International Journal of Cancer Therapy and Oncology
www.ijcto.org

Development and biodistrubition modeling of ^{99m}Tc-DTPA

Mohammed Rabie Bricha[1], El-Mehdi Hamzaoui[2], Kaddour Ben-Hamou[1], Youssef Aboussaleh[3], Abdelhalim Mesfioui[4], Abdellah Ettabia[1], Hassan Moulahid[1], Brahim Zoubir[1], Ahmed El-Yahyaoui[5]

[1]*Department of Radiopharmaceuticals Production, National Centre for Nuclear Energy, Sciences and Technologies, Rabat, Morocco*
[2]*Department of IT Data Centre, National Centre for Nuclear Energy, Sciences and Technologies, Rabat, Morocco*
[3]*Department of Compartmental Neurosciences and Nutritional Health Team, Ibn Tofail University, Faculty of Sciences, Kenitra, Morocco*
[4]*Department of Genitics - Neuroendocrinologiy and Biotechnology, Ibn Tofail University, Faculty of Sciences, Kenitra, Morocco*
[5]*Department of Radiochemistry and Nuclear Chemistry, Mohammed V University, Faculty of Sciences, Rabat, Morocco*

[Presented at the 4th African Regional IRPA congress (AFRIRPA04), which was held from September 13-17, 2014 in Rabat, Morocco. This paper was reviewed and accepted by the scientific committee of the 4th African Regional IRPA congress]

Conference Proceeding

Abstract

Purpose: In this study, the team modeled the biodistribution and the efficiency of two ^{99m}-technetium diethylene triamine penta acetate (^{99m}Tc-DTPA) based radiopharmaceuticals. **Methods:** The first radiopharmaceutical (DTPA-CNESTEN) is developed at the laboratories of the radiopharmaceutical production unit of the National Center for Nuclear Energy, Sciences and Technologies (CNESTEN-Morocco), and the second one is the commercial DTPA (DTPA-ref). Freeze-dried kits were successfully radiolabeled (radiochemical purity >95%) with the ^{99m} Tc. Then drugs were injected to male BALB/c mice. In each 2 min, 5 min, 15 min, 1 h and 2 h time points after injections we evaluate tissue's distributions characteristics. At the end, an automatic modeling of the data were recorded from thyroid, blood and urinary excretion kinetics and biodistribution in mice using both DTPA kits. The study aimed to extract the parameters of the function used to fit the recorded data. **Results and Conclusion:** the team concluded that the biodistribution of ^{99m}Tc-DTPA can be modeled using a combination of two exponential parts. Moreover, the resultant plots showed that there is strong correlation between the formula found in literature and the one derived on the basis of the fit of data sets in this study. In addition, it was found that the biodistribution behaviors of the developed kit and the commercial one were very close. The obtained results suggest that the developed DTPA has practically the same kinetics as the commercial one.

Keywords: 99m-Technitium; Biodistribution; Data Fitting; DTPA; Modeling; Urinary Excretion Kinetics; CNESTEN

Introduction

Radiopharmaceuticals are radioactive compounds used for imaging and diagnosis of human diseases. Some of the radioactive are used for cancer therapy (about 5%).[1, 2] A radiopharmaceutical has two components: a radionuclide and an agent (the pharmaceutical), which directs the radionuclide to a receptor antigen, ionic pump, or other site of interest within the body. Some radiopharmaceuticals are simple, such as the ionic form of the radionuclide, while most radiopharmaceuticals have a complex chemical structure where the radionuclide provides a signal, indicating the site of localization of the carrier molecule.[2, 3]

Diethylene triamine penta acetic acid (DTPA) is one of pharmaceuticals. It used in different complexes for different objective, for examples: [111In-DTPA-DPhe1] octreotide is used for diagnostic stomastatin sintigraphy [4, 5, 6, 7], Diethylene triamine penta acetic acid neolactosyl human serum albumin labeled with technetium-^{99m} (^{99m}Tc-DTPA-LSA kit) afford the opportunity of hepatic receptor imaging.[8] Diethylene triamine penta acetate galactosyl human serum albumin (GSA) is used as human hepatic asialoglycoprotein receptor-binding radiopharmaceutical in Japan since 1992.[8] In constant, DTPA is an efficient candidate when it is radiolabeled with a therapeutic radioisotope such as ^{177}Lu or ^{90}Y.[9, 10, 11, 12]

Although this molecule has a negligible bridging capacity.[13] It is used as a simple radiopharmaceutical (technetiated radiopharmaceutical). ^{99m}Tc-DTPA is the most commonly used radiopharmaceutical for renography.[14]

The commercial DTPA kits are usually made of pentasodium or calcium trisodium salt of DTPA (diethylene triamine penta acetate. The freeze- dry kitcontains appropriate amounts of stannous chloride dehydrate in lyophilized form. Radiolabeling is performed by adding oxidant-free $^{99m}TcO_4^-$ to the kit vial and mixing. In this study, attempt was made to fit the

Corresponding author: Mohammed Rabie Bricha; Department of Radiopharmaceuticals Production, National Centre for Nuclear Energy, Sciences and Technologies, Rabat, Morocco.

***Cite this article as**: Bricha MR, Hamzaoui EL-M, Ben-Hamou K, Aboussaleh Y, Mesfioui A, Ettabia A, Moulahid H, Zoubir B, El-Yahyaoui A. Development and biodistrubition modeling of 99mTc-DTPA. Int J Cancer Ther Oncol 2015; 3(3):3318. **DOI:** 10.14319/ijcto.33.18*

ISSN 2330-4049

obtained experimental data in order to establish the developed DTPA biodistribution mathematical model. Also, the team had compared the behaviors of the developed kit (DTPA-CNESTEN) and the commercial one (DTPA-ref).

Methods and Materials

Preparation of kit for ^{99m}Tc-DTPA

Reagents

- Ethylenediamine pentaacetic acid (DTPA)
- Stannous chloride dihydrate: $SnCl_2.2H_20$;
- Hydrochloric acid HCl;
- Sodium hydroxide: NaOH;
- Water for injection;
- Nitrogen gas.

Technitium-^{99m}

Technetium-^{99m}-pertechnitate was obtained from a commercial ^{99m}Mo/^{99m}Tc generator (10 GBq) (schering/CIS- biointernational).

Gamma counter

The gamma counter used is NaI detector type COBRA 5002 from Packard.

Radiolabeling

Radiolabeling of DTPA-CNESTEN and the DTPA-ref, l, were performed by reconstituting the freeze-dried kit using 5 mL of freshly eluted $^{99m}TcO_4^-$ solutions. The $^{99m}TcO_4^-$ solutions were eluted from ^{99m}Mo/^{99m}Tc generator containing a maximum of 40 mCi of activity. The ^{99m}Tc-DTPA labeled in this manner should be stable for over 4 hours after labeling.[15, 16]

Animals

To evaluate tissue's distributions characteristics of DTPA-CNESTEN and DTPA-ref, biodistribution studies were performed using male BALB/c mice, average weight about 50-60 g. The animals were carried from Pasteur Institute- Casablanca. During this study, they were reared in our laboratory and they had free access to food and water at all times. The room temperature was about 20 °C.

Quality control

Radiochemical purity

Radiochemical purity (RCP) is an important quality parameter for radiopharmaceuticals, as their radiochemical form determines their biodistribution.[14, 17] For this reason, the use of radiopharmaceutical in vivo needs RCP testing to be carried out just before administration to the patient.[16, 18,19]

To determine radiochemical purity labeling efficiency of the product, European Pharmacopeia's chromatographic method, which is fast and easy, has been used.[14] This method involves ascending instant thin layer chromatography (ITLC). The ITLC strips were utilized as the stationary phase and two different solvent systems as indexed in European Pharmacopeia 2008. The procedure involved spotting about 2 µl sample of ^{99m}Tc DTPA onto chromatography strips 10 cm in length. After developing in the solvent, the strips were cut into two portions and the activity in each portion was measured in the form of counts using a gamma counter. The percentage activity at the origin and the front were determined. The complex ^{99m}Tc DTPA remained at the origin and free technetium traveled with the solvent (methyl-ethyl- ketone MEK) front (Rf = 0.9 - 1.0). The percentage of colloid was determined using 0.9% solution of sodium chloride as the mobile phase (the ^{99m}Tc-DTPA and TcO_4^- have about the same Rf in this system). Complexes were successfully labeled (RCP > 95%) and stable about 24 hours.

Biodistribution study

Biodistribution studies regroup almost all of studies using radiopharmaceuticals. It is an important analytical step in preclinical studies.[20]

After successful operations of radiolabeling, dilution (with 0.9% NaCl solution) to obtain a volumetric activity about 400 µCi / 5.4 mL and RCP was above 95%. Mice were weighed and injected with 0.1 mL of the radiolabeled compound in the tail vein. After injections, the animals were kept in separately numbered beakers. And their urine was collected.

The injected activity is calculated by taking the difference between the weight of the syringe before and after the injection. At the end of 2 min, 5 min, 15 min, 1 h and 2 h time points, the animals were killed. They were dissected and blood sample was first taken by heart puncture. Then the other organs of interest were carefully dissected, placed in individual disposable plastic tubes and accurately weighed. The focus of this study was thyroid, blood, and urine. At least three animals were studied at each time point. After killing each mouse, the tail was removed and kept separately. The activity in the organs and the tail were measured in the gamma counter.

The total retained dose (%TRD) estimated as follows:

$$\%\ \mathrm{TRD(organ)} = \frac{A}{B} \times 100$$

Where, "A" denotes the activity or counts in the organ of interest and "B" represents the activity or counts in all organs and the carcass except for the tail.

To accurately estimate the activity and to account for decay corrections in the ^{99m}Tc activity, standard solutions of the radiopharmaceuticals were prepared.

 ISSN 2330-4049

Data Modeling

Radiopharmaceuticals administered to humans disappear from the biological system through fecal or urinary excretion. This biological disappearance follows a bi-exponential law [21, 22], expressed as:

$$y = a\,e^{bt} + c\,e^{dt} \quad (eq.1)$$

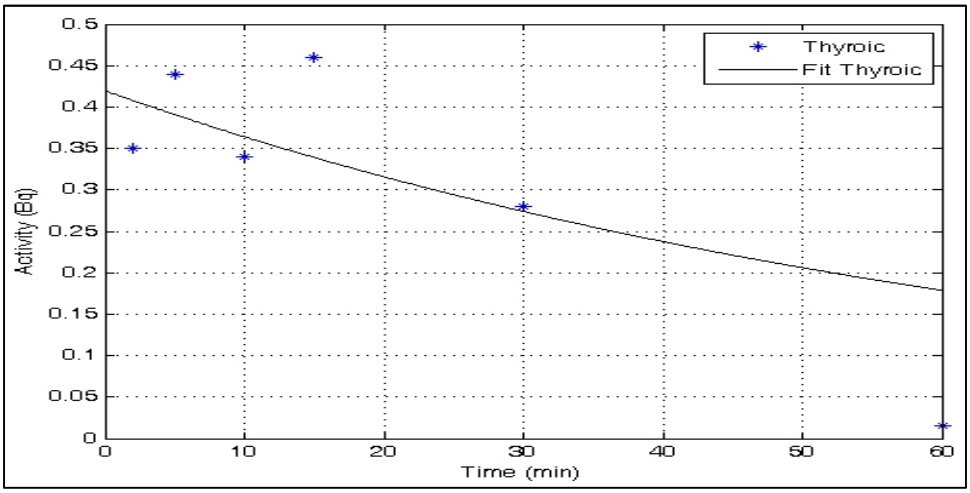
where, "t" designate time, "a" and "c" the intercepts and "b" and "d" are the slopes of the fast and the slow components of the plasma disappearance curve.

In this work, the team used the Open Curve Fitting Tool 3.3.1, incorporated under the MATLAB R2013b platform [23], to compute the best values of the exponential parameters *a*, *b*, *c* and *d* with 95% confidence bounds. The optimal values of these parameters correspond to the minimum of the mean square error, which can be defined as the differences between the original data y (response value) and the predicted response value $\hat{y}$ at each predictor value.

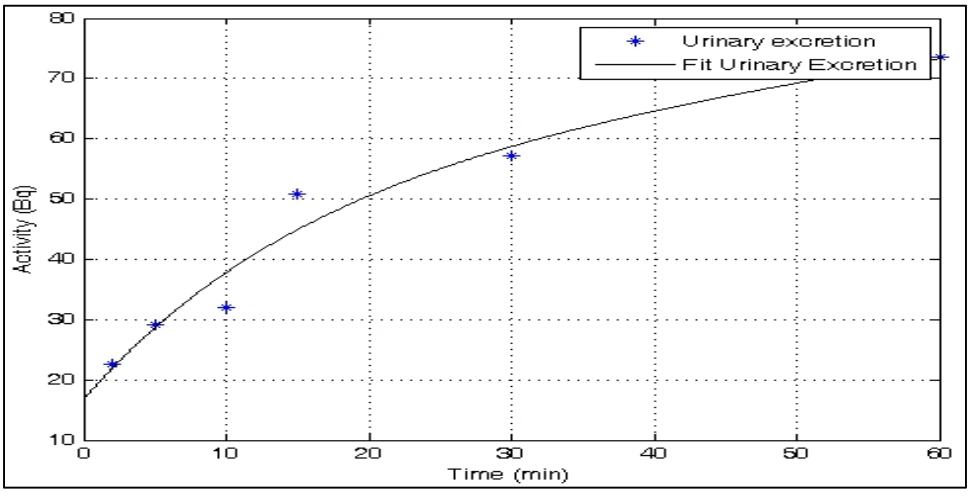
For both sets of data, the mean values of the parameters defined above were calculated. The **Table 1** below shows the obtained values of the model parameters; whereas **Figure 1** illustrates the plot of the experimental data and the corresponding mathematical model.

TABLE 1: Parameter values of the bio-distribution data fitting model.

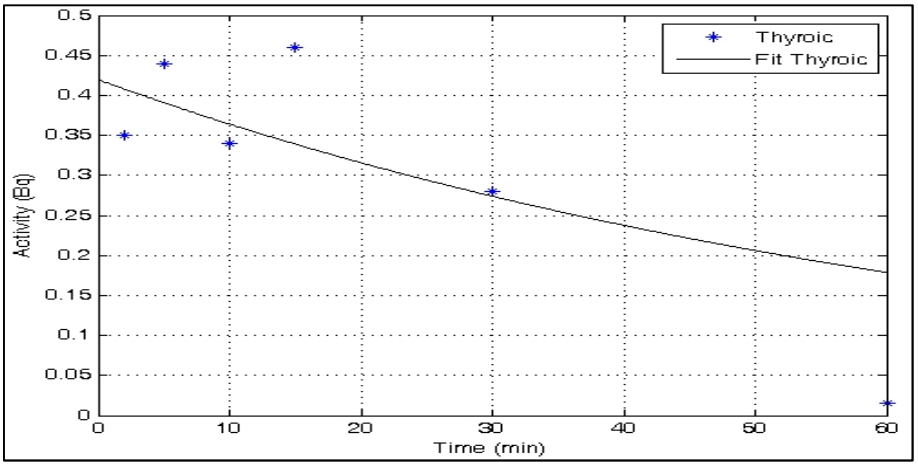
Biological parts	Model Coefficients				MSE
	a	b	c	d	
Blood	14.23	-0.03257	0	-0.03257	2.7603
Urinary excretion	57.08	0.004396	-40.31	-0.06128	5.9184
Thyroid	0.4199	-0.01421	0	-0.01421	0.1542



(a)



(b)



(c)

FIG. 1: Plot of the experimental data and the corresponding mathematical model in different biological parts. (a) Blood (b) Thyroid (c) Urinary excretion.

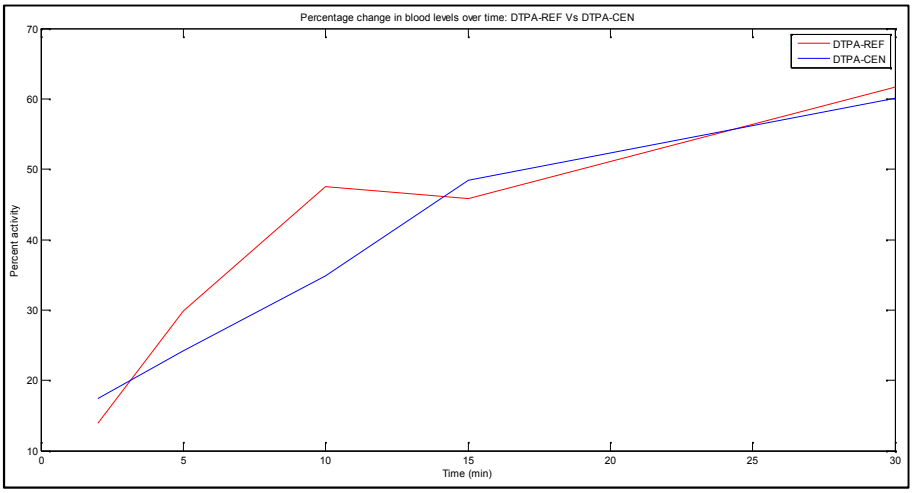


FIG. 2: Comparison of the developed DTPA and the commercial one in blood.

The comparison of the results obtained for the developed DTPA at the CNESTEN and the commercial one are illustrated in the **Figure 2** below. This figure shows that the developed medicine has the same behavior as the commercial one.

Conclusion

In this study, the team concluded that the biodistribution of DTPA developed at the CNESTEN, can be modeled using a combination of two exponential parts. In addition, the graphics representations of the modeled data show that the drug evolution in both thyroid and blood has an opposite sense with its evolution in the urinary excretion. However, the obtained results proved that the developed DTPA has practically the same kinetics as the commercial one.

Conflict of interest

The authors declare that they have no conflicts of interest.

The authors alone are responsible for the content and writing of the paper.

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