, and extrapolated data from animal to human.

II. Materials and Methods

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

The low-LET β-emitting ^{177}Lu was obtained by the neutron irradiation of 1 mg of enriched Lu_2O_3 at TRR, and then was dissolved in 200 μL of 1.0 M HCl to be inform of [^{177}Lu]chloride in optimal grade and diluted to the appropriate volume with ultra-pure water. The radionuclidic purity of the

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solution was measured by γ-ray spectroscopy on an HPGe detector basing on the major photons of ^{177}Lu. The radiochemical purity of the solution using ITLC method was performed on the above-prepared quality control mixture. For this purpose, 5 µl of the solution was spotted on Whatman No. 3 paper and developed in two solvent systems [A: 10 mmol.L-1 diethylene triamine pentaacetic acid (DTPA) at pH=5 and B: 10% ammonium acetate:methanol (1:1)].

^{177}Lu-DOTATOC was prepared according to the previously reported literature [15]. 115 µg/µl DOTATOC was added to the vial containing 7 mCi of $^{177}LuCl_3$, while the pH of the mixture was set to 4 utilizing 0.4 M sodium acetate. The reaction mixture was heated in 90 °C water bath for 30 min. The radiochemical purity of the mixture was then surveyed by ITLC method.

B. Biodistribution of ^{177}Lu-DOTATOC in Mice Bearing Breast Adenocarcinoma Tumors

A bolus of ^{177}Lu-DOTATOC (100 µL, 3.7MBq) was injected via the tail vein in mice and animals were euthanized at 2, 4, 24, 48, 72, and 168 h by CO_2 asphyxiation.

For pharmacokinetics studies, samples of blood and tissues (heart, kidneys, spleen, stomach, intestine, lung, liver, skin, bladder, bone, muscle, thyroid, adrenal and pancreas) of interest were excised, weighted, and counted for radioactivity in a p-type coaxial HPGe detector. Data were calculated as percent injected dose per gram tissue (%ID/g) with the aid of suitable standards of the injected dose.

C. Biodistribution Modeling of ^{177}Lu-DOTATOC

A biodistribution model can anticipate the time course of radioactivity concentration in a tissue region from knowledge of the local physiological variables and the input function (%ID/g of tissues). Compartmental modeling is the most commonly used method for eliciting the behavior of the radiopharmaceutical absorption, mathematically [16].

Estimation of the absorbed dose data will be more accurate if the data points of the activity–time curve increase. This model is a substitute way to the direct calculation of cumulated activity. In subsequence, the biodistribution modeling will be benefiting for molecular imaging and in vivo dosimetry.

In this part of work, using ANACOMPTM software, the equations derived from time dependent model of all the tissues were obtained. In fact, these equations produce numerous points (or % ID/g data) on the time–activity curves of each tissue, which it increases the acceleration of absorbed dose calculations as well as high accuracy.

D. Dosimetric Calculations

The calculated mean value of the percentage injected activity per gram of tissue (%IA/g) for the organs in mice was extrapolated to uptake in organs of a 73-kg adult, using the following formula:

$$\left(\frac{\%\mathrm{IA}}{g_{organ}}\right)_{human} = \left(\frac{\%\mathrm{IA}}{g_{organ}}\right)_{mouse} \times m_{human\ organ} \times \frac{M_{\mathrm{mouse}}}{M_{\mathrm{human}}} \quad (1)$$

where m is the organ mass and M is the total body weight $\frac{\%\mathrm{IA}}{\mathrm{g}}$.

The extrapolated values (%IA) in human organs at 4, 24, 72 and 168 h were fitted with the exponential equations of compartmental model and the equations were integrated to obtain the number of disintegrations in the source organs. The integrals (MBq.s) for all organs were evaluated and used for the dosimetry evaluation.

For instance, below formulation is related to accumulated activity in blood of mice:

$$\tilde{A} = \int_4^{168} 0.593e^{-0.07005\mathrm{t}} + 1.001e^{-0.02047t} - (0.2801)e^{(-0.4806\mathrm{t})} + (0.173)\,e^{(-0.6926\mathrm{t})} + (0.07991)e^{-0.8485\mathrm{t}} - 1.18\,e^{-0.03629\mathrm{t}} + (0.03313)\,e^{-0.594\mathrm{t}}\ .e^{-\lambda t}\,.dt$$

The absorbed dose in human organs was calculated by RADAR formalism based on biodistribution data in the rats [17]:

$$D = \tilde{A} \times \mathrm{DF} \quad (2)$$

where DF and $\tilde{A}$ is:

$$\mathrm{DF} = \frac{k\ \sum_i n_i E_i \varphi_i}{m} \quad (3)$$

$$\tilde{A} = \int_{t_1}^{\infty} A\,(t)dt \quad (4)$$

where the product $n_i\ E_i$ is the average energy per decay (0.147 MeV), ϕ_i is the absorbed fraction in the target, m is the mass of the target region (kg), and k is some proportionality constant ($\frac{\mathrm{mGy.kg}}{\mathrm{MBq.s.MeV}}$), and A(t) is the activity of each organ at the time t.

In this method the uptake per organ is extrapolated one-to-one from mouse to human. The extrapolated human source organ residence times were used as input in the OLINDA/EXM dosimetry software to calculate the absorbed doses per administered activity in humans [18].

III. Results and Discussions

A. Preparation and Quality Control of ^{177}Lu-DOTATOC

^{177}Lu was prepared with the specific activity of 2.6-3 GBq.mg^{-1} and radiochemical purity of 98%. Photons of 112 and 208 keV energy was observed while counting the samples on an HPGe. Thin layer chromatography showed the radiochemical purity of greater than 99% for ^{177}Lu-DOTATOC.

B. Biodistribution of ^{177}Lu-DOTATOC in Mice Bearing Breast Adenocarcinoma Tumors

After intravenous administration of ^{177}Lu-DOTATOC, it was highly accumulated in the tumors in 24 h p.i. (1.123 %ID/g), and approximately 63% of it was retained in 168 h p.i. (0.71 %ID/g). Radioactivity in the kidney reached a maximum level of 4.252 %ID/g at 4 h. After the kidneys as excretory organs, radioactivity was maintained at higher levels

in the tumor (1.094 to 1.123 %ID/g) and in the pancreas (0.599 to 0.752 %ID/g) before 24 h and then gradually decreased. Radioactivity in the liver was maintained at higher levels (0.424 %ID/g) before 4 h and gradually decreased with time. Low levels of radioactivity distribution were found in the skin, muscle, and spleen. While tumor uptake decreases slightly with time, tumor to blood and tumor to muscle activity ratio reach to the maximum amounts in 168 h post injection, that it is an important option for planning of the treatment and even imaging.

For better conclusion and ensure of acceptable positioning of complex, the accumulation of ^{177}Lu-DOTATOC species in main organs of normal rats [15] and tumoral mice is compared in Fig. 1.

As it can be seen in this figure, in both states, the complex is washed out from the circulation immediately, while the initiate uptake in tumoral mice is so less than other state. Kidney excretion as the main excretion route can be observed for both species that occur due to the water solubility for ^{177}Lu-DOTATOC, but its uptake in tumoral mice is higher than normal rats. A major difference in liver uptake is observed for both of them. Albeit, the major differences in spleen and liver uptake are observed for the two states but that is to say ^{177}Lu-DOTATOC has almost no significant and alarming spleen and liver accumulation.

Also, the radiolabeled compound has higher accumulation in tumoral term rather than normal term in pancreas as sstr-positive tissues at all times after injection. Thus, it goes without saying that adenocarcinoma tumor with high expression of sstr, has authority to accumulating of the complex more than other organs.

C. Biodistribution Modeling of ^{177}Lu-DOTATOC

The compartmental model was used to produce a mathematical description of the complex behavior in each organ and elicitation of exponential equation in order to obtain integrated data of accumulated activity and finally their utilization of them for dosimetry. The following equations (Table I) and figures (Fig. 2) of modelling were obtained for each organ. In each case, t=0 corresponds to the time of injection.

D. Dosimetric Calculations

The organ radiation absorbed doses for the administration of ^{177}Lu-DOTATOC to humans, as determined using compartmental modelling data from the residence times in mice, are shown in Table II.

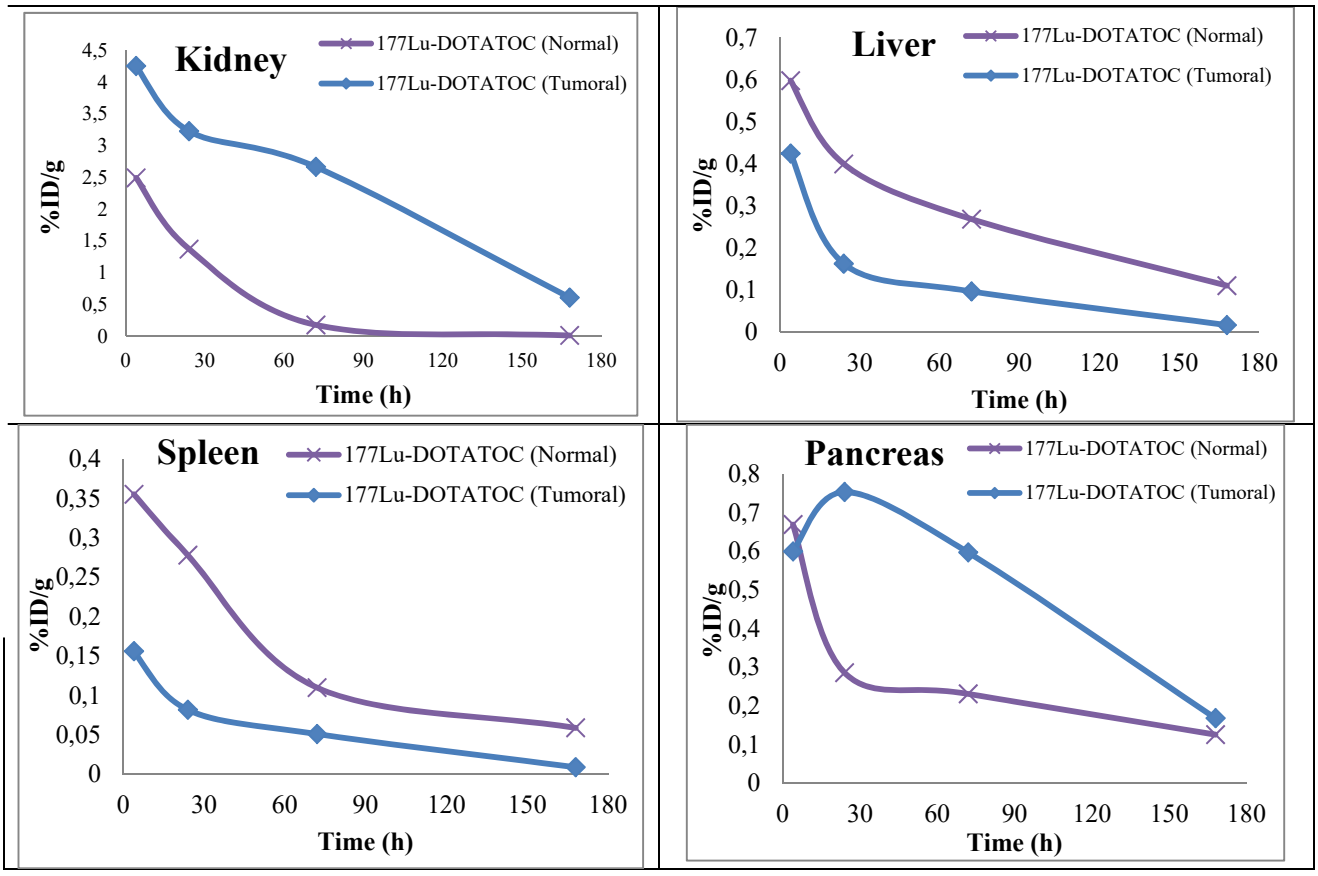

Fig. 1 Biodistribution of 177Lu-DOTATOC in normal and tumoral mice

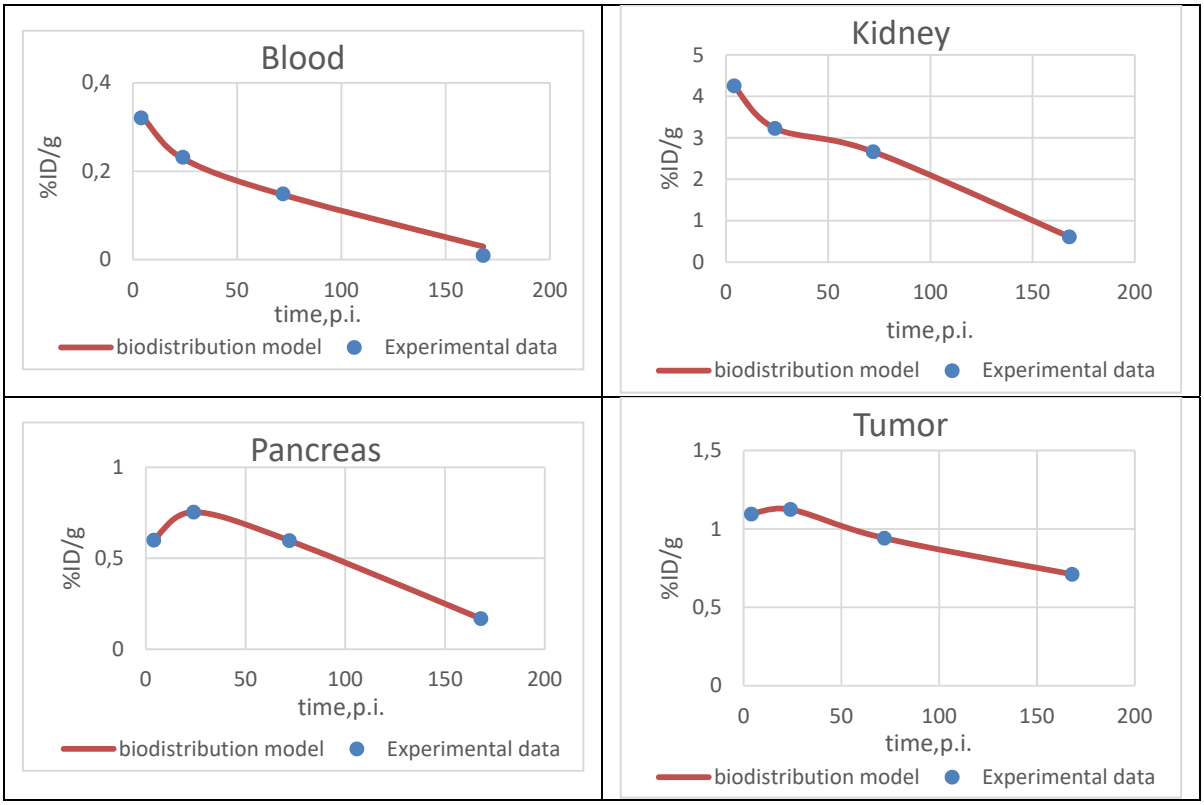

Fig. 2 Temporal behavior of biodistribution of ^{177}Lu-DOTATOC in mice organs

TABLE I
MATHEMATICAL RELATIONSHIPS DERIVED FROM BIODISTRIBUTION MODELING OF ^{177}LU-DOTATOC

Blood: $f_1 = 0.593e^{-0.07005t} + 1.001e^{-0.02047t} - (0.2801)e^{(-0.4806t)} + (0.173)\,e^{(-0.6926t)} + (0.07991)e^{-0.8485t} - 1.18\,e^{-0.03629t} + (0.03313)\,e^{-0.594t}$	**Liver:** $f_2 = -1.088e^{-0.3238t} - 1.115e^{-0.3277t} + (1.611)e^{(-0.2032t)} - (0.3391)\,e^{(-1.032t)} + (0.2145)e^{-0.01222t} + 0.363\,e^{-0.6094t} + (0.8404)\,e^{-0.5956t}$
Heart: $f_3 = -1.01e^{-0.03606t} + 0.622e^{-0.07656t} + 0.04146e^{(-0.3147t)} - 0.2766\,e^{(-0.175t)} + 0.1017e^{-0.5437t} + 0.8331\,e^{-0.02019t} - 0.06458\,e^{-0.7588t}$	**Kidney** $f_4 = 0.593e^{-0.0041t} - 2.365e^{-0.03t} - 0.7973e^{(-0.006t)} + 2.474\,e^{(-0.099t)}$
Tumor: $f_5 = 1.164e^{-0.00501t} + 0.0384e^{-0.01403} + 0.07462e^{(-0.00599t)} - 0.2356\,e^{(-0.09907t)}$	**Pancreas:** $f_6 = 1.323\,e^{-0.005t} - 0.3186e^{-0.014t} - 0.135e^{(-0.006t)} - 0.3814\,e^{(-0.099t)}$
Bone: $f_7 = -1.31e^{-0.3535t} + 0.4578\,e^{-0.6187t} + 0.2319\,e^{(-0.004966t)} + 0.5195e^{(-0.6168t)} + 0.4017\,e^{-0.3426t} - 0.2713\,e^{-1.041t} + 1.16\,e^{-0.2287t}$	**Skin:** $f_8 = 0.3199e^{-0.3147t} + 1.325\,e^{-0.01866t} - 0.1506\,e^{(-0.7771t)} + 0.404\,e^{(-0.313t)} - 0.7947\,e^{-0.1585t} - 1.07\,e^{-0.03107t} - 0.04161e^{-0.461t}$

TABLE II
CALCULATED ORGAN RADIATION DOSE ESTIMATES OF ^{177}LU-DOTATOC FOR HUMAN

Target	Estimated dose (mSv/MBq)
Adrenals	0.000659
Brain	0.000347
Breasts	0.000228
GB Wall	0.000518
LLI Wall	0.032473
Small Int	0.000443
Stomach Wall	0.003169
ULI Wall	0.000391
Heart Wall	0.007951
Kidneys	0.013684
Liver	0.008331
Lungs	0.008284
Muscle	0.005749
Ovaries	0.000548
Pancreas	0.039803
Red Marrow	0.020837
Bone	0.154402
Spleen	0.004221
Testes	0.000332
Thymus	0.000381
Thyroid	0.000418
UB wall	0.000401
Uterus	0.00044
Total Body	0.007342

Dosimetry was analyzed by the OLINDA/EXM software. Extrapolated radiation dose for a 73-kg male adult. GB: Gallbladder Wall; LLI, lower large intestine; Int: Intestine and content; ULI, upper large intestine, UB Wall: Urinary Bladder Wall.

IV. Conclusion

^{177}Lu-DOTATOC is a promising potential candidate for targeted therapeutic radiopharmaceutical for treatment of sstr-expressing tumors specially breast adenocarcinoma tumors. Also, it was shown that the compartmental model may be an approach for estimation of the human absorbed dose beneficially after i.v. administration of ^{177}Lu-DOTATOC with the time integrated data from the equations of modelling. The dosimetric calculations delivered the safe and appropriate dose for therapy of SSTR-positive tumors although further biological studies in other appropriate animals are still needed.

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