LABELING OF SOMATOSTATIN WITH ^{99m}Tc USING DTPA and D-PA AS BIFUNCTIONAL AGENTS

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1. INTRODUCTION

Peptides have been preferred for diagnostic and threpautic purposes with regards to their small sizes, easy preparing, ability to attach bifunctional agents from their –C and –N terminals in nuclear medice for last decades[1].

Somatostatin(SST) is a peptide hormone containing 14 aminoacids with a short biological half-life and it has a wide spectrum in life sciences. Somatostatin and its analogs have been radiolabeled with such radionuclides as ^{99m}Tc, ^{111}In, ^{123}I, ^{64}Cu, $^{66/67/68}$Ga, ^{18}F, ^{186}Re[1-2].

In designing new peptide radiopharmaceuticals, ^{99m}Tc has been preferred for most applications due to its optimal emission of gamma ray energy and its suitable half-life for imagining purposes[3-8].

By the way of BFCA approach appears to be the most suitable for preparation of the ^{99m}Tc-labeled peptide radiopharmaceuticals, especially those for clinical use, because of its convenience, well-defined labeling chemistry and feasibility of kit formulation. A major limitation of this method is the lengthy and costly synthesis of the BFCAs.

Many radiopharmaceuticals have been obtained by using DTPA, DOTA, HYNIC, DADT. DTPA has been preferred by many researchers as a BFCA for labeling somatostatin analogs with various radionuclides[1,9-10]. DTPA is a popular bifunctional agent to label somatostatin analogues. Octreotide is most widely used somatostatin analogue and ^{111}In-DTPA-octreotide is the first improved peptide radiopharmeceutical having large area for imaging tumor[11-13].

In our previous study, SST was labeled with ^{99m}Tc using D-Penicillamine (D-PA) as a BFCA and its labeling conditions and stability were compared with DTPA[14]. The aim of this study is to investigate radiopharmaceutical potential of ^{99m}Tc-D-PA-SST on male Albino Wistar rats and make a comparasion via ^{99m}Tc-DTPA-SST complex.

2. MATERIALS AND METHODS

$Na^{99m}TcO_4$ was supplied from Department of Nuclear Medicine of Ege University. D-PA, DTPA were purchased from Sigma. Somatostatin-ucb was a present from UCB Pharma Ltd. Water was purified by filtration through a Waters-Millipore purification system. All other chemicals were supplied from Merck Chemical Co. and Aldrich Chemical Co.

Labeling procedure

The radiolabeled compounds ^{99m}Tc-D-PA and ^{99m}Tc-DTPA were prepared using stannous chloride reduction method and labeling pH was adjusted to 7.

The preparation of ^{99m}Tc-D-PA-SST and ^{99m}Tc-DTPA-SST complexes were carried out using stannous chloride reduction and BFCA labeling method at 80°C described as previous study[14]. Labeling of both complexes were performed with 55.5 MBq (1.5 mCi) $^{99m}TcO_4^-$.

Quality control procedures

Thin Layer Radiochomatography (TLRC): Physiological serum was used as developing medium. When the solvent reached the desired distance along the strip in the developing medium, the strips were taken from the TLRC tank and dried at room temperature. Each TLRC sheet was covered by a cello-band after its development and was cut into 0.5 cm wide strips, which were counted by using detector. TLRC chromatograms were obtained by plotting counts versus distance. The R_f values and labeling yields were derived from these plots.

Electrophoresis: Electrophoresis was performed with a Gelman Electrophoresis Chamber. Cathode and anode poles and application points were indicated on cellulose acetate strips and these strips were moistened

with physiological serum. The application point was at zero point of the electrophoresis paper. They were placed in the electrophoresis chamber after the samples were set on the strips. Standing time and applied voltage were one hundred and twenty minutes and 250 volts, respectively. The developed strips were dried and cut into 1 cm pieces. They were counted.

For all chromatographic method Cd(Te) detector equipped with a RAD 501 single-channel analyzer was used to count the radioactivity.

High Performance Liquid Radio Chromatography (HPLRC) (model LC-10Atvp Shimadzu): Eliminating of possible uncertainties was also performed using HPLRC.

Biodistribution studies on rats

The protocol was approved by Institutional Animal Review Committee. ^{99m}Tc labeled product was sterilized by passing through a 0.22 μm membrane filter. 200 ng in 0.2 mL volume per rat was then injected into the tail vein of male Albino Wistar rats that were 24 weeks of age and had a weight range of 110 - 150 g. The number of animals used for each study was 3 per group at each time point. The rats were sacrificed under intense ether atmosphere in 30th, 120th and 300th minutes after injection and their organs were removed. The activities were counted and the percent of activities per gram of organ weight (% injected activity/g organ) was then determined.

In vivo blocking experiments: Ten micrograms of cold ligand (SST) was prepared under the same conditions as ^{99m}Tc labeled ligand and injected into the rats 15 min before ^{99m}Tc-ligand to determine whether uptake in SST receptors expressing target tissue is specific. The same procedures were repeated as indicated above.

Statistical analysis: Differences in the mean values of measured activities were evaluated statistically by SPSS 10 program (Univariate Variance Analyses and Pearson Correlation). Probability values <0.05 were considered to be significant. Pearson correlation was carried out between organs for ^{99m}Tc-D-PA-SST and ^{99m}Tc-DTPA-SST.

3. RESULTS AND DISCUSSION

Labeling efficiencies were higher than 98%. Labeled products were also stable more than 5 hours at room temperature. According to electrophoresis studies both labeled complexes had an anionic structure. High Performance Liquid Radio Chromatography (HPLRC) was also established for elimination of possible uncertainties.

Biodistribution studies of both ^{99m}Tc-D-PA-SST and ^{99m}Tc-DTPA-SST were performed on Albino Wistar rats. The activity per gram organ was calculated and time-activity curves were plotted as shown in Figs.1 and 3. Figs.2 and 4. represented receptor blocking studies.

Although five different somatostatin receptors are known and they are found in different part of brain, pancreas, gastointestinal system and lymphatic tissue[1], we could not observe a significant correlation between saturated and unsaturated biodistribution studies for ^{99m}Tc-D-PA-SST. However there is considerable relation for ^{99m}Tc-DTPA-SST complex and it could be receptor specific for kidney, pancreas, s. intestine, stomach and spleen.

According to biodistribution studies ^{99m}Tc-D-PA-SST exhibited longer uptake time in organs and its clearence time was longer when compared to ^{99m}Tc-DTPA-SST complex. As a result, using of D-PA as chelating agent can enhance the biological half life of somatostatin which has short biological half life. D-PA was proposed as a BFCA in previous work[14] so we concluded that ^{99m}Tc-D-PA-SST complex may suitable for in vivo studies in present study.

In conclusion, these results can be improved in further studies and ^{99m}Tc-D-PA-SST may be used as a peptid-based radiopharmaceutical for diagnostic purposes.

4. REFERENCES

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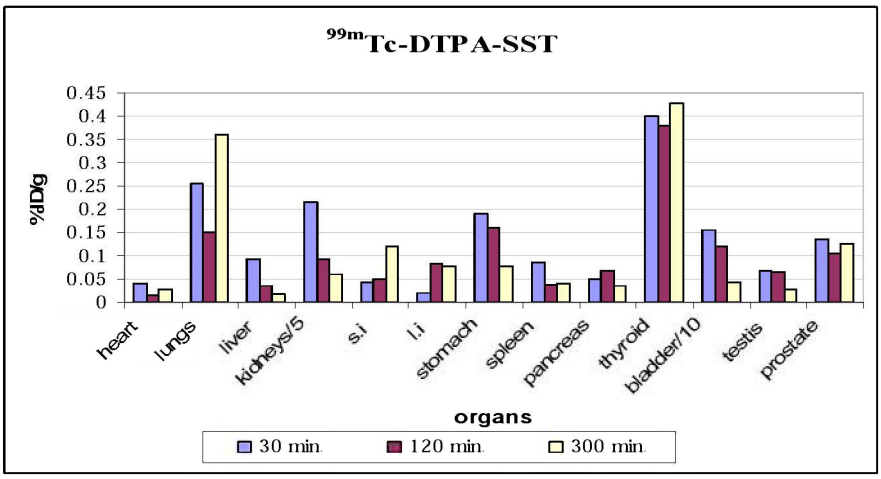


Figure 1. Biodistrubition of ^{99m}Tc-DTPA- SST complex for some organs.

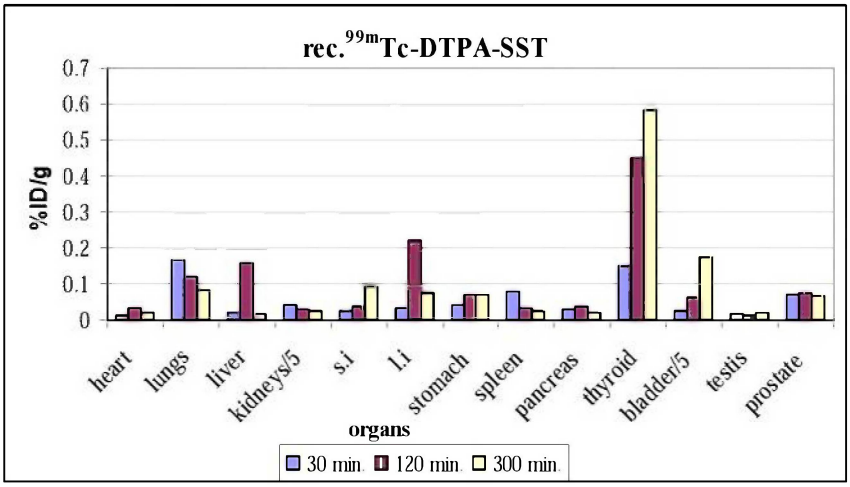


Figure 2. Biodistrubition of receptor blocking study of ^{99m}Tc-DTPA- SST complex for some organs.

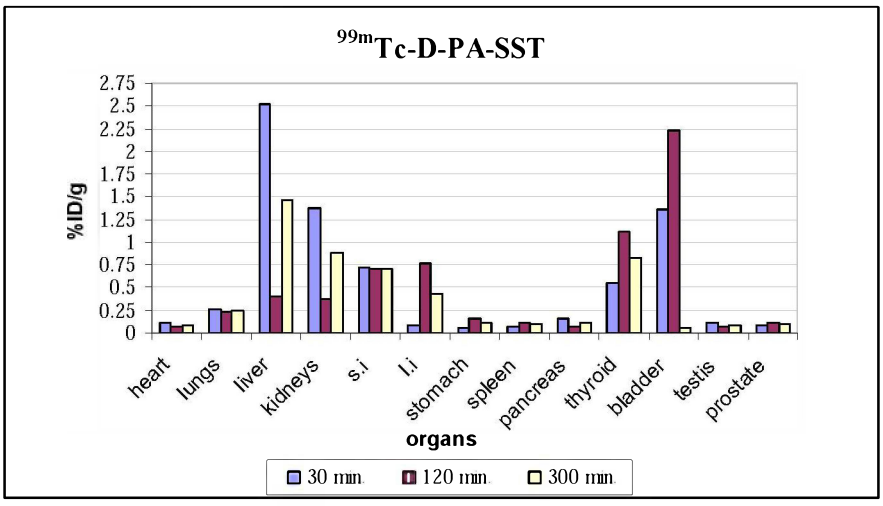


Figure 3. Biodistrubition of ^{99m}Tc-D-PA- SST complex for some organs.

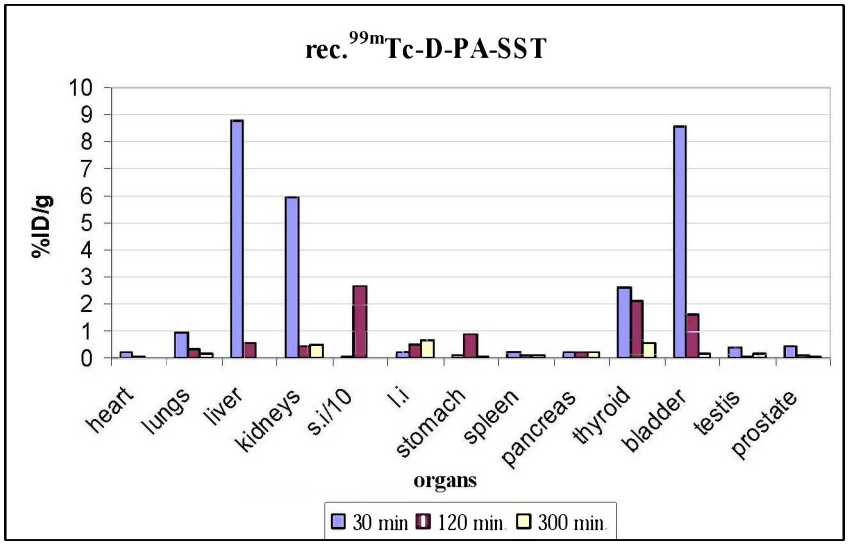


Figure 4. Biodistrubition of receptor blocking study of ^{99m}Tc-D-PA- SST complex for some organs.